ROLE OF NUTRACEUTICALS AND THEIR RELEVANCE IN SECONDARY COMPLICATIONS OF TYPE-2 DIABETES MELLITUS USING DIFFERENT ANIMAL MODELS

Thesis submitted to

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For the award of the degree of

Doctor of Philosophy

In the Faculty of

Pharmacy

by

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(Registration No: KLEU/Ph.D./15-16/ DO1215012)

Under the Guidance of

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OCTOBER-2020

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LIST OF ABBREVIATIONS

Sl. No.	Abbreviation list	
01	AAFCO	Association of American Feed Control Officials
02	ACh	Acetylcholine
03	ADP	Adenosine diphosphate
04	AGE	Advanced glycocylation end product
05	ALT	Alanine transaminase
06	ALP	Alkaline phosphatase
07	ANOVA	Analysis of variance
08	AST	Aspartate aminotransferase
09	ATP	Adenosine triphosphate
10	BRB	Blood retina breakdown
11	B.W.	Body weight
12	Ca^{+2}	Calcium
13	CAT	Catalase
14	CCl_4	Carbon tetrachloride
15	CF	Compare
16	CML	Carboxymethylsin
17	CPCSEA	Committee for purpose of control and supervision of experiments on animals

18	**C-RP**	**C-reactive protein**
19	**CVD**	**Cardiovascular disease**
20	**DCM**	**Diabetic cardiamyopathy**
21	**DNA**	**Deoxyribonucleic acid**
22	**DM**	**Diabetes mellitus**
23	**DN**	**Diabetic neuropathy**
24	**DR**	**Diabetic retinopathy**
25	**EC**	**Effective concentration**
26	**ELISA**	**Enzyme linked immune sorbent assay**
27	**EMC-D**	**Encephalomyocarditis infection**
28	**FBG**	**Fasting blood glucose**
29	**FDA**	**Food and drug administration**
30	**GDM**	**Gestational diabetes mellitus**
31	**GIT**	**Gastro intestinal tract**
32	**GLP-1**	**Glucagon like peptide -1**
33	**GLUT-2**	**Glucose transporter 2**
34	**GP**	**Glycogen phophorylase**
35	**GSH**	**Gluthathione**
36	**GSH-Px**	**Gluthathione peroxidise**
37	**H_2O**	**Water**
38	**H_2O_2**	**Hydrogen peroxide**

39	**HbA1c**	**Glycosylated haemoglobin**
40	**HCl**	**Hydrochloric acid**
41	**HDL**	**High density lipoprotein**
42	**HOMA-IR**	**Homeostasis model assessment list**
43	**HRV**	**Human rhino-virus**
44	**IAEC**	**Institutional animal ethics committee**
45	**IC**	**Inhibitory concentration**
46	**ICAM-1**	**Intracellular adhesion molecule 1**
47	**IFG**	**Impaired fasting glucose**
48	**I.P.**	**Intra peritoneal**
49	**IR**	**Insulin resistance**
50	**IS**	**Insulin sensitivity**
51	**IGT**	**Impaired glucose tolerance**
52	**K^+**	**Potassium**
53	**KCL**	**Potassium chloride**
54	**LDL**	**Low density lipoprotein**
55	**LHD**	**Lipid hydroperoxide**
56	**MDA**	**Malondialdehyde**
57	**Na^+**	**Sodium**
58	**NAD**	**Nicotinamide adenosine dinucleotide**
59	**NO**	**Nitric oxide**

60	**NOS**	**Nitric oxide synthase**
61	**NPDR**	**Non-proliferative diabetic retinopathy**
62	**NSP**	**Non-saturated polysaccharide**
63	**O_2**	**Oxygen**
64	**OECD**	**Organization for economic co-operation and development**
65	**OGTT**	**Oral glucose tolerance test**
66	**P.O.**	**Per oral**
67	**PDR**	**Proliferative diabetic retinopathy**
68	**PHF**	**Polyherbal formulation**
69	**PKC**	**Protein kinase C**
70	**PPAR-γ**	**Peroxisome proliferator-activated receptor gamma**
71	**PPBG**	**Post-prandial blood glucose**
72	**PRC-β**	**Protein kinase-C beta**
73	**PUFA**	**Poly unsaturated fatty acids**
74	**RNA**	**Ribonucleic acid**
75	**RNS**	**Reactive nitrogen species**
76	**ROS**	**Reactive oxygen species**
77	**RS**	**Reactive species**
78	**SGLT2**	**Sodium glucose co-transporter 2**
79	**SGOT**	**Serum glutamatic oxaloacetic transaminase**

80	**SGPT**	**Serum glutamic pyruvic transaminase**
81	**SOD**	**Superoxide dismutase**
82	**STZ**	**Streptozotocin**
83	**STZ-NA**	**Strpetozotocin-nicotinamide**
84	**T2D**	**Type 2 diabetes**
85	**T1DM**	**Type 1 diabetes mellitus**
86	**T2DM**	**Type 2 diabetes mellitus**
87	**TC**	**Total cholesterol**
88	**TG**	**Triglyceride**
89	**TNF-α**	**Tumor necrosis factor alpha**
90	**VEGF**	**Vascular endothelial growth factor**
91	**VLDL**	**Very low density lipoprotein**
92	**WHO**	**World health organization**
93	**ZO**	**Zucker obese**

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ABSTRACT

Objective: This study aimed to evaluate the role of Polyherbal formulation (PHF) in secondary complications of type 2 diabetes mellitus using animal models.

Background: Nutraceuticals refers to natural functional/ medicinal foods or bioactive phytochemicals that have health promoting, disease preventing or medicinal properties. Herbal drugs are gaining acceptance worldwide because of their effectiveness, lesser side effects and relatively low cost. In view of the benefits that it offers, several proprietary PHF are available for use, therefore, investigating the role of PHF for their therapeutic utility is important and desirable to provide a rational basis for its employability in metabolic disorders like diabetes mellitus.

Materials and Methods: All chemicals and reagents were of analytical grade. Biochemical estimations were performed using commercial test kits and procedure suggested is followed. Histopathological investigations were performed by competent pathologist, blind to treatment protocol. Rodents used for these investigations were procured from licensed animal breeder and CPCSEA guidelines were followed during experimentation and maintenance of laboratory animals during the entire course of investigation. IAEC clearance was taken prior to all animal experimentations**.** Role of PHF on antidiabetic and several secondary complications was assessed in two animal models viz., streptozotocininduced and fructose induced diabetes in rodents. Diabetic animals were treated with two preselected doses based on the oral acute toxicity profile of PHF. Simultaneously, a vehicle control non diabetic control and diabetic / positive control and standard drug treated (metformin) groups were employed for comparative purpose. Following once a day, oral PHF administration done as per standard protocol, various biochemical parameters to assessed to evaluate the effect of PHF on various parts of the

body and histopathology study was done to correlate the biochemical and pathophysiological changes / restoration of organ / structural integrity, if any, due to PHF treatment. All data were expressed as Mean ± SEM of n=6, followed by one-way ANOVA and applicable post – hoc using Prism statistical software. All comparison was made against positive control group.

Results: Statistically significant, dose dependent changes in the antioxidant enzymes / MDA levels observed in the PHF treated diabetic animals, compared to untreated diabetic animals (in both models) clearly establishes a protective effect resulting from the PHF treatment**.** In addition, analgesic effect in diabetic neuropathy, reversal of neuropathic damages in sciatic nerves, nephropathy and retinopathy. Biochemical changes, taken with restoration of structural integrity / changes in PHF treated animals suggests a significant change compared to untreated diabetic animals.

Conclusion: In both animal models employed for investigations**,** PHF possess not only antidiabetic property, is also protective against secondary complications of diabetes via antioxidant mechanism and further studies are required for bioactivity guided drug discovery to isolate lead compounds, which may be responsible for the observed protective effects and its use in diabetic patients to protect against secondary complications.

Keywords: Polyherbal formulation, Nutraceuticals, Diabetes mellitus, Streptozotocin, Fructose, Metformin.

1.0 INTRODUCTION

Patients, worldwide, are challenged in combating the disease "Diabetes mellitus (DM)", affecting their day-to-day life at an alarming level. Commonly known as a "Metabolic disorder", DM progresses from an early asymptomatic stage to a "multiple-organ-damage" state, requiring the intervention of Pharmacology. This metabolic syndrome, profoundly affects the quality and longevity of life. Both clinicians as well as patients are challenged with facing a heterogeneous disorder; because as the complications progress, the response to treatment varies with each individual patient over the due course of time.[1]

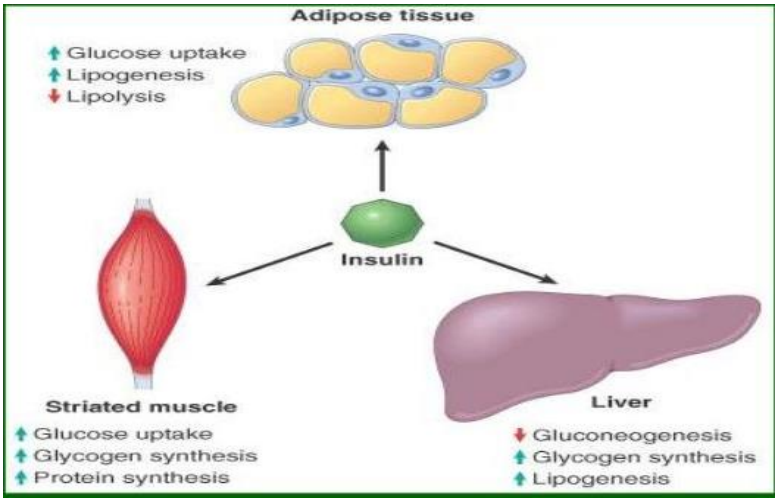


Figure 1: Role of insulin[1a]

Secondary complications due to diabetes mellitus: Liver and skeletal muscles of a diabetic patient are not able to store glycogen and tissues lose the ability to utilize glucose. Chronic hyperglycaemia may turn into multiple organ damage and diabetic associated complications not excluding hypertension, Lipid profiles that increases risk of heart diseases. This metabolic syndrome shares a common phenotype of hyperglycaemia as a consequence of its absolute lack of insulin as in case of T1DM or resistance to insulin as observed in case of T2DM.[2] Consistent chronic hyperglycaemia enhances toxic effects in a number of tissues, especially

in neurons as they are more susceptible to glucose uptake. Following are secondary complications in brief that arise due to chronic hyperglycemia.[3]

Cardiopathy: (Coronary illness and stroke) Having hypertension/elevated cholesterol builds the danger of heart assault, stroke and other heart ailments.

Nephropathy: Hyperglycaemia unchecked for years can irreversibly damage renal function.

Neuropathy: (Nerve damage) having high glucose over significant lots of time can harm the nerves. There are various sorts of nerve harm; well known among others is diabetic peripheral neuropathy. The side effects can incorporate, torment, affectability, deadness and weakness.

Retinopathy: Hyperglycaemia that remains untreated with drugs and appropriate life style changes leads to retinopathy- early onset of cataract, glaucoma etc.

Gum disease: High glucose expands the hazard for bacterial diseases, bringing about poor gum condition.

As these secondary complications are well established concepts, no details are given.

DM is a kind of condition which does not have any permanent cure, but it needs a change in life-style in order to it should not worsen the disease to its associated complications as mentioned above. The change in life style and compliance with the medications and self-monitoring of blood glucose at home are among important steps in the management of diabetes. At present, Haemoglobin A1c (HBA1c) is one of the standard tool which is widely used for monitoring blood sugar levels in patients.

Considering the lethality of diabetes associated complications, complementary and alternative beneficial products have made nutraceutical particularly appealing amongst medicine practitioners and patients. Although, nutraceuticals were in the scenario since a long time, it

was only recently proven scientifically.[4] Nutraceuticals can be of both plant and animal origin. Most of the nutraceuticals have plenty's of medicinal properties.[5] Nutraceutical considered as a one of the source of safe health promotion, mostly in amelioration of life threatening diseases, as they also possess anti-oxidant activity.[6-13]

Glycosylated Haemoglobin (HBA1c)

Within body, when there is exorbitant sugar, it will bind with proteins. The platelets that circle in the body live for around a quarter of a year prior to the cease to exist. The point at which sugar ties to haemoglobin proteins, resulting in glycosylated haemoglobin (HBA1c) and its assessment gives an estimate of average blood glucose level in the preceding three months.

Criteria as per American Diabetes Association:

Normal range	(<5.9%)
Poor control	(8%)
Good control	(<7%)

By estimating HBA1c it have advantage that it will give sensible and well firm perspective going on through the span of time (three months). There is an immediate relationship between A1c levels and normal glucose levels as pursues.

A1c is a screening tool, which gives idea to physician about if someone is diabetic or not if there is increase in values, because there are no such guidelines in use.[14,15]

There are four prominent speculations have been proposed in order to clarify how hyperglycaemia may prompt incessant entanglements of diabetes mellitus and these are; End products of advanced glycosylation – AGEs and augmented glucose metabolism via Sorbitol pathway, increase the arrangement of di-acyl-glycerol prompting actuation of protein kinase

(PKC) and raised dimensions of blood glucose increment the transition by hexosamine pathway, which lead to generation of which leads to generation of a substrate (Fructose 6 Phosphate) for 0- glycosylation and proteoglycan products. In diabetes, there is a condition known as expanded free radical creation, which is a result of disparity between the radical creating and radical scavenging systems promotes production of potentially toxic reactive oxygen species. It might be only incorporate not that it will expanded non-enzymatic and auto-oxidation glycosylation yet in addition associated with metabolic stress which results from change in vitality digestion, dimensions of provocative go betweens and the status of cell reinforcement protections. In spite of presently available traditional and newly anti-diabetic agents from various sources, diabetes and its associated entanglements keep on being a noteworthy weight on total population.

1.1 BACKGROUND

Conventional Therapy:

Agreement on treatment of T2D is that way of being the board is bleeding edge of treatment choices. Notwithstanding exercise program, weight control/dietary arrangement and restorative nourishment treatment, oral glucose bringing down medications and insulin infusions contain the traditional treatments. Pharmacological treatment is demonstrated for when there is FBG level surpasses >140 mg/dL and PPBG level surpasses >160 mg/dL.[16]

Need and Scope of Alternative Medicine:

Due to the inclusion of various adjustments in way of life, T2DM has been turn into noteworthy medical issue in developing nations. Way of life changes include predominantly following an exacting eating regimen, eliminating starches (sugar) and working out (at any rate strolling for at least 20 to 40 minutes per day). Mediation of pharmacological treatment will start at point when there is negligence in control of blood glucose by edible and exercise. [17] These medicines have their own downsides extending from advancement of obstruction and antagonistic impacts to absence for response in a huge fragment ill populace. Besides, available anti-diabetic agent have no glucose-bringing down specialists sufficiently control the hyperlipidemia that is a hazard factor for diabetes mellitus.[18] The impediment of presently accessible oral anti-diabetic agents either as far as viability/wellbeing combined with the rise of the malady into worldwide pandemic have supported elective treatment that can oversee diabetes all the more effectively and securely.

Herbal Medicines:

Herbal preparations are generally considered as safe, effective, relatively less toxic for variety of medical conditions including several metabolic disorders. Herbs that is appropriate for

human beings is result of native knowledge of indigenous that is most suitable for human consumption.[19,20]

A large number of the medicinal products are now easily available to physicians which are having a long history as utilised by home grown medicines, including. WHO estimation point to growing inclination towards herbal medication by world population – utilizing the same as part of essential human health services. Pharmaceuticals are restrictively costly for the greater part of the total populace, half of which lives on under $2 every day. In correlation, home grown prescriptions can be developed from seed or accumulated from nature for almost no expense. Herbal medicine is a noteworthy segment in all customary medication frameworks and a typical component in Siddha, Ayurveda, Homeopathic, Naturopathic, Traditional Chinese Medicine, and Native American Medicine.[21,22]

Among the different dynamic mix right now are employed. Currently less than 10%, has proved and a positive correlation exists between their utilization and restorative use – i.e., underutilized. It's interesting to note that large majority of herbs with reported restorative properties are from underdeveloped countries. Around seven thousand isolated compounds of proven therapeutic properties are of plant origin and have also found its place in modern pharmacopoeias.[23-25]

According to ancient literature, in excess of 800 plants are accounted for to have antidiabetic properties[11]. Ethano-pharmacological overviews show that in excess of 1200 plants are utilized in customary prescription for their unified hypoglycaemic activity.[26] Indian Materia Medica has referenced for various dravyas, which have been accounted for viable in Madhumeha.[27]

The indigenous or traditional diet routine may not be helpful in bringing/ lowering down the glucose level to a similar degree as available anti-diabetic agents does, still it has different impacts, which might valuable for administration of the aliment and complications.[28] In

diabetes, some of the home grown remedies options are demonstrated to give symptomatic alleviation and aid the avoidance of the diabetes associated complications. A few plants have been demonstrated in recovery of ß-cells and in conquering opposition.

Notwithstanding keeping up ordinary glucose level, a few plants likewise reported to have antioxidant and cholesterol bringing down activity. Administration of T2DM is conceivable with medication which can bring down the glucose level at one side and re-establish the liver glycogen level on another side. Current arrangement/ available medication, no such medication, have dual properties.[29] Blood glucose lowering properties of herbs /natural concentrates and related proprietary have been confirmed by animal models, usually chemically induced, mimicking human diabetes. Interestingly, *Galega officinalis* is the source of Metformin.[30-34]

Out of the 1200 customary plant medications for diabetes that have been accounted for, just few these have gotten logical and restorative assessment to evaluate their viability. All plants have indicated differing level of hypoglycaemic and anti-hyperglycaemic activity.

Table 1: Herbs used for anti-hyperglycaemic activity[35]

Herbs used for treating Diabetes	Common Name	Part Used
Allium sativum	Garlic	Bulb
Allium sepa	Onion	Bulb
Aloe vera	Ghikumari	Leaf
Azadirachta indica	Neem	Leaf
Brassica juncea	Indian mustard	Leaf
Caesalpinia bonducella	Kanderi	Bulb
Cajanus cajan	Tuar	Seed
Coccinia indica	Kundru	Fruit
Eugenia jambolana	Amrut	Fruit
Ficus benghalensis	Banyan tree	Leaf
Gymnema sylvestre	Gurmar	Leaf
Momordica charantia	Bitter lemon	Fruit
Mucuna pruriens	Kiwanch	Leaf
Murraya koeingii	The curry tree	Leaf
Ocimum sanctum	Tulsi	Leaf
Pterocarpus marsupium	Banda	Leaf
Swetria chirayita	Chirata	Leaf
Syzigium cumini	Jamun	Fruit
Tinospora cordifolia	Giloe	Leaf
Trigonella foenum-graceum	Fenugreek	Seed

Nutraceuticals[36]**:**

The impediment of the medication accessible in worldwide market as far as safety has supported development of elective therapies for the treatment and the board of numerous sicknesses. Elective treatment incorporates restorative plants, nutraceuticals, fragrant healing and so forth. From a more extensive point of view, nutraceuticals are nourishment or part of sustenance that assume a significant job in changing and keeping up typical physiological capacity in people. Nutraceuticals extend from segregated supplements, dietary enhancements and herbal products, explicit weight control plans and processes sustenance's, for example, oats, soups, and drinks.

According to Association of American Feed Control Officials (AAFCO) 1996, 'Nutrient' signifies feed part structure and dimension, bolster a real existence of person or a creature while 'Nutraceutical' signifies any non-dangerous, no-toxic nourishment segment or enhancements, herbal products, probiotics and prebiotics, restorative sustenance's that has logically demonstrated the medical advantages including the aversion and treatment of ailment. Items or isolated/ bioactive molecules separated or decontaminated from nourishment are sold in therapeutic forms not more often than not connected with sustenance. A nutraceutical has a physiological advantage in that it gives insurance against incessant infections.

The vast majority of the nutraceuticals for consumption are known to have numerous restorative impacts with no symptoms, which is one of the prime reasons why they are in the new developing elective methodology for treatment of numerous ailments. The move in light of a legitimate concern for researchers internationally towards nutraceuticals is additionally because of different components like expanding extra cash, ideal estimating condition development in pharma retail chain and increment in human services uses, , and yet there is

absence of institutionalization and mindfulness, high valuing, advertising and appropriation which go about as constraining elements.

Categories of nutraceuticals:

Nutraceuticals arranged in various types relying on comprehension and application. Based on nourishment sources, mechanism of action, concoction nature and so forth nutraceuticals are categorised. They are as follows:

Dietary fibers:

Dietary strands which are not hydrolysed by enzymes are fundamentally plant items omitted by stomach related tract, yet are processed by micro flora. Non-starch polysaccharides (NSP) generally include dietary filaments. Organic products, oats, grain and beans are not many models that are wealthy in solvent strands.

Probiotics:

Probiotics are live microbial sustenance supplement, when consumed in adequate quantity improves intestinal microbiota and includes various species of *Lactobacilli*, Gram positive cocci, *Bifibacteria*. Probiotics are available for oral consumption either in the form of powder, liquid, gel / paste and even granular forms. Particular probiotics like lactose prejudice which are commonly used to treat diarrhoea. Probiotic are non-pathogenic, non-lethal and impervious to acidic medium, bind to epithelia tissue in gut, in this manner creating antibacterial substances. Studies demonstrate that if probiotics are organized, it may lead to reduction foundational conditions, for example, sensitivity, malignancy, a few different contaminations of the ear, urinary tract.

Prebiotics:

Prebiotics, constituents of several ingredients, influence the host by affecting gut bacteria. These are made up of short chain polysaccharides human gut can't easily process. Vegetables like beans, fruit like pears are rich in Prebiotics. An average amount of 5– 20 g in combination with insulin significantly influence development of bifido group of microorganisms.

Polyunsaturated fatty acids: (PUFAs)

PUFAs, also Known as "essential fatty acid" as they a pivotal role in physiological process and are externally supplemented. PUFAs are classified in: Unsaturated fats namely n3 and n6 (Omega 3and 6). Vegetables are rich source of linoleic acid.

Antioxidant vitamins:

Vitamin C, E and carotenoids are antioxidant vitamins and are protective against oxidative stress hence can be useful against several disease conditions such as diabetes mellitus, cancer, to name a few. Tocophenols, tocotrienols, vitamin E are also equally effective, especially selenium produce synergistic effect against lipid peroxidation.

Polyphenols:

These are secondary metabolites of plant constituents of large group of plant based chemicals. Among 8000 classes of polyphenols flavones and flavnones are being most important ones. Flavonoids and phenolic acids are the most effective. Among them, the most interesting one is dietary polyphenols which produce extensive antioxidant effect in both *in vitro* and *in vivo* models of diseases or pathological processes. Being antioxidants and anti

inflammatory in nature play a significant role in counteracting effects of DM on various body parts.

Spices:

Spices are one of add-on food product using back thousands of year for enhancing food taste. In tropical countries there is varied quantity and types of spices used. Recent research indicated that dietary spices even very small quantity has wide medical benefits and beneficial to us. Volatile oils like terpenes and other constituents are known to be responsible for such health benefits. In a large portion of the cases, flavors and herbs are innocuous, when taken as nourishment in little sums, however may display poisonous quality, when utilized as drug, on account of their relative higher portion regulated, or fairly because of the potential outcomes of their co-operations with other pharmaceutical medicines. Some studies also suggest that excessive consumption may leads to deleterious effects on human health condition.

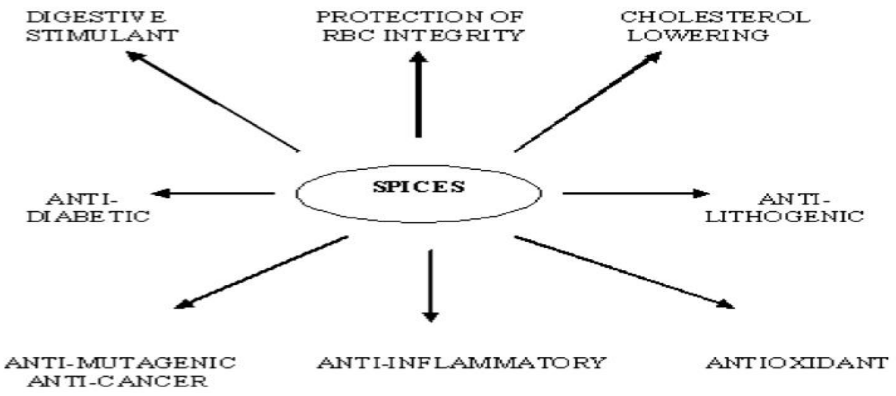


Fig. 2: Summary of potential health benefits of spices

Figure 3: Relation between Nutraceutical, Pharmaceutical and Nutritional supplement

Diabetes and nutraceuticals

T2DM associated with obesity continue to these are despite variety of medication usage for treatment and to counteract secondary complications. T2DM, thus represents a finanicial burdent to patients and family members and general public substantially.[37,38,39, 40] WHO perceives DM as a worldwide health problem and also associated with early dath and disability.

Considering the secondary entanglements that emerge after a long haul diabetes treatment, the initial step to accomplish satisfactory glycemic control and avoid secondary complications of DM in preclinical animal models. Unfortunately, the success of preclinical studies can't replicated in a well structured clinical trials.[41-43] Isoflavones, are notable phytoestrogens. Essentially, soy isoflavones have considered the more and it has additionally seen that their utilization prompted lower frequencies of death rate of T2D, coronary illness, osteoporosis and certain cancers.[44]

Various examinations have demonstrated that omega-3 unsaturated fats can diminish glucose resilience in patients slanted to diabetes.[45]

Lipoic acid, which is cell reinforcement is additionally utilized regularly for the treatment of diabetic neuropathy and has shown a long haul adequacy as dietary enhancement for the aversion of diabetes patients from secondary complications.[46] Dietary filaments from

psyllium have been utilized broadly as pharmacological enhancements and nourishment fixings.[47] Various examinations have additionally shown that various plant concentrates, for example, *Teucrium polium*, cinnamon and bitter melon demonstrate their capability for anticipate or treatment of diabetes.[48-50]

Use of nutraceuticals in T2DM-

Insulin resistance (IR) is a significant indication of diabetes mellitus type 2 related with heftiness. The basic instruments of insulin opposition are as yet being assessed and in a manner stays unanswered. Major organs, tissues and muscles engaged with glucose digestion incorporate fat tissue, skeletal muscle and liver which consequently assume a crucial job in insulin obstruction. Several Studied have appeared that IR and insulin obstruction associated with oxidative stress levels is engaged in the pathology of IR and there are studies showed that associate insulin obstruction with mitochondrial dysfunction, for example, decreased mitochondria number and ATP creation. The statement of qualities associated with oxidative phosphorylation, in patients with diabetes is altogether diminished in the skeletal muscle. Mitochondria are the real site of reactive oxygen species generation in the body. In the event that the effectiveness of oxidative phosphorylation decreases, more O2–is created to the detriment of ATP, in this manner lessening oxidative harm by improving mitochondrial work which is by all accounts a discerning method to forestall and treat insulin resistance.[51]

Various customary therapeutic plants have been accounted for to be helpful in diabetes, which may demonstrate to be new anti-diabetic medication and can counter the costly treatments which are accessible in market around the world. It is realized that India is a one of the rich wellspring of therapeutic plants and Ayurveda and Siddha arrangement of meds are proof that various plant concentrates have been observed to be valuable for the treatment of diabetes. The bit of leeway that a conventional restorative plant has fewer symptoms with various remedial activities because of the nearness of various bioactive mixes when

contrasted with the admission of medications accessible on long haul. Also, diet and spice treatments have turned into a noteworthy methodology as of late for the administration of diabetes and a lot of work is being finished with *Momordica charantia* Linn. *Coccinia indica* W. and, A. and *Lagerstroemia speciosa* Linn. These herbs have shown critical anti-diabetic activity[52-55] in any case, their anti-oxidant activity in DM has not been completely contemplated however. Remembering these actualities, this study taken up to evaluate synergistic antidiabetic action of these plants and look at its potential in streptozotocin-nicotinamide instigated diabetes and fructose induced diabetes in rodents.[56]

There is a disarray identified with the wording of nutraceuticals, for example, phytochemicals, pharma sustenance's, restorative nourishments, useful nourishments, dietary enhancements, originator nourishments, and so forth. There is slender line which partitions their compatible utilization by various individual's apathetic events. Pharmaceuticals are for the most part considered as meds which are utilized fundamentally to treat illnesses, where as nutraceuticals are the substances which are generally considered to avert infections and furthermore gives sustenance as well.[57] Both like Pharmaceuticals and nutraceuticals can fix and avoid disease(s) nonetheless, only that first have administrative assent as nutraceuticals are as yet being concentrated widely.[58] Pharmaceuticals are mixes which typically have patent insurance because of costly testing. Be that as it may, nutraceuticals needn't bother with this testing documents.[59]

Numerous leafy foods are known to possess nutraceuticals of medical importance. Thus, these leafy vegetables have proved to be valuable in the effective management of elevated cholesterol level. Hypertension and DM. One of the outstanding nutraceutical is Echinacea. [60, 61, 62]

Encouraging results of studies strongly encourages utilization of nutraceuticals and warrants further detailed investigations.

The components of activity of nutraceuticals are not yet completely comprehended is as yet being examined. Be that as it may, they may be engaged with various natural procedures which incorporate enactment of various pathways.[63]

In context with the previously mentioned realities this work will discover the pharmacological potential of the poly-herbal formulations based on their anti-diabetic properties. This postulation empowers us to comprehend the job of nutraceuticals and their importance in secondary complications of T2DM utilizing distinctive animal models.

1.2 JUSTIFICATION

Globalization, better-life, modernity, human progress, industrialization and some more "needs" of humankind may leads to various nourishment that are water, air and earth contaminations/defilements beginning at the miniaturized scale to the full scale level misusing the broad utilization of different synthetics. Decimations that were caused to the nature, thus reflected/built up it's unsafe and deplorable impacts to humanity back as expanded rate of different sicknesses or medical difficulties. This demands better medicinal services which, thus, have significantly expanded at the expense of therapeutic consideration.

Individuals have endeavoured to accomplish a superior personal satisfaction using organic products and other plant products and different types of health treatment.[64-66] Henceforth, expanding requests for nutraceuticals, phytonutrients and it's respective administrations, makers, advertisers, and related authorized experts have duplicated gigantically.

Plants are a standout amongst the most significant assets of human sustenance's and medications. Wellbeing advancing nourishments, it also has gotten broad consideration from both wellbeing experts and the general population. New ideas have showed up with this pattern, for example, nutraceuticals, dietary treatment, phytonutrients, and phytotherapy.[67-69]

These useful or restorative nourishments and phytonutrients or phytomedicines assume positive jobs in upgrading wellbeing and improving resistant capacity to avoid explicit ailments and furthermore hold incredible guarantee to lessen reactions and social insurance costs.[70-72]

Numerous individuals trust these medications are more natural than utilizing physician prescribed medications. They feel dietary enhancements will enable them to feel more grounded and more beneficial, give them more vitality and avoid disease. A few people go to these items when they feel standard medications for their particular ailments have fizzled.

A lot of ebb and flow research is centred on conventional home grown concentrates. Researchers are looking at cases connecting these concentrates with wellbeing improvement and anticipation of interminable infections. At any rate to some degree, this speaks to a push to legitimize homeopathic cures and Eastern prescription. The touchy interest development for biological active elements which will used as nutraceuticals and utilitarian sustenance's much of the time referred to wellbeing concerns, for example,

- Cardiovascular ailments
- Blood and other cancers
- Female health issues
- Skin problems
- Brain and psychological maladjustments
- Respiratory problems
- Sexual problems
- Diabetes mellitus

Future issues and recommendations of progress in the way of life can anticipate the sicknesses like metabolic disorders. One of the arrangements in the way of life change is changes in their eating regimen. The key issues for Nutraceuticals are

- Establishment of logical evaluation standard for avoidance of maladies
- Establishment of evaluation framework for infection aversion by human preliminaries
- Establishment of consistent framework to exchange organizes from essential research to industrialization.

Nutraceuticals are not really a solitary material; thusly, the normal impact for the anticipation of an illness may be the mind boggling activity of a few parts which are available in the product, it is likewise important to think about protection impacts for various kinds of nourishment. Consequently, it is important to lead biomarker inquire about for counteractive

action of target ailments. Subsequently, it is likewise important to characterize the estimation strategy for biomarkers and institutionalize indicators.[73,74]

At last, we re-hover back 2500 years prior expressed by Greek doctor Hippocrates. "Give sustenance a chance to be thy drug and prescription is thy nourishment"

2 REVIEW OF LITERATURE

DIABETES MELLITUS (DM):

DM is a disorder of metabolism portrayed by increase in sugar level in blood, unusual lipid, and protein digestion alongside explicit long haul intricacy influencing the retina, kidney, and sensory system.[75]

DM significantly affects the wellbeing, personal satisfaction and future of patients just as on the human services framework. Impaired Glucose Tolerance (IGT) was first instituted in 1979 by the World Health Organization (WHO).

Epidemiology:

DM has been perceived as a becoming overall pestilence by numerous wellbeing support gatherings including WHO.[76] The measurements are disturbing; 30 million individuals were determined to have diabetes worldwide in 1985 and by 1995 the number raised to 135 million, and presently there will be somewhere in the range of 300 million constantly 2025 as anticipated by the WHO.[77] At present, there are in excess of 34 million type 2 diabetic patients in the developed world.

The occurrence of IGT and T2D is increasing every year that the significant hazard factors for these conditions are winding up progressively common. These hazard components incorporate stoutness, an expansion in the mean age of the populace, and progressively inactive ways of life.

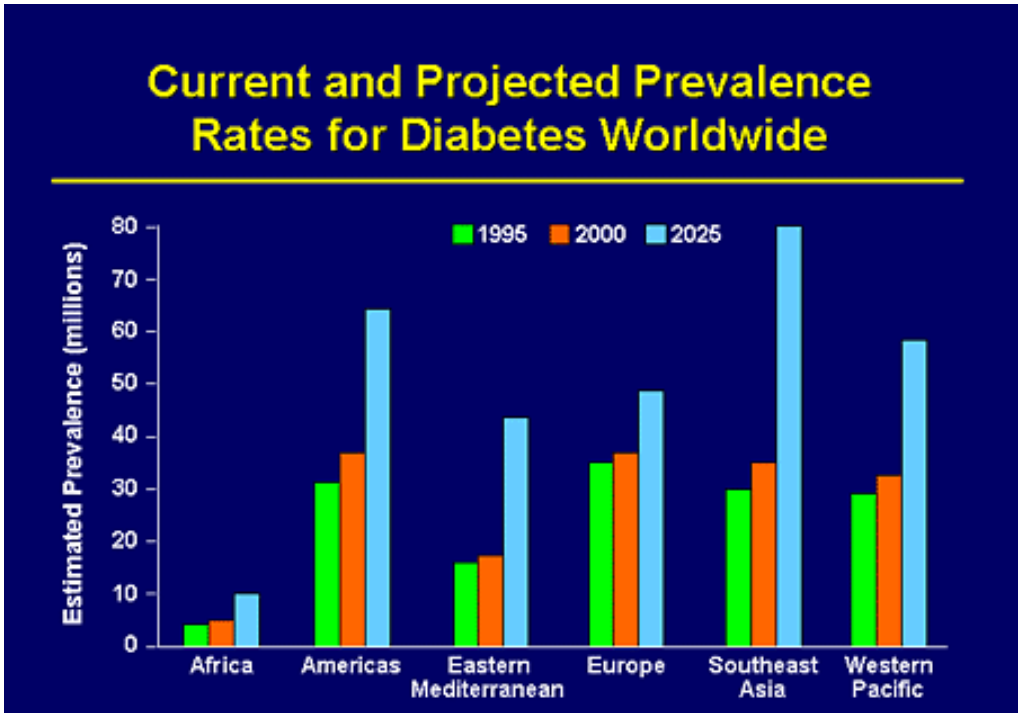


Figure 4: Current and projected prevalence rates for diabetes worldwide[7]

Present and anticipated pervasiveness rates for DM worldwide since 1995 to 2025 has been disturbing as the above diagram speaks to. The evaluated commonness in 2025 particularly for Southeast Asia is 80 million, trailed by 50 to 65 million for America and Western Pacific individually. About not more than 1000 subjects at the age group of 40-79 years from a cross segment group from overll public, assessed for occurrence of IR.

Stationary way of life, lack of physical wellness, both are consider as a factors responsible for conversion of IGT to T2D. Studies have additionally shown that even humble measures of weight reduction effect sly affect glucose control.

Pharmacological Treatment analog, recent advancements and limitations[78,79]

Classification of Oral Anti-diabetic Drugs

1. **Sulfonylureas :** act by promotes insulin release**.**

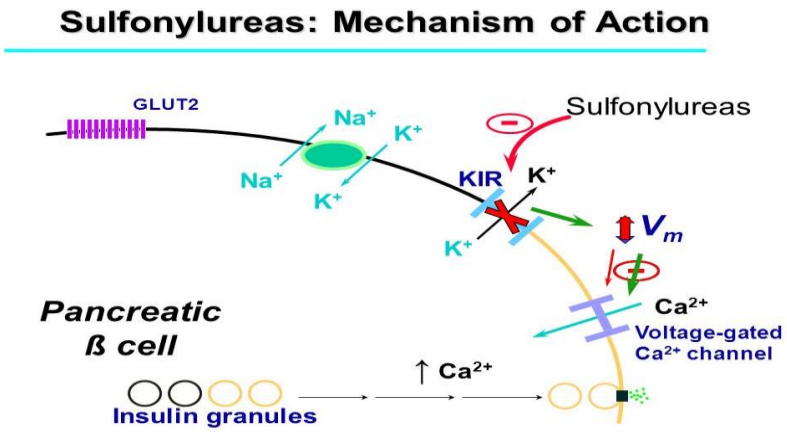


Figure 5: Mechanism of Action of Sulfonylureas

Adverse effects - Hypoglycemia

First generation sulfonylureas Viz., Tolbutamide, Acetohexamide and Chlorpropamide

Second generation sulfonylureas Viz., Glibenclamide / Glyburide, Glimepiride, Glipizide.

2. **Meglitinides:** act by animating the pancreas to deliver more insulin.

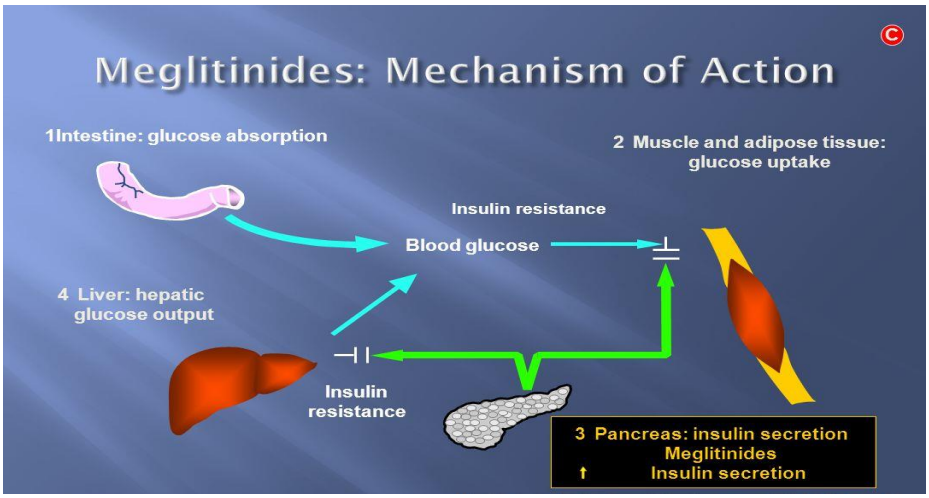


Figure 6 : Mechanism of Action of Meglitinides

Adverse effects - Hypoglycemia (low blood sugar)

- Repaglinide
- D-Phenylalanine Derivative – Nateglinide

3. **Biguanides:** It will make liver to decrease the production of glucose.

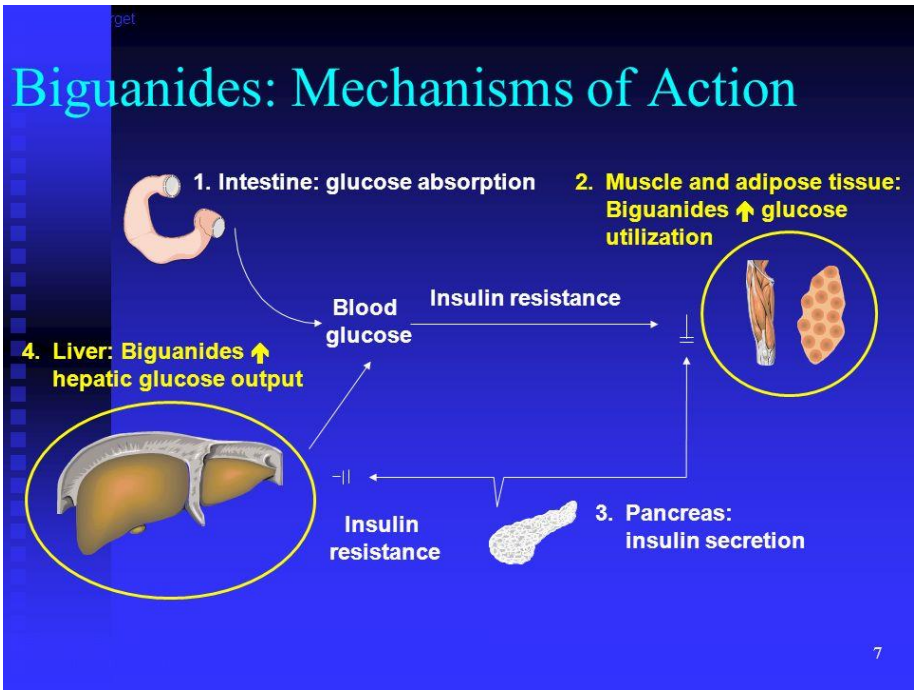


Figure 7: Mechanism of Action of Biguanides

Adverse effects - Diarrhea, metallic aftertaste, nausea

- Metformin

4. **Alpha Glucosidase Inhibitor:** act by abating the retention of carbohydrates (sugar) ingested.

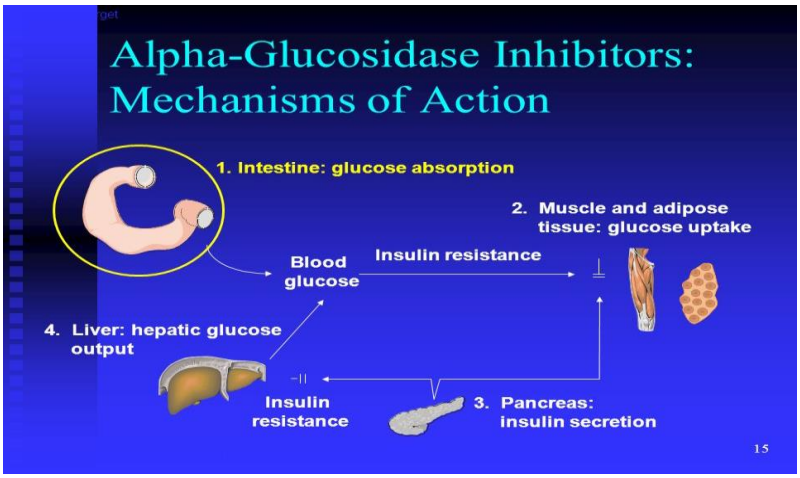


Figure 8: Alpha-Glucosidase Inhibitors-Mechanism

Adverse effects - Bloating and flatulence.

- Acarbose

5. **Thiazolidinedione Derivatives:** act by expanding the insulin affectability of the body cells and reducing the effect of liver to produce glucose.

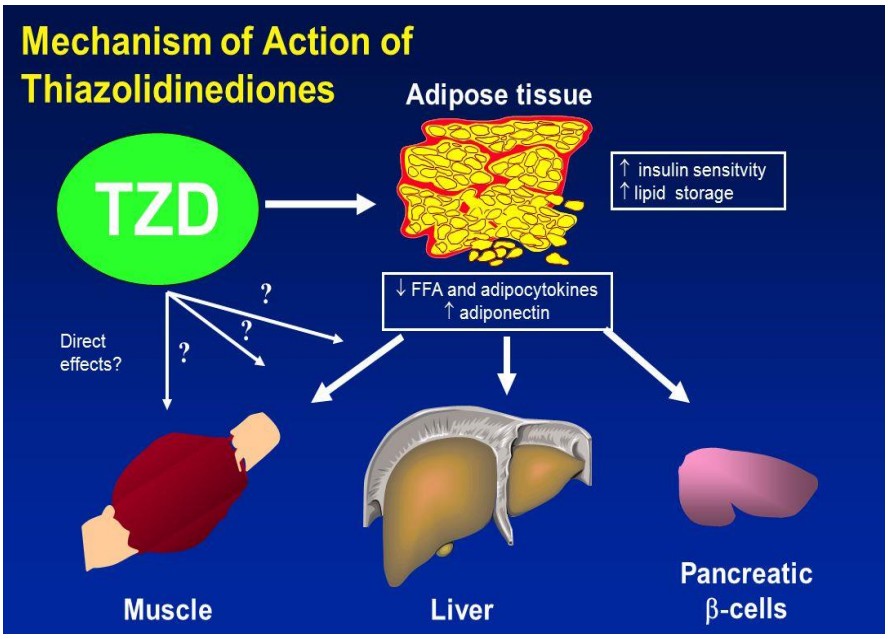


Figure 9: Mechanism of Action of Thiazolidinediones

Adverse effects - swelling caused by retention of water, increase in body weight.

- Rosiglitazone
- Pioglitazone
- Troglitazone

6. **Dipeptidyl Peptidase-4-Inhibitor:** act by increasing the impact of incretins in the maintaining the blood glucose levels.

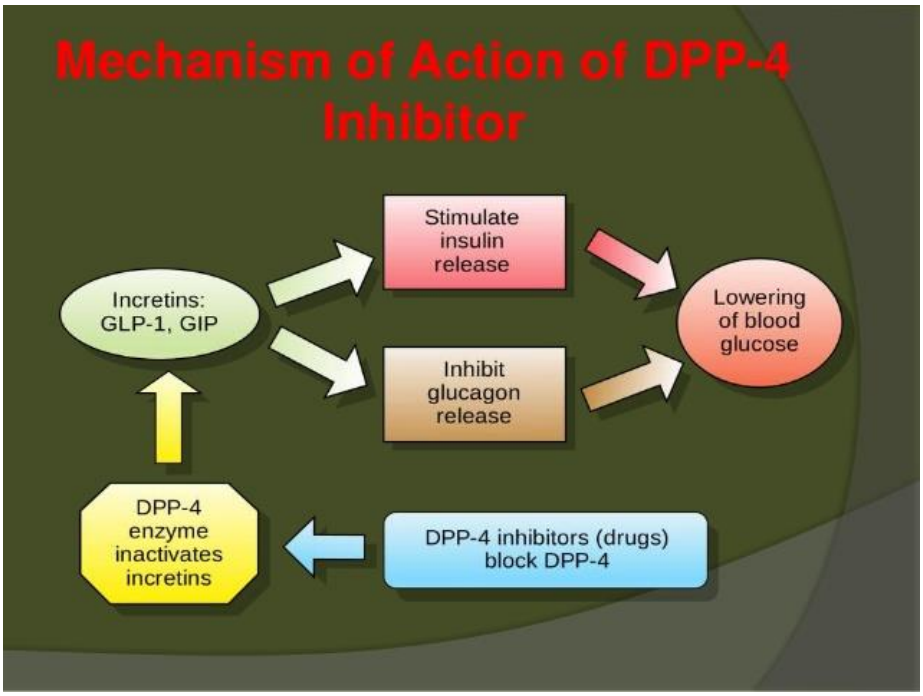


Figure 10: DPP-4 Inhibitor-Mechanism

Adverse effects - Pharyngitis, headache.

- Sitagliptin
- Pramlintide
- Exenatide

7. **Glucagon-like peptide-1 (GLP-1) agonist:** act by impersonating the impact of certain incretines engaged with the control of glucose.

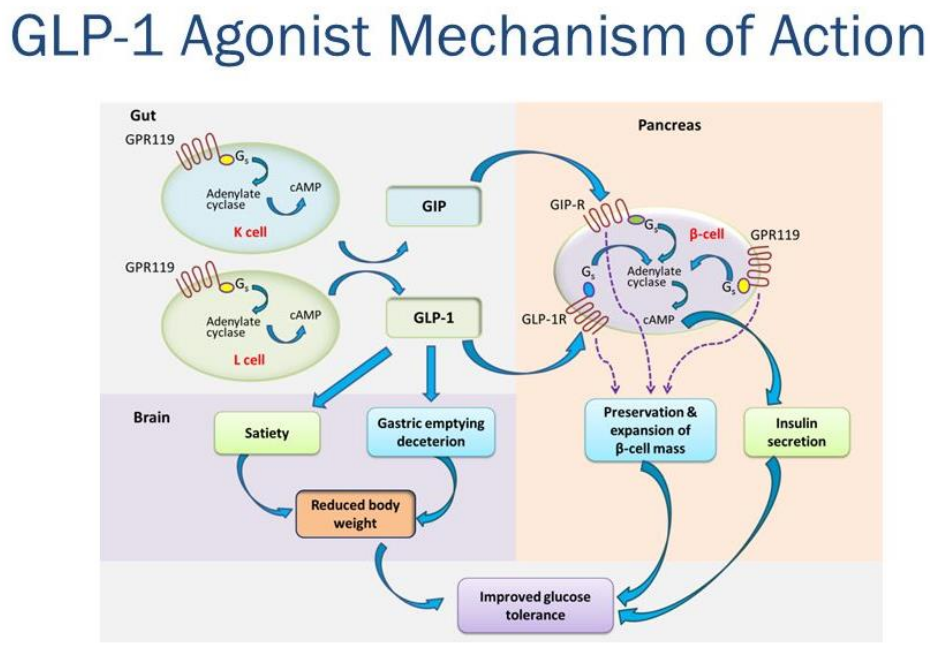


Figure 11: GLP-1 Agonist- Mechanism

Adverse effects - Nausea, diarrhea, vomiting.

- Exenatide (Byetta®)

8. **Sodium glucose cotransporter 2 (SGLT2) inhibitors:** act by disposing of glucose in the urine.

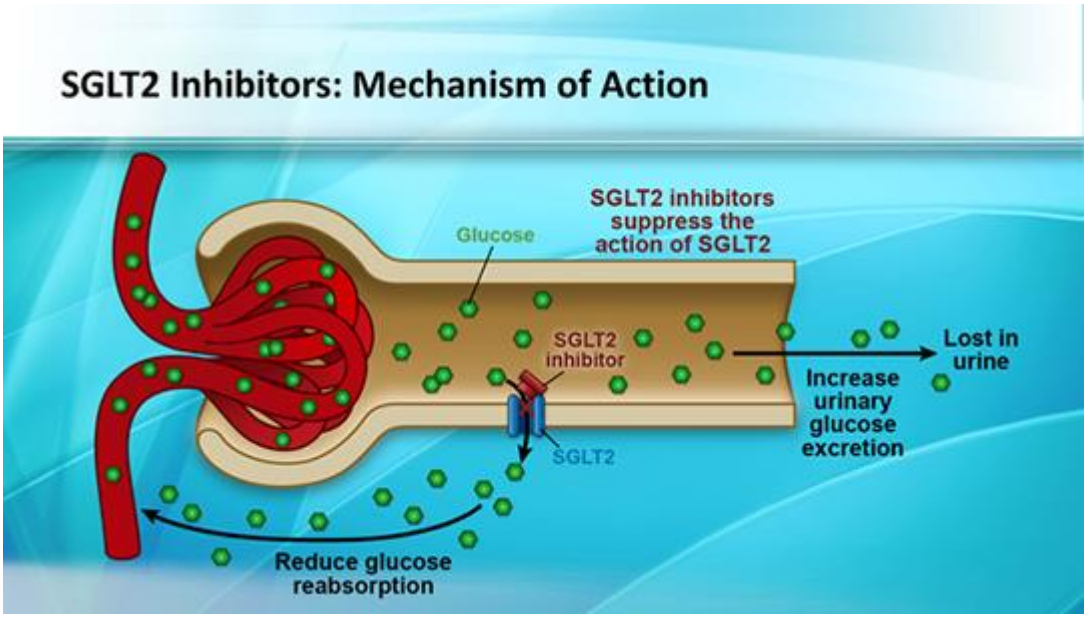


Figure 12: Mechanism of Action of SGLT2 Inhibitors

Adverse effects - Genital and urinary infections, more frequent urination.

- Canaglifozine
- Dapagliflozine
- Empagliflozine

Eight groups of antidiabetics (orally effective) are routinely employed in the successful management of T2DM. Oral treatment is suggested for such patients whose food habit and physical activity unable to reduce glycemic control. Albeit, beginning reaction might be great but as time gone their side effects make oral hypoglycemic medications less viability in a noteworthy level of patients.

INSULIN: [80,81]

Insulin, one of the oldest treatment for diabetes typically given by subcutaneously route. Some of the common reactions of insulin are gain in body weight and reduction of sugar levels. Fiery insulin treatment may likewise convey an expansion in atherogenesis.

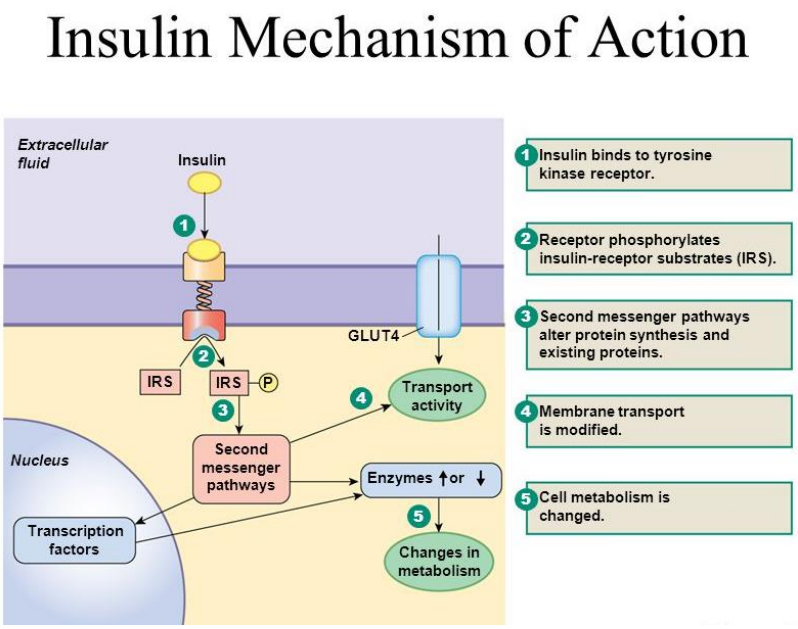


Figure 13: Mechanism of Action of Insulin

Pathogenesis of T2DM

The history of T2DM has been all around depicted in various populaces. T2DM patients acquired qualities from guardian's tissues impervious to insulin. IR especially in liver, β-cell and in muscle disappointment speaks deeply pathophysiologic surrenders being developed of T2DM. Factors responsible for destruction in β-cells are age; genes, IR, lipo-lethality, gluco-poisonous quality, amyloid testimony and irregular incretin. The dynamic decrease in insulin discharge, which diminishes β-cell mass of pancreas, nearness to IR contributes to change the condition of the irregular blood glucose level end with clear diabetes. 3 fundamental

deformities of metabolism describe the ailment: IR, insulin secretary imperfection that isn't immune system interceded, and an expansion in glucose generation by the liver.

INSULIN RESISTANCE (IR):

To maintain the whole body homeostasis of glucose typically depends on secretary reaction of insulin with the help of beta cells of pancreas. Normal body tissues get affected by mass activity of glucose into hyper-insulinemia, hyperglycemia. In, this way consolidated impacts of insulin advances transfer of glucose by 3 firmly component, (i) Concealment into generation of endogenous hepatic glucose.

(ii) Glucose take-up into splanchnic tissues in addition of G.I.T.

(iii) Glucose take-up by muscle of peripheral tissues.

INSULIN SERETORY DEFECT THAT IS NOT AUTOIMMUNE-MEDIATED

β-cells of pancreas produces Insulin, a peptide hormone. Also helps glucose ingestion flow from skeletal muscle and fat tissues.

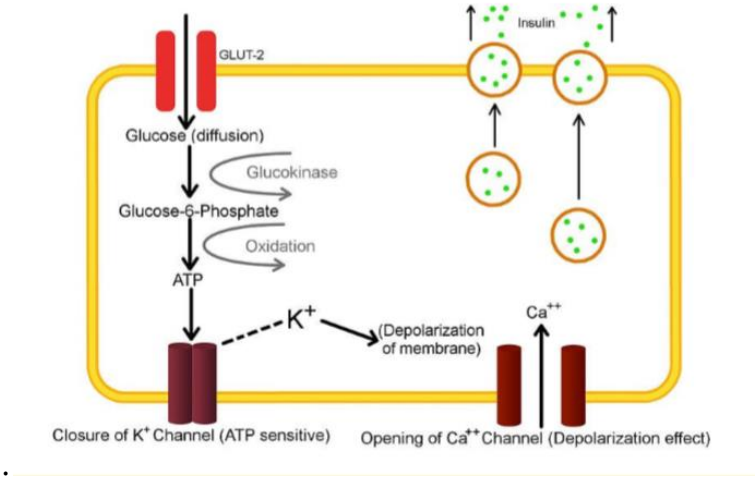


Figure 14: Insulin secretion

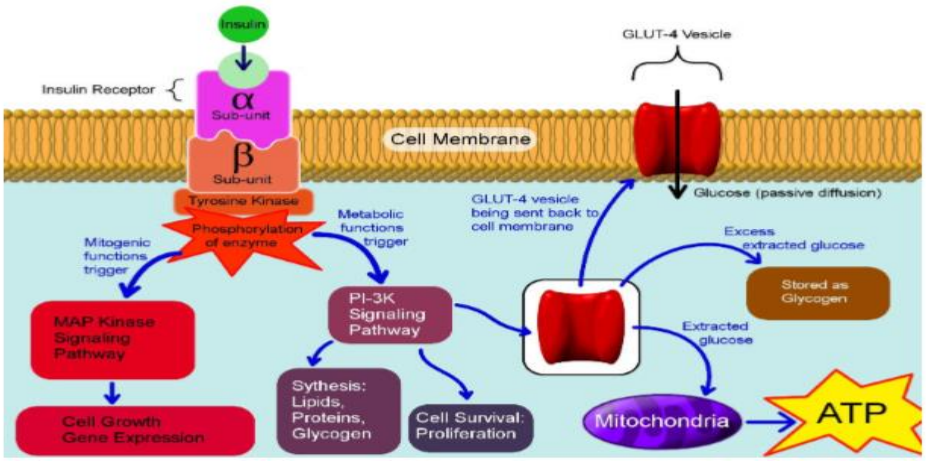


Figure 15: Insulin Function

β-cells of pancreas discharge insulin in 2 stages, as two phase way. First stage loss is considered as a free indicator for T2DM in beginning only. Patients who are having T2DM, the reclamation of this stage stifles the hepatic glucose yield will be reclamation in this stage, which leads to improve in blood glucose.

Decrease in pancreatic capacity will be supported by mainly two factors, which are known as glucose toxicity and lipid toxicity.

Gestational DM (GDM) is one of the commonness, which found in 3% in every pregnant lady. GDM is consider as the second-stage reaction of insulin in ladies with OGTT, but still the main stage for discharge of insulin decreased with glucose in intravenous form and later it has a pinnacle ascend along with OGTT. Decreased in IS up to 70% by demonstrated in two gathering. It is having advantage that it will return back to normal with ordinary OGTT, but this scenario will not be for ladies who have GDM. In a similar period, last gathering additionally exhibited determined and intemperate proinsulin discharge. Ladies who are suffering from GDM also have significant increment in the level of insulin discharge along with OGTT. In any case, this ascent is less in ladies with GDM contrasted and pregnant ladies who hold typical OGTT.

Accordingly, blood glucose level after 2 hour of glucose given by oral route, consider as the one of the most dominant burden for the advancement for the advancement of DM of Type 2. This is identified by the brokenness of IR and β-cells, which are significant imperfections being developed of the ailment. Insulin discharge is the later stage is impeded in IGT subjects. Last gathering subjects exhibited additionally in high IR in muscle and lower IR in hepatic, while IFG subjects showed higher IR in hepatic or close ordinary IS in muscle.

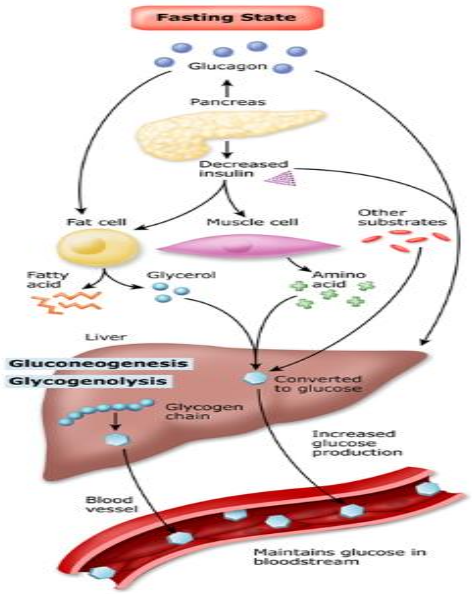


Figure 16: Gluconeogenesis (Glucose production by the liver)

PATHOGENESIS OF SECONDARY COMPLICATIONS OF DM[82-84]

DIABETIC RETINOPATHY:

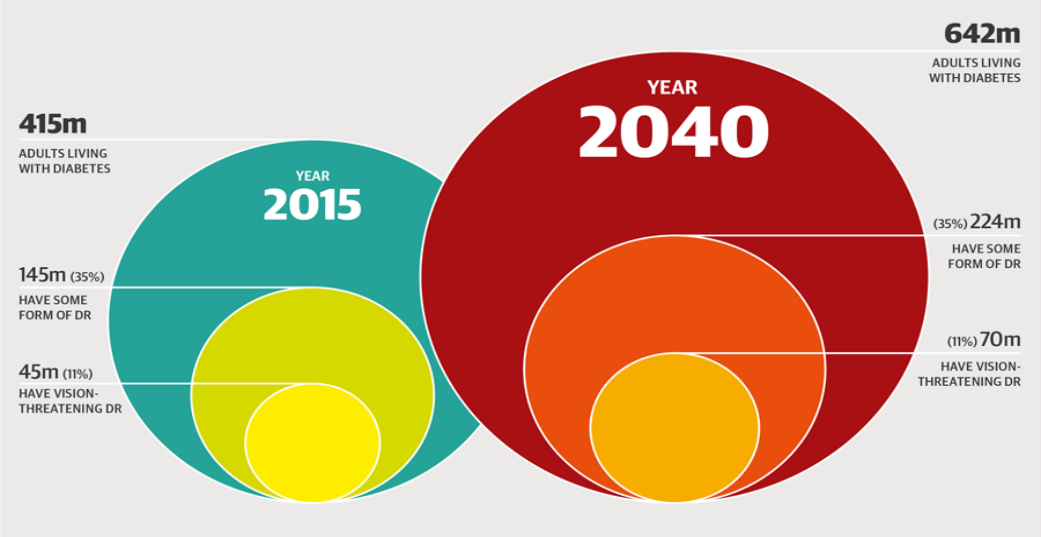


Figure 17: Global prevalence of people with diabetes and diabetic retinopathy

Of all the types of optional difficulties of DM, Diabetic Retinopathy (DR) is on the ascent as appeared in the figure above. Individuals with diabetes can have an eye disease called retinopathy, when elevated blood glucose affecting veins of retina, which subsequently swells / closes, preventing further blood flow

Phases of diabetic eye infection:

There are two primary phases of diabetic eye malady;

1. NPDR (non-proliferative diabetic retinopathy):

It is the early stage (remove for beginning of eye), when retina swells as the blood spills. Macular oedema results from swollen macula and many diabetics it is known to result in loss of vision. Macular ischemia results in foggy vision in diabetics

PDR (Proliferative Diabetic Retinopathy):

PDR is a progressive form of diabetic eye damage and starts appearing when neovascularization commences and newly formed vessels frequently enter the vitreous. PDR is likely to hinder normal vision.

DIABETIC NEPHROPATHY:

Kidney ailment which is one of the associated complications of diabetes is the main source of kidney failure. Right around 33% of individuals with diabetes people creates diabetic nephropathy. Individuals with diabetes and kidney disease regret more by and large than individuals with kidney sickness alone. This is on the grounds that individuals with diabetes will in general have other long-standing ailments, similar to hypertension, elevated cholesterol, and vein illness (atherosclerosis). Individuals with diabetes likewise are also have renal-related issues, likewise, contamination and nerve harm to bladder.

In type 1 diabetes kidney ailment is different from as in T2D. In type 1 diabetes after 10 years of finding diabetes, kidney sickness will identified. In T2D, a few patients as of now have kidney illness when they are determined to have diabetes.

DIABETIC NEUROPATHY:

Diabetes may harmful for nerves also, which is painful known as neuropathy. It is mainly due to high blood sugar level if it persists for longer duration and can be in several ways. PERIPHERAL NEUROPATHY: Feet and legs are most affected parts. Rarely arms, abdomen, and back got affected.

AUTONOMIC NEUROPATHY: Autonomic neuropathy affects GIT - mainly stomach, urinogenital system and vasculature.

PROXIMAL NEUROPATHY: Partially affects proximal regions of the legs and is also likely to lead to weakness of legs.

FOCAL NEUROPATHY: It will appear immediately in head, torso, or leg. It causes muscle weakness or pain.

OTHER DIABETES NERVE DAMAGE: Carpal tunnel syndrome which is very common type of entrapment syndrome which is identified by numbness and tingling of hands and also pain sometimes in hands.

Comparison between populations:

Reason for these metabolic deformities and in this manner the reason for T2D, is to a great extent obscure. Unmistakably, T2D has a solid hereditary segment and is discovered all the more much of the time in families and ethnic gatherings, for example, Hispanics, African Americans, Pacific Islanders, and American Indians. Hopeful qualities yet not distinguished as the reason for T2D, and almost certainly, the sickness is the aftereffect of multi-hereditary imperfections. Components that add to IR incorporate heftiness, maturing, and a stationary way of life. Other procured factors that may add to the IS imperfection incorporate incessant gluco-lethality and raised free unsaturated fat dimensions.

As referenced over, T2D a metabolic deformities, which can be due to insulin obstruction, ß - cell brokenness, weakened hepatic glucose creation and still some discussion exists that

either IR or deficient insulin discharge which will happens initially in pathogenesis of diabetes. In any case, agreement developed that opposition of insulin is one of the essential deformity in T2D.[85]

ROLE OF FREE RADICALS IN DIABETES:

Role of free radicals is well established in the vascular complications of DM, especially of T2DM i.e., convincing role of oxidative stress.[86] In diabetics, elevated ROS concentration can be related to imbalance between generation of ROS and its effective removal of them by endogenous enzymes namely SOD, CAT and GSH-Px .[87]

PATHOPHYSIOLOGY OF OXIDATIVE DAMAGE IN DIABETICS

Literature review reveals the definitive role of elevated levels of free radicals resulting in oxidative stress in the pathogenesis and progress of both insulin and non-insulin dependent diabetes.[88] It is reported that lipids, specifically Apolipoprotein portion of LDL has a definitive role.[86] In DM, mitochondria are a primary repository of oxidative stress. During oxidative digestion in mitochondria, part of used oxygen is diminished in water, and remains part will get converted into oxygen free radical ($O_{\cdot}$) which is a significant ROS that is changed over to different RS, for example, $ONOO^-$, OH and H_2O_2.[89] ROS/RNS are two different ways by which insulin signaling twisted. Insulin resistance remains a key factor in T2DM.[90]

OXIDATIVE STRESS AND DIABETIC COMPLICATIONS

Numerous confirmation by investigations given connection among diabetes and oxidative stress. It is trusted in the beginning, movement generally diabetic complexity, free radicals should have noteworthy job because of their capacity to harm lipids, proteins and DNA.[91] Assortments of obsessive conditions are prompted by oxidative pressure, for example, rheumatoid joint inflammation, DM and malignancy.[92] Oxidative stress and free radical

prompted intricacies from DM incorporate coronary supply route sickness, neuropathy, nephropathy, retinopathy[93]and stroke.[94] *In-vivo* studies bolster the job of hyperglycemia in the age of oxidative pressure prompting endothelial dysfunction in veins of diabetic patients.[95] Increment in the dimensions of glucose and insulin alongside dyslipidemia in patients experiencing diabetes creates full scale angiopathies that reasons oxidative stress prompting atherosclerosis.[96]

BIOMARKERS FOR OXIDATIVE STRESS IN DIABETES MELLITUS

Proteins

With some amino acid, ROS responded *in vitro,* delivering changed, denatured and proteins, which is non-working that in further might in charge of oxidative stress.[97] As per investigations by *in vitro* myeloperoxidase catalyzes change of L-tyrosine to 3,3-dityrosine which fills in as a crosslink between polypeptide chains of the equivalent or various proteins making it an advantageous biomarker for protein oxidation.[98]

Lipids

Lipid profile is significantly altered in DM.[99] Studies have reported that poly unsaturated fats of cell layers are most susceptible to free radical damage due to its proximity. [100] Lipid hyperperoxides through halfway extreme responses produced unsaturated fats; create exceptionally receptive and dangerous lipid radicals that structure new LHP.[101] Lipid peroxidation is a basic biomarker, which is most investigated region of research with regards to ROS.[102] MDA is product of lipid peroxidation that can be employed to assess the extent of lipid peroxidation due to oxidative stress.[103]

Vitamins

Vitamin A, C and E are among nutrients, which undergoes antioxidant process by performing detoxification of free radicals. Vitamin E body dimensions have been accounted either to be

expanded or diminished by diabetes. Various studies reported Vitamin E pernicious impact on diabetes incited changes in vascular.[88]

Glutathione (GSH)

Diabetes leads to changes in glutathione peroxidase and glutathione reductase action and these compounds found in cell which uses peroxide, converts into water and further changing over to glutathione disulfide again into glutathione. Change in their dimension leads to inclined of cells, leads to oxidative stress and causes damage to cell.[88]

Catalase (CAT)

Catalase is controller of hydrogen peroxide, more in amount causes genuine harm to lipids, DNA and RNA. It converts H_2O_2 into H_2O and O_2. Beta cells of pancreas consist of mitochondria, experiences oxidative stress, if there is overabundance of ROS, which leads to breakdown of beta cells and eventually diabetes, if there is arise an occurrence of lack in catalase.[104,105]

Superoxide dismutase (SOD)

This offers first line of protection against ROS interceded cell damage by extension of superoxide, the essential ROS in oxygen digestion, to atomic oxygen and peroxide. [106]

Preclinical screening animal models for anti-diabetic activity assessment:[107]

Concentrates on animal models of diabetes have contributed fundamentally in understanding the etiology alongside pathogenesis of diabetes. Understanding the connection between the disease procedure in the animal and human is of extraordinary esteem. *In vivo* animal models have been employed to explore and understand the damage caused by diabetes on various parts of the body, besides, it additionally support advancement and assessment of fresher specialists for treatment of diabetes.

The majority of studies carried out and reported in the field of Ethnopharmacology used chemical induced screening model for induction of diabetes. Streptozotocin 69% and alloxan 31% are the most frequently used chemicals for the study of numerous aspects of the diabetic condition in animals. The dose of the diabetogenic agents varies depending on species, route of administration and nutritional state of animal. A vast variety of animal models are available for the experimental work on diabetes, and they are generally classified on the basis of their clinical similarities to the human disease.

I. **Chemically induced diabetes**- Animal Models for Type-1 & T2D

Diabetogenic chemicals that effectively produces diabetes in laboratory animals by either damaging β-cells of the pancreas, temporarily production of insulin and or release also known to significantly influence its action on target organ.

1) Streptozocin (STZ) induced diabetes:

   Streptozotocin, a broad spectrum antibiotic was originally a cytotoxic / anticancer antibiotic isolated from *Streptomyces archromogens* (1959) was found to be specifically toxic to β-cells of pancreas, Rakieten and collaborators originally announced diabetogenic impact of antibiotic Streptozotocin. Acceptance of diabetes

in lab animals, as often as possible in rodents, by STZ has transform into a profitable device in diabetes research being adequately utilized by numerous examiners.

2) Alloxan induced diabetes:

Ordinarily, alloxan infusion causes hyperglycemia and glucosuria that was at that point revealed in differentanimal species, for example, in bunnies, in dogs, in rodents, and in different species. Guinea pig discovered protection from alloxan for improvement of diabetes. The portion of alloxan differs dependent on species and foundation of organization. Triphasic reaction of alloxan was seen in the vast majority of the species: An underlying increment in blood glucose pursued by a decrease in blood glucose because of arrival of insulin from beta cells again pursued by a constant increment in blood glucose levels. Alloxan typically delivers most extreme cell lethality inferable from its transformation to anionic radicals (free radical instigated harm).

3) Goldthioglucose obese diabetic mouse model:

4) Atypical antipsychotic-induced diabetic model:

5) Miscellaneous chemical induced diabetogenic animal models:

Dithizone induced diabetes model.

II. **Surgically induced diabetes**: Complete or partial pancreatectomy

III. **Genetically induced diabetic animal model**:

Rodents have been utilized to display unconstrained DM on a genetic premise. Modification in the quality structure to animate diabetes in animal models demonstrated deformities in the leptin pathway, different transformations in the gene structure of mouse brought about leptin insufficiency. Since the innovation of leptin and its down guideline signal transduction cascade, new understanding of the

hereditary qualities conduct of diabetic and fat creature ailment models were determined.

(A) Zukker Diabetic Fatty Rat:

(B) Goto-Kakizaki rat:

(C) LEW.1WR1 rats:

(D) NONcNZO10 mouse:

(E) C57BL/6J mice:

(F) Kuo Kundo mice:

(G) Tsumara Suzuki Obese Diabetes mice:

(H) db/db mice:

(I) Obese rhesus monkey (Macaca mullata):

IV. **Virus induced diabetic animal model:**

A viral contamination and immune system ailment that cause pancreatic beta cell demolition causing decline in insulin delivering cells which lessens dimensions of coursing insulin and increments in plasma glucose levels. Contamination of encephalomyocarditis infection (EMC-D) creates pancreatic beta cell harm to instigate insulin subordinate diabetes in mouse strains is like that of human. Cyclosporine treated animal indicated expanded seriousness and rate of diabetes.

V. **Hormone induced diabetic animal model:**

Corticosteroids and growth hormone in exploratory animals produce hyperglycemia. Repeated / continuous use of this growth hormone is known to produce diabetes resembling human diabetes – as a side effect. In the rodents, guinea pig and bunnies, hyperglycemia created by organization of corticosteroid. Corticotrophin, animated by adrenal cortex has the ability to emit glucocorticoids which prompt steroid incited diabetes.

VI. **Insulin Antibodies-induced diabetes**

VII. ***In-vitro* models for diabetes.**

PLANTS INVOLVED IN ANTI-DIABETIC THERAPY

Poly-herbal formulation:

According to antiquated writing, in excess of 800 plants are accounted for to have antidiabetic properties.[108] Ethanopharmacological studies show that in excess of 1200 plants utilized for conventional medication in their partnered hypoglycaemic movement.[109] Indian Materia Medica has been referenced various types of dravyas have been accounted for successful in Madhumeha.[110]

The indigenous diet routine only may not be valuable in bringing down the glucose level to a similar degree as traditional agents do, yet it has different impacts, which could helpful for administration of illness and associated complexities.[111] In diabetes, natural choices which demonstrated to give symptomatic help and aid the aversion of its associated complications. A few herbs additionally have been demonstrated for recovery of ß-cells and in conquering obstruction. Notwithstanding keeping up ordinary glucose level, a few herbs are likewise answered to have antioxidant activity and cholesterol bringing down activity. The administration of T2DM is conceivable with medication that can bring down the glucose level at one part and re-establish the glycogen level in liver then again. In present day arrangement of medication, there is no such medications available, which have both of the properties.[112] Some home grown concentrates confirmed in human and preclinical models of T2DM in animals and regular medications to bring down blood glucose levels from active constituents. Metformin, An earliest oral hypoglycaemic agent (Glyophage) from *Galega officinalis* is still prescribed in the management of T2DM.[113-117]

Table 2: MEDICINAL PLANTS HAVING ANTIDIABETIC ACTIVITY[117a]

Plant Species (Family)	**Part Used**	**Plant Species (Family)**	**Part Used**	**Plant Species (Family)**	**Part Used**
Aloe vera, A.barbadensis, Aloe arborescens (Liliaceae)	Dried sap	*Cyamopsis tetragonolobus* (Leguminosae)	Fruits and seeds	*Myrtus communis* (Myrtaceae)	Leaves, branch-lets
Artemisia herba-alba (Compositae)	Stem and leaflets	*Dioscorea japonica* (Dioscoreaceae)	Tubers	*Neurolaena lobata* (Compositae)	Leaves
Allium cepa (Liliaceae)	Bulbs	*Dioscorea dumetorum* (Dioscoraceae)	Tubers	*Opuntia streptacantha* (Cactaceae)	Stem
Aconotum carmichaelii (Ranunculaceae)	Roots	*Eriobotrya japonica* (Rosaceae)	Leaves	*Phyllantus niruri* (Euphorbiaceae)	Leaves
Bridelia ferruginea (Euphorbiaceae)	Leaves	*Eugenia jambolana* (Myrtaceae)	Seeds	*Plantago psyllium* (Plantaginaceae)	Mucilage
Bauhinia canicans (Leguminosae)	Leaves	*Euphorbia prostrata*	Aerial parts	*Poupartia birrea* (Anacardiaceae)	Leaves
Bumelia sartorum (Sapotaceae)	Root bark	*Ganoderma lucidum*	Fungus	*Rubus fruticosus* (Rosaceae)	Leaves

		(Polyporaceae)			
Capsicum annuum (Solanaceae)	Fruits	*G. sylvestre* (Asclepiadaceae)	Leaves	*Psidium guajava* (Myrtaceae)	Fruits and Leaves
Cecropia obtusifolia (Moraceae)	Leaves	*Hammada salicornica* (Chenopodiaceae)	Whole plant	*Poterium ancistroides* (Rosaceae)	Aerial parts
Centaurea corcubionensis (Compositae)	Leaves and flowers	*Hedyotis biflora* (Rubiaceae)	Whole plant	*Salvia lavandulifolia* (Labiatae)	Aerial parts
Cluytia richardiana (Euphorbiaceae)	Whole plant	*Leucaena leucocephala* (Leguminosae)	Seeds	*Swietenia mahagoni* (Meliaceae)	Bark
Coccinia indica (Cucurbitaceae)	Leaves and roots	*Lupinus albus* (Leguminosae)	Seeds	*Swertia chirata* (Gentianaceae)	Whole plant
Cuminum nigrum (Umbelliferae)	Seeds	*Lythrum salicaria* (Lythraceae)	Flower, leaves, stem	*Tecoma stans juss* (Bignoniaceae)	Whole plant
Coprinus comatus (Coprinaceae)	Fruits	*Momordica charantia* (Cucurbitaceae)	Fruits	*Tephrosia purpurea* (Leguminosae)	Seeds

Teucrium oliverianum (Labiatae)	Aerial parts	*Tillandsia usneoides* (Bromeliaceae)	Whole plant	*Trigonella foenum* (Leguminosae)	Seeds
Pterocarpus marsupium (Leguminosae)	Bark	*Coix lachryma-jobi* (Gramineae)	Seeds	*Zizyphus rugosa* (Rhamnacae)	Bark
Bixa orellana (Bixaceae)	Seeds	*Eleutherococcus senticosus* (Araliaceae)	Aerial parts	*Lithospermum erythrorhizon* (Boraginaceae)	Roots
Anemarrhena asphodeloides (Liliaceae)	Rhizomes	*Ephedra distachya* (Ephedraceae)	Roots	*Coptis chinensis* (Ranunculaceae)	Aerial parts
Atractyloides japonica (Compositae)	Rhizomes	*Momordica Cochinchinensis* (Cucurbitaceae)	fruits	*Lathyrus japonicus* (Leguminosae)	Seeds
Centaurea seridis (Compositae)	Aerial parts	*Ficus religisa* (Moraceae)	Bark	*Galenga Officinalis* (Leguminosae)	Seeds
Coffea arabica (Rubiaceae)	Green beans	*Cornus officinalis* (Cornaceae)	Beans	*Lepidium ruderale* (Cruciferae)	Aerial parts

Till today, 400 or more than 400 conventional plant medications accounted for diabetes, albeit just few these have gotten logical and restorative assessment to evaluate their adequacy. Coming up next is a summary of a few of the most considered and usually utilized therapeutic herbs.

NUTRACEUTICALS:

In simple terms, Nutraceuticals means, NUTRITION + PHARMACEUTICAL: Food stuffs (Dietary supplements) that provide health benefits.

Nutraceuticals are sustenance supplements that give therapeutic or medical advantages including the avoidance as well as treatment of a malady. Nutraceuticals have a preferred position over drugs as they keep away from reactions. Nutraceuticals, based up on their characteristic source and synthetic gathering, classifies into three key terms in particular, supplements, herbals and dietary enhancements. The most quickly developing sections of the business are dietary enhancements and regular/natural items. FDA approved "dietary enhancements" as nourishments so as to guarantee that they are sheltered. Government of India passed Food safety and standard act to direct the expanding nutraceutical business. Home grown nutraceuticals are utilized as an incredible source in keeping up wellbeing and to act against intense and interminable ailments, along these lines advancing ideal wellbeing, life span, and personal satisfaction.

Categories of Nutraceuticals:

These are classified based upon natural sources, chemical constituents and as per pharmacological conditions of the product. They can be obtained from natural (plants, animals, microbes) or synthetic sources.

1. **Nutrients with established** n**utritional functions**: Feed constituents that are rich in minerals, fats, proteins, carbohydrates, and vitamins.
2. **Dietary Supplements**: Products which contains more than one ingredient, for example: Vitamins, Minerals, herbs and an amino acid.
3. **Nutraceuticals**: Any food component which is non-toxic in both preparation as pharmaceutical product and as well as nutrient and it has proved scientifically its health benefits, including cure, prevention and for treatment of diseases.
4. **Herbals**: Herbs product can be used in the extract or concentrate form, which will be used for treatment of various acute and chronic diseases.[118]
5. **Synthetic Reagents** Synthetic reagents like Pyruvates, precursors of steroidal hormones and chondroitin sulphate are examples of are now popular used as a nutritional supplement for sports purposes, weight loss supplements and as dietary supplements.

Advantages of Nutraceuticals

1. Promotes better wellbeing and counteracts sicknesses.
2. Reduced reactions and expands medical advantages.
3. Provides dietary enhancements normally that are effectively accessible, financially savvy and monetarily moderate.
4. It gives sustenance for populaces exceptional needs (eg: supplement thick nourishments for mature people).[119]

Drawbacks of Nutraceuticals

1. **Bioavailability**: These are excreted out from body very early after ingestion and do not provide complete medicinal benefits leading to poor bioavailability.

2. **Placebo Effect**: Sometime body is able to recover itself, eliminating the need to use nutraceuticals.
3. **Lack of regulations**: By the international market, nutraceuticals product claims to use organic ingredients, but due to inadequate regulation, it may compromise the product safety and effectiveness.
4. **Transparency:** Problem encountered with these types of products is that they do not provide adequate information to consumers about the product safety and effectiveness, side effects and Drug-drug interaction.[120]

Dietary Supplements, Health and Education Act (DSHEA-1994):

These products are planned for enhancements of diet regimen, which contains one of dietary element, for example it can be nutrient, mineral, and herb. These substance use by man especially for enhancement of diet regiment expanded day by day consumption. These are available in various dosage forms.[121]

Dietary enhancements are not always represented as regular nourishments. The producer of a dietary enhancement is in charge of guaranteeing that the dietary enhancement is protected before it is showcased.[122]

Energy Value Estimation:

Calorific value of sample can be determined (in Kcal) by multiplying percentage of carbohydrate, crude lipid and crude protein by recommending factor (3.57, 8.37 and 2.44), which is used for analysis of vegetable.[123,124]

Phytonutrients:

They are fundamentally plant supplements with specific natural exercises in advancing human wellbeing.[125] Notwithstanding these, phytonutrients additionally fill in as particular development factor and maturation substrates for useful microbes, specific inhibitors of harmful intestinal microscopic organisms, foragers of responsive or lethal synthetic compounds and as ligands that anguish or irritate cell surface or intracellular receptors.[126]

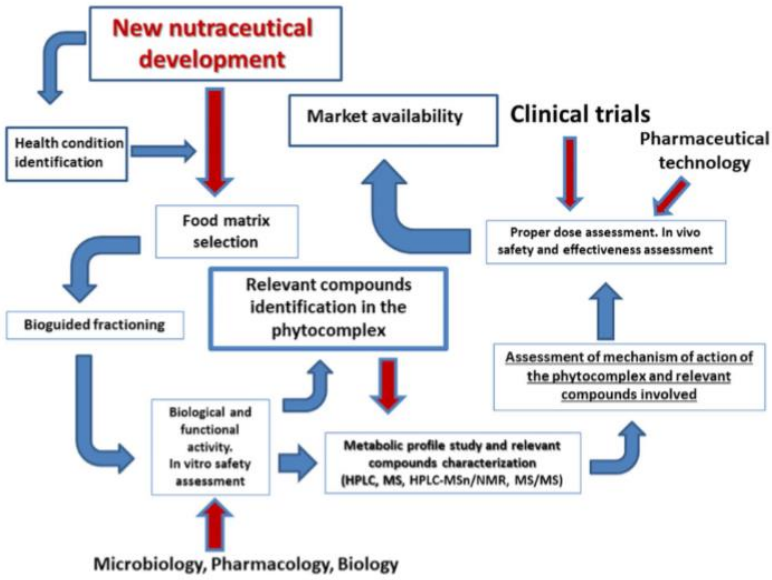


Figure 18: Developmental stages of a nutraceutical

Beneficial effects of Nutraceuticals in Diabetic Therapy:

Quercetin, a flavonoids reported to have antidiabetic activity, act by regeneration of pancreatic islets and mostly it will result in increase release of insulin in STZ- induced

diabetic rodents. In one more study, it was found that it stimulates release of insulin and enhanced Ca2+ uptake from separated islets cells, suggest a place for flavonoids in noninsulin-dependent diabetes.[127-129]

Cereals:

From staple food to nutraceuticals, many cereals, which are used as staple food in worldwide, have different nutraceutical properties.

1. Millets: These are the one of highly nutritious, least allergenic, Water soluble B group of vitamins, especially niacin, B6 and folic acid, calcium, iron, potassium, magnesium and Zinc. Their seeds are also rich in phytochemicals and phytic acid which helps in lowering cholesterol and phytate, which is associated with treatment of cancer.
2. Wheat: It is especially used as a food ingredient; very minute quantity of the grain is used for enhancing health and prevention of chronic diseases. FDA permits foods with at least 51% whole grain must display mentioning that the consumption is associated with health benefits.
3. Barley: It is also the one of the most widely used traditional food grain and used for malt and beer production. The active component in barley is β-glucan which may decreases the chances of coronary heart disease, diabetes and heart related problems.
4. Rice: Rice is considered as one of the important sources of energy is known for its hypoallergic status, with high nutritional source, kernel of which is made up of hull, bran, embryo and 70-72% endosperm. Nutrition wise is a rich source of bran (up to 5%) and oil (12-18.5%). Rice bran which lowers the bad cholesterol in the blood and increases the levels of good cholesterol in the blood. Rice bran also contains various minerals like; phosphorous, potassium, magnesium, calcium and manganese. Metabolic

antioxidants are also present which controls body weight. Essential fatty acids are found to improve health of eyes.

5. Buckwheat: It has nutritional benefit and is easily digestible. Buckwheat is a rich source of mineral and iron; which have a key role in prevention of hypertension and anemia.[130-131]

CURRENT GLOBAL STATUS AND DEMAND FOR NUTRACEUTICALS MARKET IN DIFFERENT COUNTRIES:

The global nutraceutical industry that functions under 3 main segments such as functional foods, dietary supplements and herbal products.[132-133] In 2007, sale of nutraceuticals were expected to touch $74.7 billion at an AAGR of 9.9%, followed by assumption that this will help recover world economy of 2003 and put an end of competition in pricing.[134]

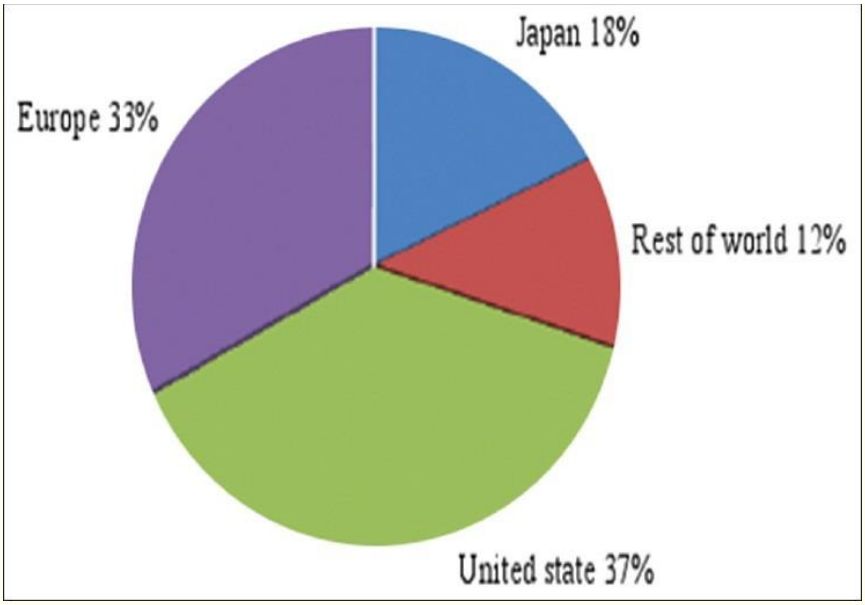


Figure 19: Nutraceutical market in different countries

85% of total globe market accounts for vitamins and minerals, 10% and 5% accounted for antioxidants and herbal extracts. Among worldwide US is in first position followed by India and China in second position for nutraceutical market.

Nutraceutical ingredients demand has rise up to $ 15.5 billion from 5.8% annually. Among worldwide India and China are the fast moving markets of nutraceutical. Herbal and non-herbal extracts are used worldwide and widely used by medical professionals and increased from 6.5% annually to $1.85 billion in 2010. Nutrients, minerals and vitamins demand reached $ 9.5 billion in 2010 up to 6.3% annually from 2005. Global demand for nutraceutical vitamin ingredients increased up to 4.6% annually to $4.2 billion in 2010.[135-140]

SCOPE AND OPPORTUNITY OF INDIAN NUTRACEUTICAL MARKET

As per recent studies, growth rate of India market for nutraceutical is 21 percent annually. It is expected to reach INR 95bn in next four year from current INR 44bn.[141]

Accordingly to a concept, in India nutraceutical market is still in infancy stage, but in coming time it will grow at faster rate, when compare to CAGR of 18 percent from last 3 years. Products like dietary supplements and herbal products have most growth rate when compare to any other nutraceutical products.[142]

Reports from M/s Frost and Sullivan, a research consultant from that functional food will be one of the fastest growing products from 2015. At present, the nutraceuticals is the largest category and constitute for 64% of the nutraceuticals.[143]

FUTURE ASPECTS: Worldwide development of nutraceutical expanded, indicates that end users now looking for food with extra nutritional benefits with less processed food, it is

because of scientist believe that enzymes play a key role from frontier like Carotenoids, Lycopene.[144]

CONCLUSION:

Nutraceuticals will supplement substances which are required for healthy humans. Nutraceuticals provide energy and nutrient supplements to body, which are required for maintaining good health. It is an industry which is growing at a much faster rate. In promotion and care of human health to prevent diseases this food play an important role. It can consider as key to obtain medicinal effect with less side effects. It proves its benefit for humans to maintain an overall good health. Regulatory body implementation is necessary to promote and standardize this industry, as they are promoting health and health professionals, regulatory toxicologists and nutritionists suggests that these professionals work hard hand in hand for planning of appropriate strategy to provide health and therapeutic benefit to human beings.

Literature Review:

The Polyherbal formulation (PHF) consists of;

Aqueous extract of aerial parts of *Coccinia indica*, fruits of *Momordica charantia* and leaves of *Lagestroemia speciosa.*

a) *Coccinia indica* W.&A.

Synonym: English- Scarlet fruited gourd

Hindi- Bhimb, Kunderi

Sanskrit- Rakta phala

Kannada- Tondikay

Family- Cucurbitaceae

Fig 20: *Coccinia indica* Plant

Indigenous to different parts of India and Bengal. *Coccinia indica* is abundant in India, Asia Pacific, Africa and Australia. It is employed in Ayurveda and Unani from in the Indian subcontinent.

Leaves- 5-10 cm long and wide, brilliant green above and pale underneath, studded and here and there harsh with papillae, palmately 5 nerved from a cordate base, regularly with round organs between the nerves close to the petiole and nervules as a rule finishing off with glandular far off denticulations; gently venose underneath, applaud, or orbicular, uncaringly 5 angled projections. Leaves demonstrated that it discouraged the movement of the chemical glucose-6-phosphatase and has a anatioxidant action, which might be ascribed to its defensive activity on lipid peroxidation and to the upgrading impact on cell reinforcement resistance adding to the assurance against oxidative harm in streptozotocin diabetes.

Fruits- Fusiform, ellipsed and marginally hooked, Checked when juvenile with white streaks, splendid red when completely ready. The plump green organic product is extremely severe. Green organic product is bitten to fix wounds on the tongue.

Seed- Obovoid, rounded at apex, slightly papillose and much compresses yellowish grey and having antigibberellins activity in plants.

Root- Thick, stems are grooved, slender and glabrous. It is used for treating diabetes.[145]

Chemical constituents-[146] Phytochemical investigations confirms triterpenoids, carotenoides, flavonoids and alkaloids.

- Aerial parts are reported to have cephalandrol, heptacosane, β-sitosterol alkaloids, cephalandrine A and cephalandrine B, triacontane.
- Leaves contain phenols.
- Fruits contain taraxerone, taraxerol, β-carotene, lycopene, cryptoxanthin, β-sitosterol, β-amyrin and its acetate.
- Seeds contain lupeol, cucurbitacin B, palmitic acid, oleic acid and linoleic acid.
- Roots contain triterpenoid, saponin, coccinioside, stigmast-7-e3-one, flavonoid, glycoside ombuin 3-0-arabinofuranoside.

Literature of review was done and it exhibited pharmacological activities of *Coccinia indica* W.&A. which are mentioned as follows:-

Anti-collagenase, anti-elastase and antioxidant activities-[147] Fruit juice concentrate of *Coccinia grandis* was explore by *in vitro* enzymatic examines to emulate the breakdown of elastin and collagen filaments and was observed to be have hostile to maturing and collagenase inhibitory action. Phenolic content of the herbal concentrate can be attributed to its free radical scavenging property.

Antihyperglycemia- [148-150]

1. Statistically significant reduction in blood glucose level, after seven days of treatment of diabetic animals with ethanol extract in combination with Acarbose have been reported.
2. Dried concentrate of *Coccinia indica* at dose 500 mg/kg, b.w. for about one and half month in diabetic patients applies antidiabetic impacts.
3. Chloroform extract is reported to significantly reduce the blood glucose level in STZ-NA diabetic rats.

Hepatoprotective-[151] Leaves (ethanol extract) possess, dose dependent hepatoprotective in chemically induced hepatotoxicity.

Chemoprotective activity-[152] Methanolic extract of of *Coccinia indica* leaves reported to possess chemoprotective property, when tested in cyclophosphamide induced rodent model of cytotoxicity..

Renoprotective activity-[153] Fruits and leaves of *Coccinia indica* seprately at 10% and 5% along with AIN-76 diet found to have renoprotective activity.

Analgesia and antipyretic effect- [154] Aqueous concentrate of *Coccinia indica* at a dose of 300 mg/kg found to have anti-inflammatory, analgesic and antipyretic activities.

Protective effect in end-organ damage-[155] Combination of aqueous extract of *Abroma augusta* and *Coccinia indica* at 300 mg/kg effectively protected diabetic animals against end organ damage

Antidyslipidemic activity-[156] Ethanolic extract at 25 mg/kg body weight in dyslipidemic hamster, significantly reduced elevated lipid profile, especially of triglyceride and cholesterol in dyslipidemic hamster model.

Mutagenic effect-[157] Silver nanoparticles of aqueous extract of leaves of *Coccinia cardifolia* with crystalline size range from 20-30 nm exhibited inhibition of growth and mutagenesis on Neurospora crassa by a gradual decline in growth of Mycelia.

Anti-hyperglycaemic and anti-ureogenic activity-[158] 200mg/kg of ethanol extract of *C.indica* showed anti-hyperglycaemic and anti-ureogenic effects in the diabetic rats by significantly reducing blood glucose level.

Antidepressant activity-[159] Methanolic extract of *Coccinia indica* at 400 mg/kg dose found to have antidepressant.

Hypoglycaemic and hypolipidaemic activity-[160] Alloxanized animal treated with aqueous extract of leaves is reported to result in hypoglycaemia and hypolipidemia.

Ovicidal activity-[161] Methanolic extract of *Coccinia indica* showed promising ovicidal activity against three mosquito species like *Calex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* at concentrations ranging from 50-300 ppm.

Correction of protein metabolic disorders-[162] The composite extract of *Musa paradisiaca* and *Coccinia indica* has a protective therapeutic effect against streptozotocin induce diabetes in rats through beta cell regeneration.

Antimalarial-[163] Essential oil from leaves of *Coccinea indica* possess excellent larvicidal property and inhibits egg hatching activity , when tested using WHO protocol.

Antioxidant-[164] Leaves of *Coccinia indica* as in ethanolic extract showed antioxidant effect at 200 mg/kg demonstrated free radical scavenging activity in STZ diabetic animals.

***b) Momordica charantia* Linn.**

Synonym: English- Bitter gourd

Hindi- Karela

Telgu- Kakarakaya

Kannada- Hagalakayi

Family- Cucurbitaceae

Fig 21: *Momordica charantia* Plant

Momordica charantia is common in tropical countries, including in India, where it is popular vegetable and is a part of folklore medicine, especially for diabetes.

Leaves- Leaves are alternate, characteristically pungent and aromatic.

Flowers- Yellow in colour.

Fruit- Limited to the two closures, ribbed with noticeable triangular tubercles.[165-166]

Chemical constituents:[167-168]

Fruits-It Contains a hypoglycaemic substance β-sitosterol glucoside, charantin, , diosgenin, cholesterol, lanosterol,. Immature fruits have non-bitter and bitter momodicosides.

Seeds- It produces α and β momorcharins (glycoproteins) and also vicine as a hypoglycaemic constituent.

Our review of literature on the reported biological activities from various databases revealed the following and the findings are significant.

Anticlastogenic and Anticarcinogenic-[169] Ground freeze-dried Thai Bitter gourd fruits at a different percentage concentration possess anticlastogenic and anticarcinogenic effect agains clastogens, cyclophosphamide and 7,12- dimethylbenz (a) anthracene, in mice using the Micronucleus assay and chemically induced colon carcinoma in rodents

Cardiac fibrosis activity-[170] Extract from fruit of *Momordica charantia* at dose of 1.5 g/ kg showed beneficial effect in diabetic cardiac fibrosis in STZ induced diabetic animals.

Insecticide activity-[171] At 1.0 ppm concentration petroleum ether extract of *Momordica charantia* shows the insecticidal activity.

Antioxidant activity-[172] Extract from bitter melon showed a impaired antioxidant activity at 150 mg/kg of body weight in diabetic rats (Chemically induced).

Antiobesity activity-[173] Aqueous and ethanol extract of *Momordica charantia* at different concentration Showed antiobesity activity in high-fat diet.

Anticarcinogenic activity-[174] Extracts from fruit and leaves of *M. charantia* in the dose of 500 and 1000mg/kg bodyweight, significantly increased life span and reduced tumour volume in cyclophosphamide induced tumour in mice.

Antimalarial activity-[175] Extract in methanolic form of *Momordica charantia* showed moderate activity with IC_{50}= 12.5 nM. The observed biological activity can be related to active constituents of the extract, preventing conversion of trophozoites to next stage of the life cycle.

Antihepatotoxicity and antioxidative activity-[176] Uccche (*Momordica charantia* L.) at 10% w/w showed inhibition of inflammation in carbon tetrachloride induced model, which showed its ability to moderate the inflammation and fibrosis in liver.

Antiatherogenic activity-[177] Bitter melon at two different doses i.e. 2 and 4 g/day ameliorating CVS risk factors – a typical secondary complication better than Glibenclamide in diabetics

***In vitro* ruminal fermentation and microbial population-**[178] Results of investigation reveal that saponin restrains *invitro* maize aging in relatively larger proportion, whereas lower concentration controls microbes of rumen, especially celluocytic microbes and parasites. **Anti-white spot syndrome virus activity-**[179] TP 22C from *M. charantia* is reported to possess potent antiwhite spot syndrome virus activity in *Litopenaeus vannamei*.

Hypoglycaemic and lipid lowering activity-[180] Bitter melon juice demonstrated strong hypoglycaemic and lipid bringing down impact in diabetic animal models and furthermore increment peroxisome proliferated-activated receptor gamma (PPAR-γ) movement and abatement protein kinase C beta (PKC-β) action in kidneys of diabetic rodents.

Renoprotective effect-[181]Aqueous extract of *Momordica charantia polysaccharides* at increasing doses, it was found to be renoprotective in diabetic animals.

Antiglycation and Antioxidant activity-[182] Pulp of *M. charantia* as aqueous extract, flesh (MCF) and charantin, all these extracts were compared for the antiglycation and antioxidant properties by *in vitro*. Study indicate that all extracts in a dose-dependent manner inhibit Generation of crosslinked glycation end products and carboxymethylsine (CML) and MCF found to be the most potent extract after comparing the antioxidant activity with MCP.

Cardiovascular activity-[183] Extract of bitter melon at 400 mg/kg, b.w. up to six weeks to lean and zucker obese (ZO) rats (Male) showed improvement of cardiac functions, and controlling the level of serum cholesterol.

Antidiabetic activity-[184,185]

1. Extract of whole fruit powder of *M. charantia* in acetone at dose of 0.25, 0.50 and 0.75 mg/kg, b.w. demonstrated fall in blood glucose level from 13.3% to 50% in alloxan induce diabetes in albino rats.
2. *Momordica charantia* fruit extract in juice form at 10 ml/kg/day dose to Wistar rats shows antidiabetic and antioxidant activities.

Hypocholesterolemia activity-[186] Karela at 5g/kg, b.w. dose orally to Wistar rats show antioxidant activity and also karela ameliorated all altered genes which were induced by hypercholesterolemia confirming its hypocholesterolemic effect.

c) *Lagerstroemia speciosa* Linn.

Synonym: English- Queen Crape Myrtle

Hindi- Jarul

Tamil- Kadali

Marathi- Taman

Family- Lythraceae

Fig 22: *Lagerstremia speciosa* Plant

Leaves- Smooth, huge, spatulate, oval to elliptic applaud, 2-4 inch width, 5-8 inch length, blooms are racemes, purplish lilac or mauve-pink. It is fill in as a diuretic, decongestant and utilized in diabetes mellitus.

Bark- Smooth, dim to cream hued and strips off in unpredictable slakes. The bark is utilized as a stimulant, febrifuge and for alleviation of stomach torments.

Fruits- Tree bears large clumps of oval nutlike fruits.[187]

Chemical constituents-[188]

The plant contains triterpenoids, corosolic acid and maslinic acid. Leaves, fruits and barks contain tannins. Leaves contain alanine, isoleucine, menthoeonine, lageracetal, amyl alcohol, ellagic acid, lagertannin, lagerstroemin.

Literature of review was done and it exhibited pharmacological activities of *Lagerstroemia speciosa* Linn. which are mentioned as follows:-

Anti-fibrotic activity-[189] Alcoholic extract of *Lagerstroemia speciosa* at 100 mg/kg dose found to have anti-fibrotic activity in Carbon tetrachloride (CCl_4) induced liver fibrosis in male rodents.

Neuroprotective activity-[190] In streptozotocin induced rodent's extract of. *Lagerstroemia speciosa* at two different doses showed neuroprotective activity.

Antitussive-[191] *Lagerstroemia parviflora* extract showed maximum suppression of cough reflex at 90 min after drug administration.

Antioxidant activity-[192] Ethanoloic extract of *Lagerstroemia speciosa* Linn. showed antioxidant activity onhigher concentrationof the extract.

Antinociceptive activity-[193] Extract of *Lagerstroemia speciosa* in ethanolic form in dose dependent manner, significant analgesia was demonstrated by ethanolic extract..

Anti-inflammatory activity-[194] Ethyl acetate extract possess significant anti-inflammatory activity in acute and chronic models.

Anti-obesity activity-[195] Hot, aqueous extract of *Lagerstroemia speciosa* reported to have anti-obesity activity in obese female KK-A^Y mice.

Anti-diabetic property-[196] In alloxan treated diabetic mice, ethanolic leaf extracts of *Lagerstroemia speciosa* showed hypoglycaemic effects at two different concentration i.e. 25 and 50% and results were comparable to insulin.

Anti-diarrhoeal activity-[197] Ethanolic extract at 500 mg/kg bodyweight was reported to elicit protective effect against diarrhoea in animal model..

Cytotoxic activity-[198] Ethanol fruit extract of *Lagerstroemia speciosa* showed prominent cytotoxic activity using the brine shrimp (Artemiasalina) lethality bioassay.

Hypoglycaemic activity-[199] *Lagerstroemia speciosa* in form of aqueous extract at 100 and 200 mg/kg dose exhibited prominent hypoglycaemic effect in STZ-NA induced T2D in rats.

Antiviral activity-[200] Ellagic acid (*Lagerstroemia speciosa* leaf-derived material*)* lead molecule found to have antiviral activity, especiall for the treatment or prevention of human rhinovirus (HRV) infection.

Hepatoprotective effect-[201] *L.speciosa* in ethanolic extract form at dose of 100 and 250 mg/kg show protective effect against chemically induced hepatotoxicity in mice.

Inflammatory Bowel Disease activity-[202] Methanolic extract of *Lagerstoemia speciosa* leaves in dose dependent manner showed protection in ulcerative colitis in C57BL/6 mice induced by dextran sulfate sodium.

3.1 MATERIALS

Evaluation of antidiabetic activity:

Polyherbal formulation was a gift sample from M/s Green Chem Herbal Extracts and Formulations, Bengaluru.

Chemicals (mentioned below) were of analytical grade and were used for investigations:

1. Streptozotocin (Sigma-Aldrich)
2. Nicotinamide (Sigma-Aldrich)
3. Fructose (S.D. Fine Chem. Ltd. INDIA)
4. Intracellular adhesion molecule-1 Elisa kit (YH Biosearch laboratory)
5. Vascular endothelial growth factor Elisa kit (RayBio)
6. TNF-α kit (BioVision, USA)
7. Serum Insulin kit (Mercodia, Swedan)
8. Fasting Blood Glucose kit
9. TC kit
10. Triglyceride kit
11. HDL-C kit
12. Creatinine kit
13. Uric acid kit
14. Blood urea kit
15. HbA1C kit
16. C-RP kit
17. SGOT kit
18. SGPT kit

Diagnostic test kits (8-18) were purchased from M/s Agapee diagnostic.

Animals:

Albino Wistar rats of either sex weighing 200-250 g (toward start of investigation) for Streptozotocin induced diabetes and 150-200 gm for fructose induced diabetes were utilized for the examination. Polypropylene cages and maintained under hygienic conditions in our animal house and research facility. The confines; confine plate, sustenance containers and water bottles were disinfected at standard interims. Animals during experimentation were fed on commercial pelleted feed and water *ad libitum* and acclimatized for a week to our research facility. All methodology depicted are investigated and endorsed by the Institutional Animal Ethics Committee (IAEC) of KLE University College of Pharmacy, Bangalore, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. (01/PA/2015). The guidelines for ethics were followed strictly during experimentation.

3.2 METHODOLOGY

Phytochemical Screening:

Preliminary analysis of phytochemical was performed for the aqueous extract of Polyherbal formulation (PHF).[203,204]

Test for Alkaloids:

Test solution for following tests was prepared by acidifying the sample with hydrochloric acid (5 ml extract + 2ml of hydrochloric acid).

a) **Dragendroffs reaction:** Upon the addition of Dragendroffs reagent, formation of dark rose-coloured solution confirms alkaloids in the sample.
b) **Mayer's reaction:** Upon the addition of Mayer's reagent, dark colored solution was positive for alkaloids**.**
c) **Wagner's reaction:** Upon the addition Wagner's reagent, dark colored accelerate showed the nearness of alkaloids.
d) **Hager's reaction:** Upon the addition of Hager's reagent, a resultant yellow solution confirms alkaloid in the sample.
e) **Test for Tannins:** When sample was mixed with 1% gelatine in 10% sodium chloride, formation of white solution suggesting presence of Tannins.[205]

Test for Cardiac Glycosides:

Keller Killani test for Deoxy sugar: Along with ferric chloride treatment and upon addition of sulphuric acid, two layers formed. Reddish brown lower and bluish green upper layer confimed deoxy sugar [3].

a) **Baljet test:** Sodium picrate was added in test solution; yellow to orange color conversion indicated the presence of glycosides.

Test for Flavonoids:

a) **Shinoda test:** Filtered ethanol (95%) extract of 1 gm of sample developed red color, after addition of magnesium and 3 drops of HCl, confirming the presence of flavonoids.

Test for Saponins:

a) **Froth test:** Persistent forth development seen upon addition of sample in quantity of 0.1 gm and shaken vigoursly with 5ml of water, on setting aside for 20 minutes.
b) **Test for Steroidal Saponins:** Hydrolyzed and chloroform extract sample tested possible for sterols.
c) **Test for triterpenoidal Saponins:** Hydrolyzed and layer of chloroform extract tested possible for triterpenoids.

Test for sterols:

a) **Libbermann-Burchard test:** Sulfuric acid (2 drops) was added to sample moistened with acetic anhydride , presence of sterols indicated by green colour.
b) **Salkowski reaction:** Upon addition of drops of chloroform and concentrated sulphuric acid to 2 gm of sample, resulted in red precipitating indicating presence of sterols.

Test for carbohydrates:

a) **Reducing of Fehling's solution:** To the boiling sample, equal volumes of Fehling's A and B was added which resulted in brick red precipitate confirms reduced sugar in the sample.

b) **Molisch test:** Addition of alpha napthol and conc. Sulphuric acid to test solution resulted in purple precipitate at the junction indicating presence of sugars.

Test for phenolic compounds: Extract, when treated with 5% alcoholic ferric chloride yielded bluish black / dark green coloured solution indicating presence of phenolic compounds in the sample.

Test for Proteins and Amino Acids:

a) **Million's test:** When treated with Millions Reagent maintaining the solution on water bath, resulted in red colour indicating proteins in the sample.
b) **Biuret test:** 5 ml of test extract was treated along with equal volumes of 10% sodium hydroxide and 0.5% copper sulphate solution, drop wise. Purple solution of the resultant solution confirmed the presence of proteins.
c) **Ninhydrin test:** 0.5 ml of 0.1% Nin Hydrin solution added to 5 ml of test extract and boiled. Purple colour development indicates presence of proteins in the sample.

Test for Fixed Oils and Volatile Oils: When sample was pressed between pieces of filter paper, it resulted in oil stains and evaporation of the same at room temperature confirms the presence of fixed oils and volatile oil respectively.

Evaluation of anti-diabetic activity:

Acute toxicity study (Oral):

Oral (Acute) toxicity was performed in healthy, non pregnant, naïve albino Wistar rat weighing between 180-230 gms according to OECD test guideline 425. Test sample treated animals were monitored continuously for the signs of morbidity and for another 48 hours for morbidity and mortality.[206]

Experimental design:

Streptozotocin or Izostazin or Zanosar (STZ)

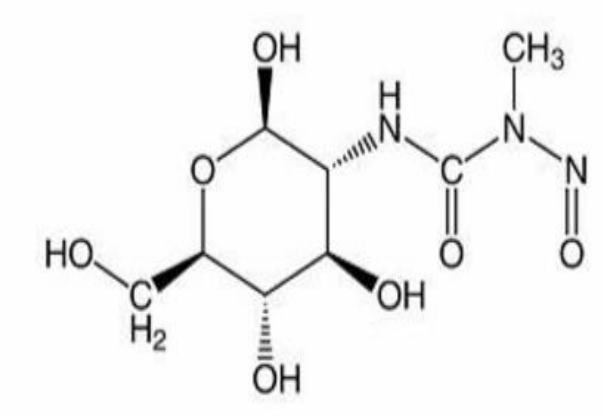


Figure 23: Structure of streptozotocin

INDUCTION OF DIABETES IN RATS USING STREPTOZOTOCIN AND NICOTINAMIDE:

Healthy, adult albino Wistar rats, aged 75-90 days and weighing between 200-250 gms were were fasted overnight and pretreated with Nicotinamide (NA) (110 mg/kg) dissolved in saline by intraperitonial route, 15 minutes prior to Streptozotocin (STZ) injection. STZ were prepared freshly by dissolving weighed quantity in 0.05 M citrate buffer (at 4.5 pH) and injected intraperitonially. All animals received 5% dextrose after 6 hours of STZ administration, to counteract severe (same time fatal) hypoglycaemia due to massive insulin release after STZinjection. STZ injection, by its cytotoxic effects on β cells of pancreas

produced diabetes in animals within 72 hours.[207] After 72 hours of STZ treatment, fasting blood glucose (FBG) was measured (by using commercial test kit) and only animals with FBG above 200 mg/dl were selected for further investigation. Diabetic animals were randomly assigned into five groups of six animals each (n=6). The treatment protocol/ schedule is as follows:

Group I – Normal control (saline treatment)

Group II – Positive control (STZ treatment): This group infused intra-peritoneally (i.p.) with STZ at a dose of 65mg/kg body weight and served as positive control group (PCG).

Group III – Aqueous PHF (STZ- NA+ Dose I): This group received both i.p. infusion of STZ at a dose of 65 mg/kg body weight and Dose I (200 mg/kg, P.O.)

Group IV – Aqueous PHF (STZ-NA + Dose II): This group received both i.p. infusion of STZ at a dose of 65 mg/kg body weight and Dose II (400 mg/kg, P.O.)

Group V – Standard group (STZ-NA+ Metformin 5mg/kg, b.w.): This group received both i.p. infusion of STZ at a dose of 65 mg/kg body weight and metformin, in a dose of 5mg/kg, b.w and filled in as standard group.

Parameters assessed

Throughout the course of experiment, body weight of all test animals was recorded at regular interval. Animals of control group were placed in enclosures independently for urine accumulation toward the finish of study. Following 2 hours of Standard and PHF treatment on the most recent day, the animals were exposed to behavioural biomarkers for development of neuropathy like Eddy's hot-plate test[208-210] and tail-flick technique[211] to survey the advancement of neuropathy. Blood was collected by retro-orbital plexus, one part of entire blood was utilized for the estimation of glycosylated hemoglobin (HbA1c) and other part of blood was centrifuged for the accumulation of serum by utilizing cooling microfuge for the estimation of blood glucose, serum insulin, triglycerides, VLDL, total cholesterol, low-

density lipoprotein, HDL and creatinine, urea, and uric acid utilizing individual test packs utilizing a auto analyzer (Recorders and Medicare Systems) and TNF-α utilizing particular Elisa kit.

Diabetic Nephropathy: Renal veins of diseased kidney secretes protein into the urine and gradually progresses into a condition referred to as diabetic nephropathy. [212]

Estimation of Biomarkers of Oxidative Stress[213]

Kidneys were isolated and kept on precooked (autoclave) rearranged Petri dish in cold conditions with ice blocks. The tissue was cross cleaved with a careful surgical tool into fine cuts in chilled 0.25 M sucrose and immediately smudged on channel paper. Sample of tissues were minced and homogenised in 10mM Tris buffer (pH 7.4) in a Teflon homogenizer, using cooling centrifuge (M/s Remi Instruments, Mumbai, India) speed at 10,000 x g. Clear supernatant sample was employed for following tests :

i. Malondialdehyde (MDA)[214]

ii. Superoxide dismutase (SOD)[215]

iii. Catalase (CAT)[216]

iv. Glutathione (GSH)[217]

Diabetic Neuropathy: Constrained blood stream and they get harmed or pass on subsequently. Diabetic neuropathy is characterised by nerve damage and hurts feet and lower regions. [212]

Assessment of Diabetes-induced Oxidative Stress in nerves:[218]

Animals were relinquished and left thigh skin parallel etched. Sciatic nerve, homogenized, centrifuged in 10% w/v arrangement, at 5000 rpm for 10 min. The supernatant was utilized for the other measure like:

i. SOD

ii. CAT

iii. MDA

Delayed Gastric Emptying:[219]

The intestinal travel of charcoal meal was dictated by modified Janseen strategy. The rodents were regulated with the charcoal feast comprising of 10% of initiated charcoal and 5% gum acacia orally (2 mL/rodent) after a medium-term fasting. The animals were sacrificed by overdose of anaesthesia 15 min after charcoal organization. The small intestine was expelled from the pyloric sphincter to the ilioceacal intersection and the separation gone by the charcoal supper was noted and communicated as level of intestinal travel utilizing the recipe given underneath:

$$\%\text{Transit} = \frac{\text{Distance travelled by charcoal meal} \times 100}{\text{Total length of small intestine}}$$

Following cleaning and estimating the length of the large intestine, 2 cm distal colon was cut and utilized for *in vitro* examinations. The distal colon was analyzed out and mounted under a resting pressure of 500 mg in an organ bath containing persistently circulated air through Tyrode's answer (40 mL, pH 7.45). Dose response curves were acquired with rising dosages of ACh. EC50 values were determined from a graph plotted utilizing percent responses against log dose.

Diabetic Retinopathy: Diseased little veins in the eye keeps leaking, thus keep spilling protein into retina. Fragile veins can promote scaring of retina and separation, thus weakening vision scarring and also retinal separation, in this manner weakening vision[10].

At the end of the studies, from mildly anaesthesia, eye samples collected and used for the estimation of vascular endothelial development factor (VEGF) and intercellular adhesion molecule-1 (ICAM-1). Samples were centrifuged (10,000 rpm for 10 Minutes) at 4^0 c and supernatant was used for the estimation of VEGF and ICAM-1levels using ELISA test kit.

Measurements of SOD and MDA levels in Serum:

End of the study, using retro orbital plexus blood collected and centrifuged to 3,500 rpm for 10 min. Serum was utilized for the assurance of SOD and MDA levels.[220]

Diabetic Cardiopathy: Diabetes promotes atherosclerosis in bigger veins due to reduced / absence of blood supply.[212]

Endogenous antioxidant systems:[221]

At the end of the examination, following standard and PHF treatment, rodents under mild anaesthesia, thoracic part of aortic tissues was extracted, cleaned and connective tissues were removed and homogenized utilizing a homogenizer. Homogenate was then centrifuged (Remi Cooling Centrifuge, Remi Instruments Limited, Mumbai-India) at 4°C 15,000 rpm for 10 minutes. Resultant supernatant was used for;

i. SOD
ii. CAT
iii. MDA

Antioxidant parameter:

Toward the end, diabetic animals were euthanized and liver tissues were extracted, washed, and solidified at - 70℃ for further investigation. Antioxidant effects of Polyherbal formulation was determined by measuring lipid peroxides and activities of antioxidant enzymes in the liver.[222]

Histopathological study was done to support the above studies.

FRUCTOSE INDUCED DIABETIC MODEL:[223]

Healthy, Wistar albino rats of either sex, in the weight range of 150-180 gm were utilized in the investigation. T2DM in the animals was induced by chronic administration of fructose (66% solution orally for about a month and a half) with the exception of normal control group. After the multi week ingestion of fructose blood glucose level was observed and diabetic rodents whose blood glucose level was in excess of 180 mg/dL were considered in the investigation and they were assigned to five groups of six animals each (n=6):

Group I – Normal control (saline treatment)

Group II – Positive control (66% fructose solution)

Group III – Aqueous PHF (66% fructose solution + Dose I 200 mg/kg, b.w.): This group received both 66% fructose solution and Dose I Per orally, for about a month and a half.

Group IV – Aqueous PHF (66% fructose solution + Dose II 400 mg/kg, b.w.): This group had both 66% fructose solution and Dose II Per orally, for about a month and a half.

Group V – Standard group (66% fructose solution + Metformin 5mg/kg, b.w.): This group received both 66% fructose solution and metformin, in a dose of 5mg/kg, b.w for about a month and a half**.**

Parameters assessed

Body weight of the animals was recorded through out the investigation. Following 2 hours of Standard and PHF treatment diabetic rodents were examined by recording parameters for evaluation of the advancement of neuropathy.[224] Using retro-orbital plexus blood was collected, one part of this was utilized for estimation of glycosylated hemoglobin (HbA1c) and other part was centrifuged, separated by utilizing cooling microfuge for the estimation of various biochemical parameteres employing individual test kits and an auto analyzer. Insulin resistance was assessed by the Homeostasis Model Assessment list (HOMA-IR) using the formula: [insulin (mU/L) x glucose (mmol/L)]/22.5.

Diabetic Nephropathy:[225]

Total nitrite in renal tissue homogenate (Griess reaction):

Total nitrite fixation level estimated in 100 mg of renal tissue homogenate. All colorimetric measures were performed utilizing an UV-visible spectrophotometer.

Diabetic Neuropathy:[224]

Appraisal of Diabetes-incited Oxidative Stress in Nerve:

At the end of the study, cut was made using scalpel blade to confine the sciatic nerve. At that point sciatic nerve was set in KCl arrangement made at concentration of 10% w/v, homogenized followed by centrifuged at 5000 rpm for 10 minutes and used for following tests:

i. SOD

ii. CAT

iii. MDA

Diabetic Cardiopathy:[226]

i) **Inflammatory marker on heart tissue:**

TNF-α level were resolved utilizing a commercially accessible ELISA pack.

ii) **Oxidative stress profile on heart tissue:**

Heart samples isolated from animals at the end of study were homogenized utilizing homogenizer and centrifuged at 15,000 rpm for 5min. Following centrifugation, the supernatant gathered and utilized for the accompanying test:

i. CAT

ii. GSH

iii. MDA

iv. SOD

Antioxidant Parameter:[227]

***In vivo* antioxidant activity:** End of examination, diabetic animals was euthanized and liver tissues were extracted, washed, and solidified at - 70℃ for further investigation. Antioxidant impact of Polyherbal formulation detailing was dictated by estimating the accompanying:

i. GSH

ii. CAT

iii. SOD

Histopathological study was done to support the above studies.

Statistical analysis:

Data obtained from all tests / estimations were expressed as Mean ±SEM of n=6 and the results were subjected to one-way ANOVA followed by Dunnett multiple comparison test. For significance results were compared with either saline /normal control or untreated pathogenic control group of animals (Positive control). All statistical was performed using Graph Pad Prism 5.

4 RESULTS

Preliminary Phytochemical screening:

Results of screening of Polyherbal formulation (PHF) was analyzed using qualitative chemical tests were tabulated [Table-3].

Table 3: Preliminary phytochemical screening of aqueous extract of PHF

Test for	Aqueous Extract of PHF
Alkaloids	+ve
Glycosides	+ve
Carbohydrates	+ve
Proteins	+ve
Flavanoids	+ve
Steroids	+ve
Saponins	+ve

Acute oral toxicity study:

No signs of morbidity / mortality were observed in treated animals with sample up to 2000mg/kg, confirming the safety of sample upto 2000mg/kg [Table 3.a.].

Table 3.a. Effect of aqueous extract of PHF 2000 mg/kg on physical and behavioural sign and symptoms

Sign and symptoms	Basal	30 min.	60min.	1 h	2h	48h
Body weight	–	–	–	–	–	–
Lacrimation	–	–	–	–	–	–
Salivation	–	–	–	–	–	–
Skin colour	–	–	–	–	–	–
Sedation	-	-	+	+	–	–
Tremors	–	–	–	–	–	–
Diarrhoea	–	–	–	–	–	–
Respiration	–	–	+	–	–	–
Result						
Number of deaths			Nil			

– = No change **+ = change**

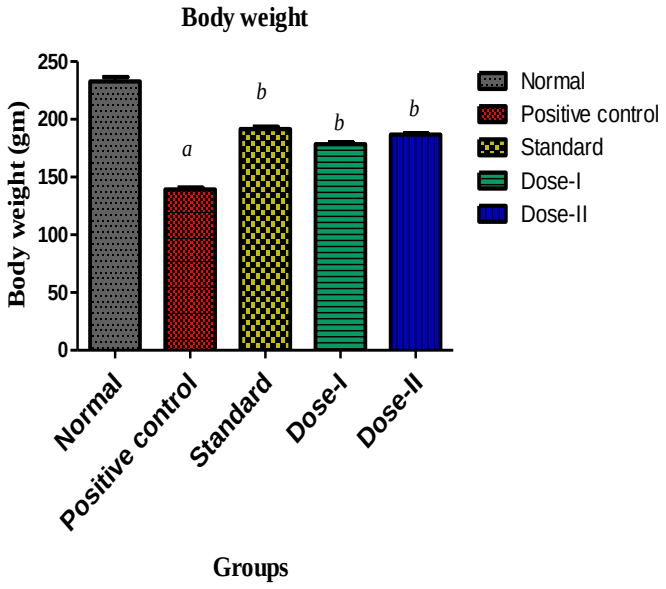
Effect of PHF treatment on body weight and volume of urine of STZ diabetic animals

Positive control animals recorded significant reduction in the body weight and increase volume of urine excreted ($p<0.001$) compared to saline / vehicle treated group of animals. Dose dependent and highly significant increase in the weight of animals and decrease urine output was recorded in PHF treated diabetic animals [Table-4].

Table 4. Effect of PHF treatment on body weight and volume of urine of STZ diabetic animals

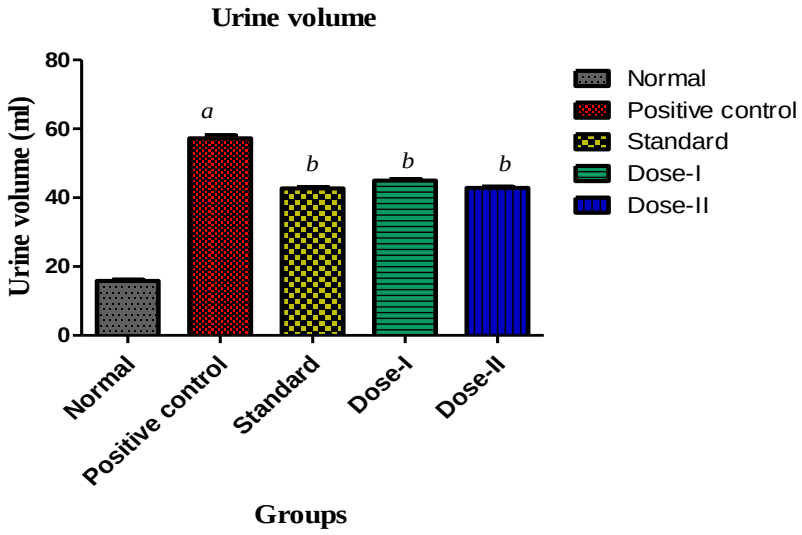
Groups	Body Weight (gm) (at the end of study)	Urine Volume (ml) (at the end of study)
Normal	232.8±3.816	15.81±0.328
Positive control (STZ)	139.3±1.476***[a]	57.23±0.812***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ STZ	191.5±1.996***[b]	42.68±0.324 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	178.5±1.335***[b]	44.99±0.385 ***[b]
Dose-II (400mg/kg, b.w.)+STZ	186.8±0.7491***[b]	42.84±0.345 ***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 24.a. Effect of PHF on Body weight in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 24.b. Effect of PHF on Urine volume in STZ induced diabetic animals

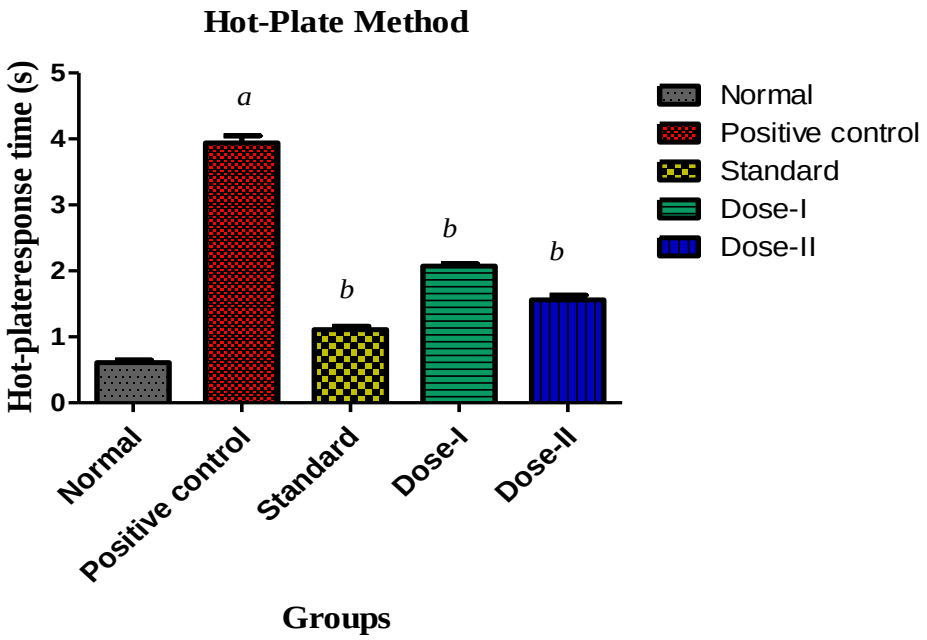
Analgesic effect of PHF in STZ diabetic animals

In both the methods employed to assess analgesic effect of PHF, untreated animals demonstrated statistically significant reduction in the latency to respond to a noxious stimuli and PHF treatment significantly reduced the latency period [Table-5].

Table 5. Analgesic effect of increasing dosses of PHF in STZ diabetic animals

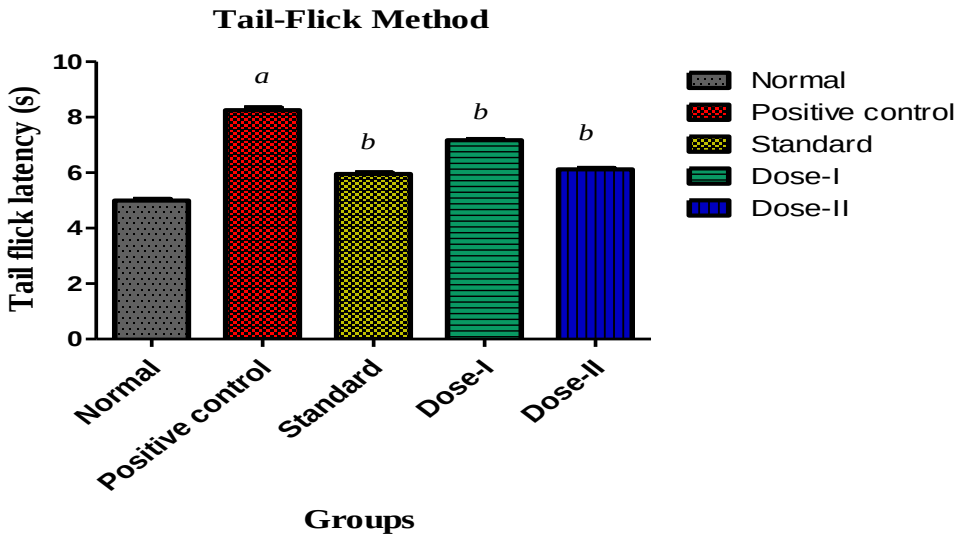
Groups	Hot-Plate Method (sec)	Tail-Flick Method (sec)
Normal	0.61±0.036	4.99±0.066
Positive control (STZ)	3.93±0.109***[a]	8.25±0.088***[a]
Standard (Metformin 5mg/kg, b.w.)+ STZ	1.11±0.048 ***[b]	5.95±0.052 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	2.07±0.034 ***[b]	7.17±0.039 ***[b]
Dose-II (400 mg/kg, b.w.)+STZ	1.56±0.071 ***[b]	6.12±0.048 ***[b]

Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 25.a: Analgesic effect of PHF in STZ diabetic animals (Hot plate method)



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 25 b: Analgesic effect of PHF in STZ diabetic animals (Tail flick method)

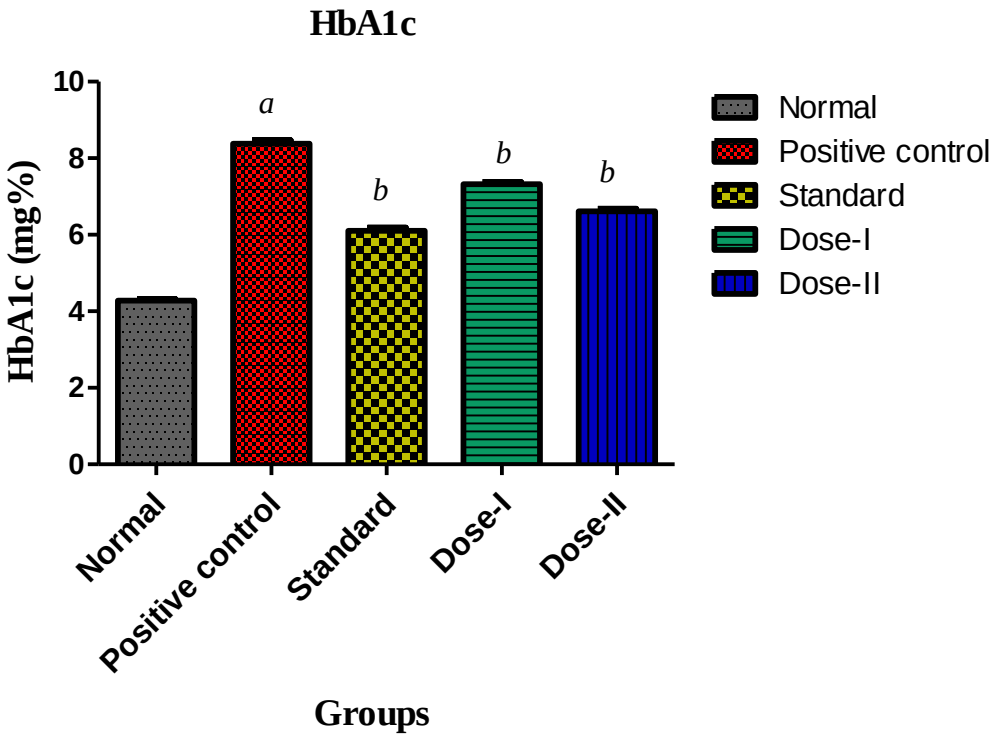
Effect of PHF on glycated haemoglobin (HbA1c) level in STZ induced animals

Elevated levels of HbA1c was recorded by STZ diabetic animals compared to non-diabetic and significant reduction ($p<0.001$) was recorded by Metformin, Dose 1 and 2 treated diabetic animals [Table-6].

Table 6. Effect of PHF on glycated haemoglobin (HbA1c) level in STZ induced animals

Groups	HbA1c (mg %)
Normal	4.275±0.049
Positive control (STZ)	8.375±0.096***a
Standard Treatment (Metformin 5mg/kg, b.w.)+ STZ	6.105±0.080***b
Dose-I (200 mg/kg, b.w.)+STZ	7.325±0.056***b
Dose-II (400mg/kg, b.w.)+STZ	6.613±0.064***b

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig.26. Effect of PHF on glycated haemoglobin (HbA1c) level in STZ induced animals

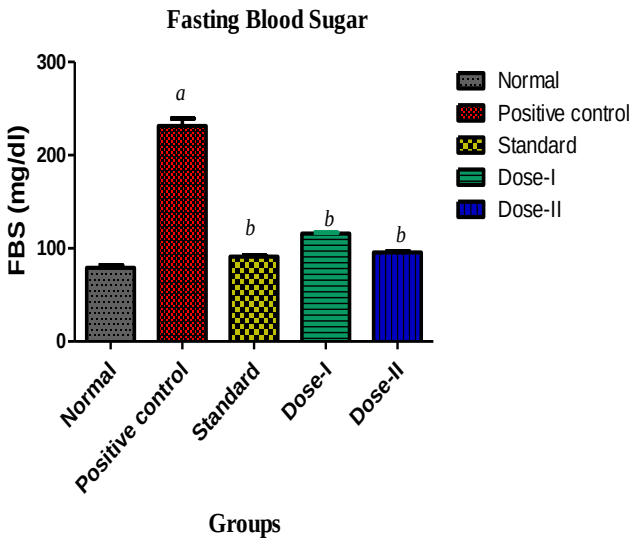
Effect of PHF on Blood glucose and Serum insulin levels in STZ induced animals

Rodents after induction of STZ recorded statistically significant ($p<0.001$) increase levels of FBS compared to normal group rodents. Diabetic rats treated along with PHF and metformin recorded statistically significant ($p<0.001$) reduction in FBS level as compared to diabetic control rats. In addition STZ injection significantly lowers insulin levels. Treatment of hyperglycaemic animals with PHF and metformin except positive control animals resulted in significant levels of serum insulin, compared with positive control group of animals [Table - 7].

Table7. Effect of PHF on Blood glucose and Serum insulin levels in STZ induced diabetic animals

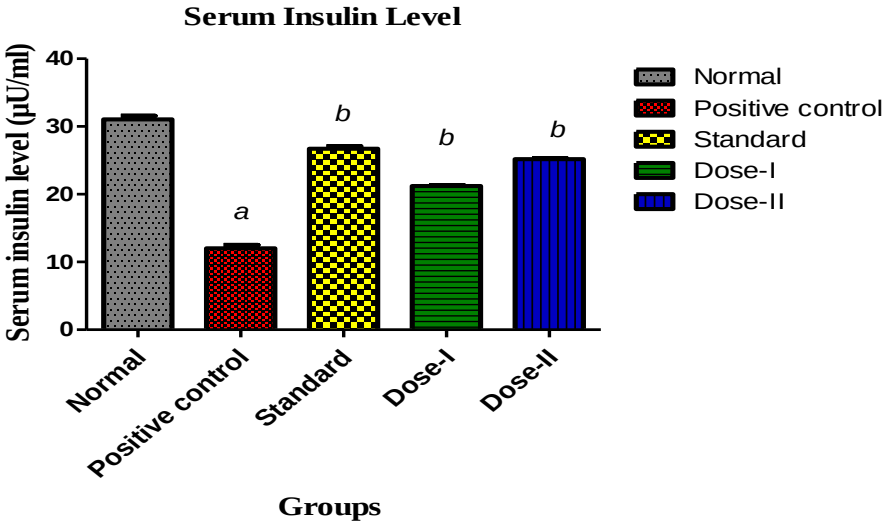
Groups	FBS (mg/dl)	Serum Insulin (µU/ml)
Normal	79.13±2.854	31.06±0.514
Positive control (STZ)	231.5±7.921***[a]	12.02±0.506***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ STZ	91.14±1.174***[b]	26.72±0.381***[b]
Dose-I (200 mg/kg, b.w.)+STZ	116.0±0.9117***[b]	21.22±0.125***[b]
Dose-II (400mg/kg, b.w.)+STZ	95.75±1.075***[b]	25.17±0.319***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 27.a. Effect of PHF on Fasting blood glucose level of diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 27.b. Effect of PHF on Serum insulin level in STZ induced diabetic animals

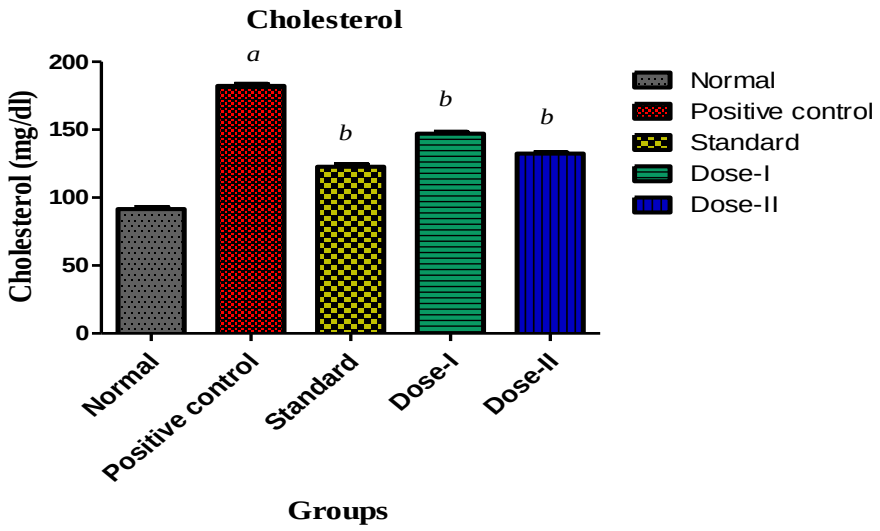
Effect of PHF on the lipid profile of STZ diabetic animals

Hyperglycaemic rats exhibited statistically significant altered lipid profile, contrasted with normal group animals. Diabetic rat's treatment with PHF and metformin treated animal's demonstrated significant change in the lipid profile of diabetic animals and so also the PHF treated diabetic animals, when compared to untreated diabetic animals [Table-8].

Table8. Effect of PHF on the lipid profile of STZ diabetic animals

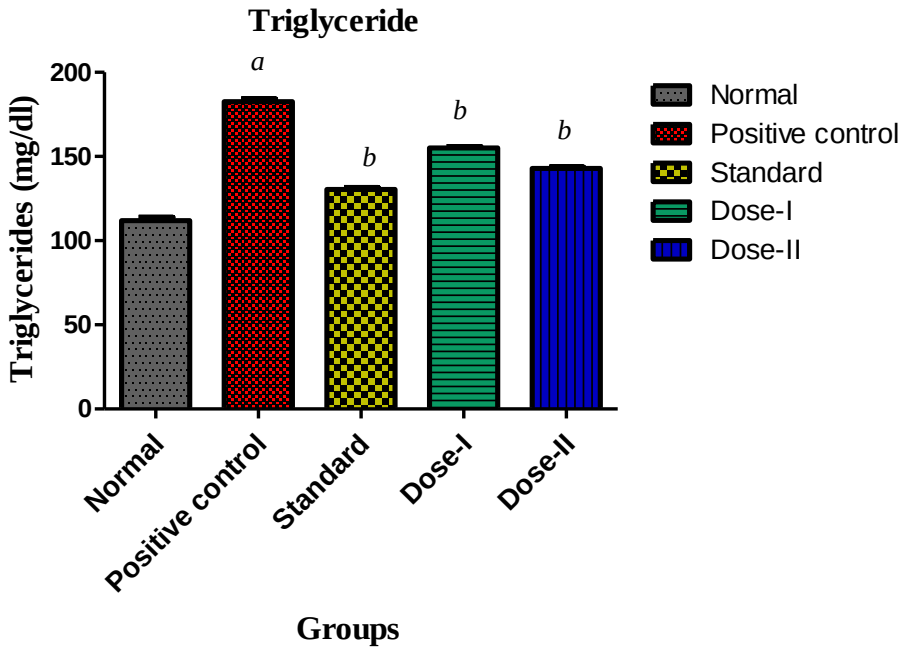
Groups	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
Normal	91.66±1.457	111.90±2.098	35.79±0.337	22.37±0.419	33.68±1.224
Positive control (STZ)	182.20±1.572 ***[a]	182.60±1.629 ***[a]	25.78±0.859 ***[a]	36.52±0.325 ***[a]	119.90±1.918 ***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	122.80±1.771 ***[b]	130.40±1.185 ***[b]	33.02±0.485 ***[b]	26.07±0.236 ***[b]	63.74±2.066 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	147.10±1.389***[b]	155.10±0.853 ***[b]	29.76±0.400 ***[b]	31.02±0.170 ***[b]	86.33±1.404 ***[b]
Dose-II (400 mg/kg, b.w.)+STZ	132.40±0.779***[b]	142.90±0.923 ***[b]	31.93±0.366 ***[b]	28.57±0.184 ***[b]	71.93±1.014 ***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



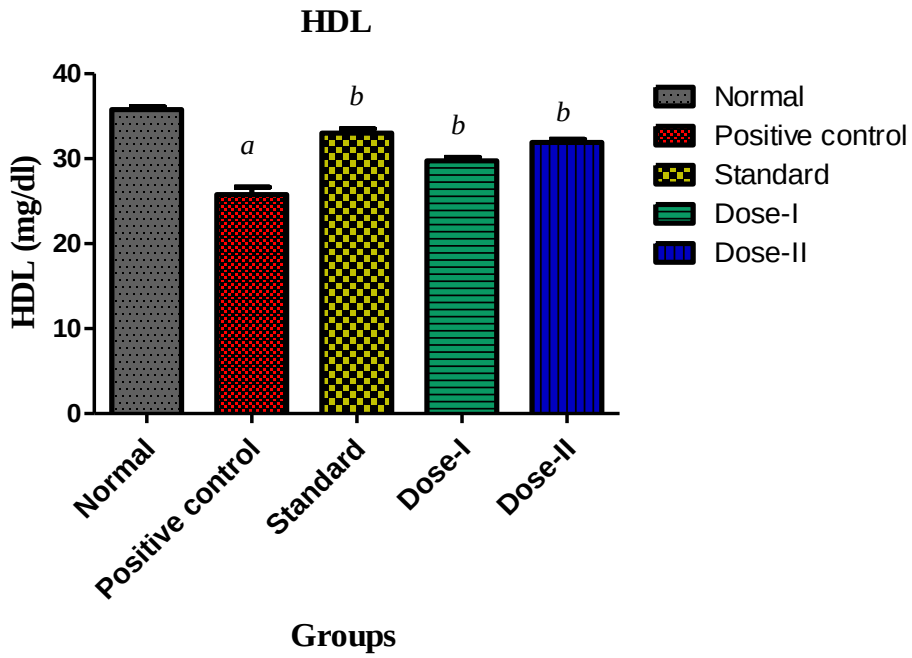
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 28.a. Effect of PHF on TC level in STZ induced diabetic animals



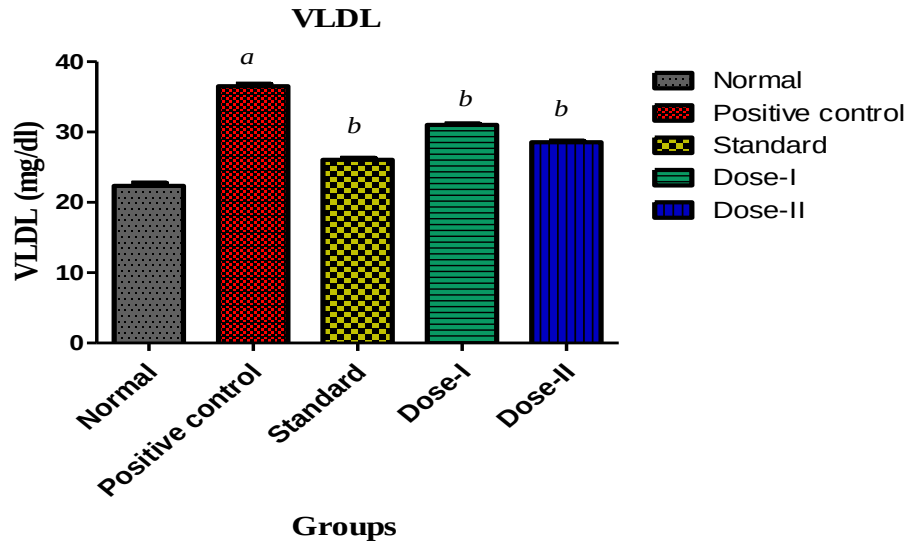
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 28.b. Effect of PHF on TG level in STZ induced diabetic animals



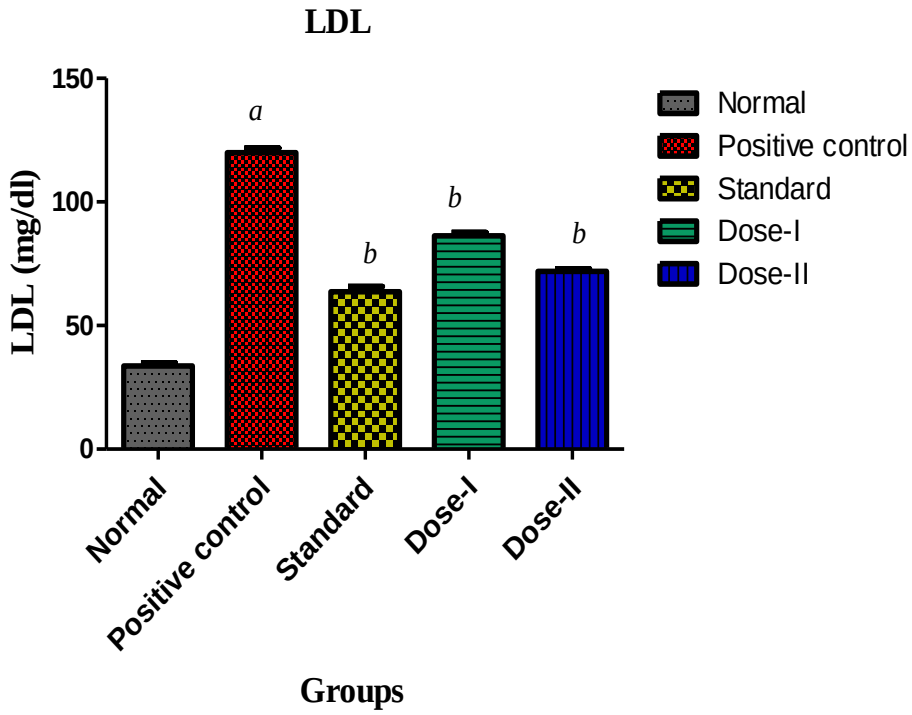
Values are expressed as mean± SEM,n=6. *** p<0.001, when compared to normal control group (a) and *** p<0.001, when compared to positive control group (b).

Fig. 28.c. Effect of PHF on TG level in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. *** p<0.001, when compared to normal control group (a) and *** p<0.001, when compared to positive control group (b).

Fig. 28.d. Effect of PHF on VLDL level in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 28.e. Effect of PHF on LDL level in STZ induced diabetic animals

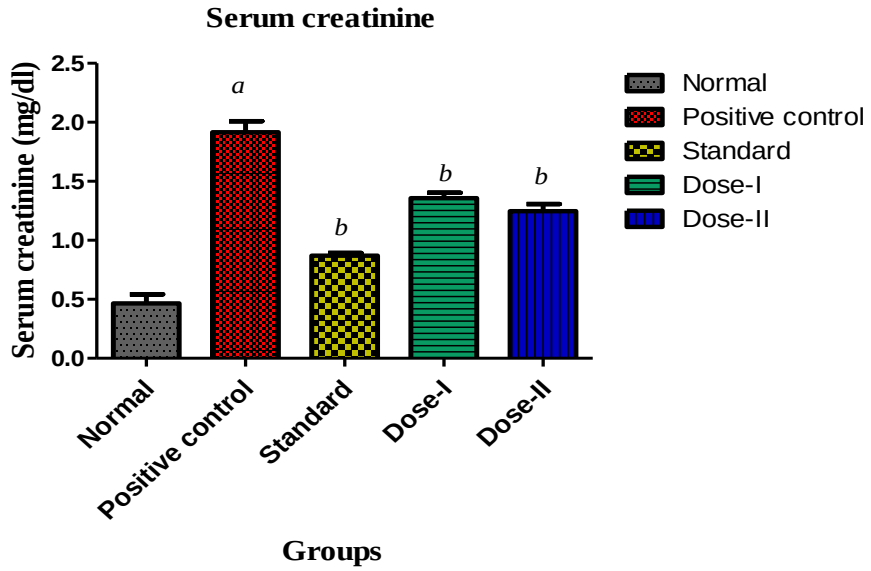
Effect of PHF on marker levels of kidney in STZ induced diabetic animals

STZ diabetic animals recorded significant reduction in the function of kidney as significantly elevated levels of serum creatinine and uric acid compared to untreated non diabetic animals. Diabetic animals treated with increasing doses of PHF resulted in statistically significant and dose dependent reduction in the serum creatinine, uric acid and urea level compared to untreated diabetic group of animals [Table-9].

Table9. Effect of PHF on marker levels of kidney in STZ induced diabetic animals

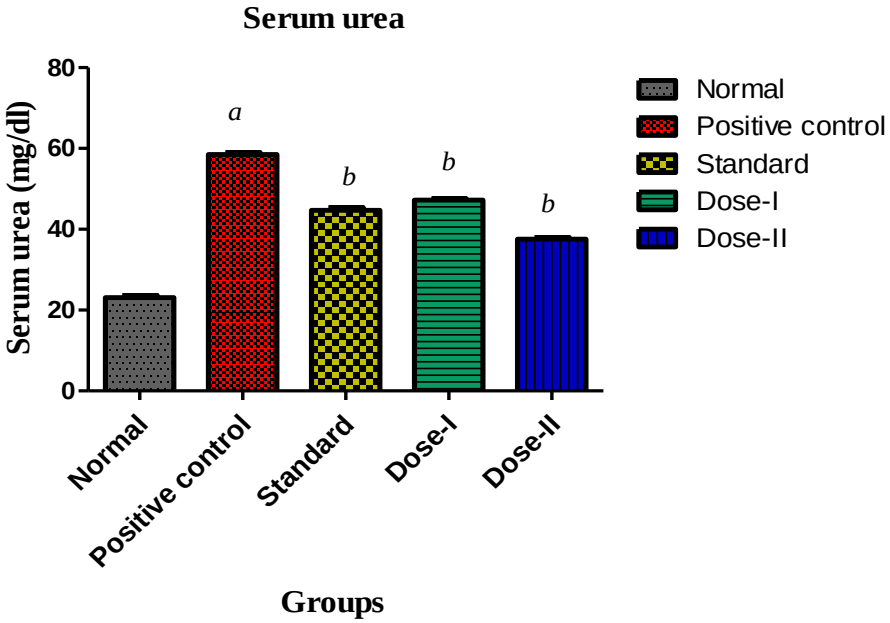
Groups	Serum creatinine(mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)
Normal	0.465±0.921	23.11±0.539	1.715±0.015
Positive control (STZ)	1.917±0.092***[a]	58.47±0.521***[a]	4.108±0.021***[a]
Standard treatment (Metformin 5mg/kg, b.w.)+ STZ	0.870±0.023 ***[b]	44.72±0.644 ***[b]	2.427±0.022 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	1.357±0.046 ***[b]	47.26±0.293 ***[b]	3.277±0.018 ***[b]
Dose-II (400 mg/kg, b.w.)+STZ	1.247±0.061 ***[b]	37.56±0.389 ***[b]	2.735±0.012 ***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



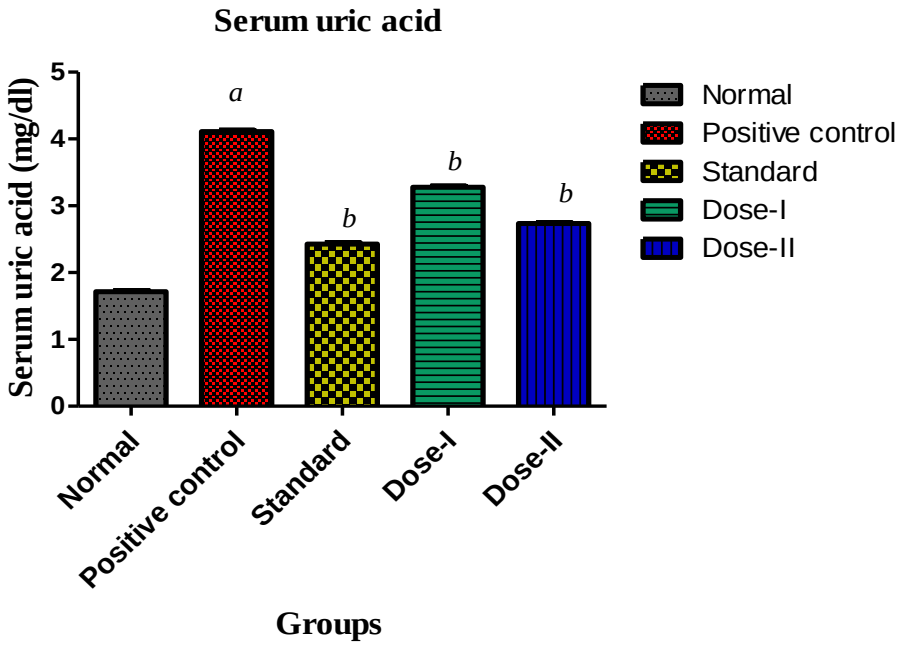
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 29.a. Effect of PHF on Serum creatinine level in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 29.b. Effect of PHF on Serum urea level in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 29.c. Effect of PHF on Serum uric acid level in STZ induced diabetic animals

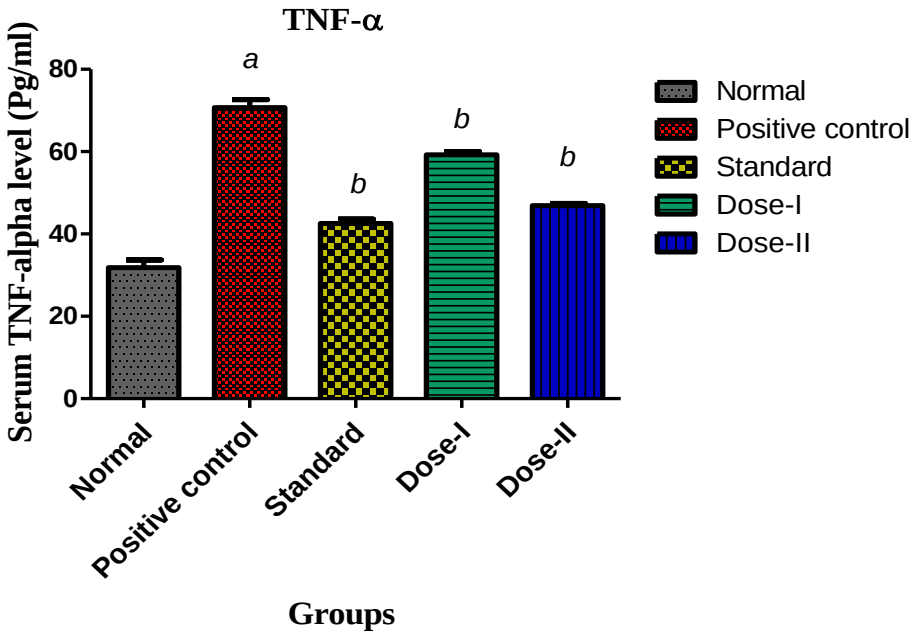
Effect of PHF on Serum TNF-α level in STZ induced diabetic animals

Diabetic animals, compared with non-diabetic animals recorded significantly elevated levels of TNF-α and PHF treatment resulted in statistically significant reduction in levels of TNF-α in a dose dependent manner [Table-10].

Table10. Effect of PHF on Serum TNF-α level in STZ induced diabetic animals

Groups	Serum TNF-α level (Pg/ml)
Normal	31.82±1.863
Positive control (STZ)	70.69±1.973***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	42.54±1.360***[b]
Dose-I (200 mg/kg, b.w.)+STZ	59.27±0.926***[b]
Dose-II (400 mg/kg, b.w.)+STZ	46.91±0.681***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig.30. Effect of PHF on Serum TNF-α level in STZ induced diabetic animals

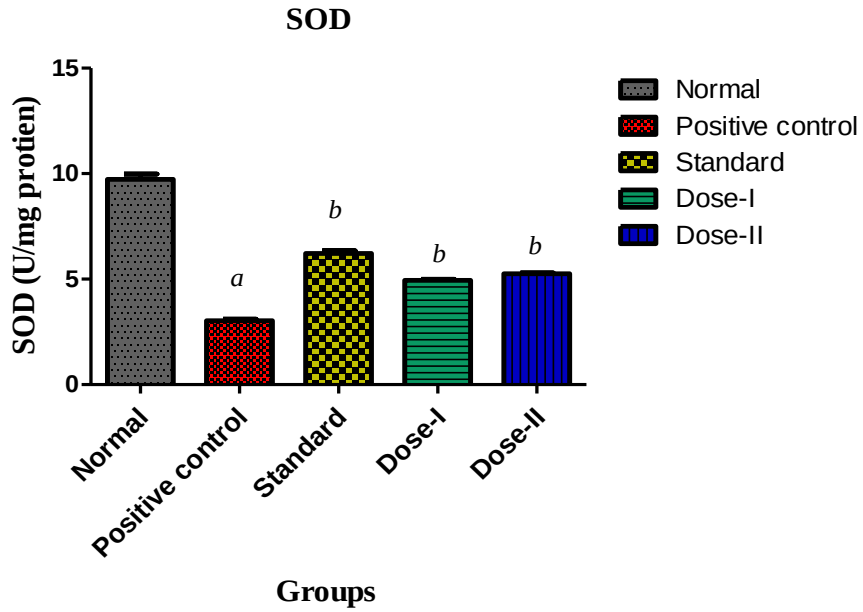
Effect of PHF on biomarker levels of oxidative stress of kidney of STZ diabetic animals

Statistically significant changes in the biomarker levels of oxidative stress in the kidney of the diabetic animals was recorded by untreated diabetic animals and the treatment with PHF resulted in significant reduction in the oxidative stress enzyme profile in a dose dependent manner [Table-11].

Table11. Effect of PHF on biomarker levels of oxidative stress of kidney of STZ diabetic animals

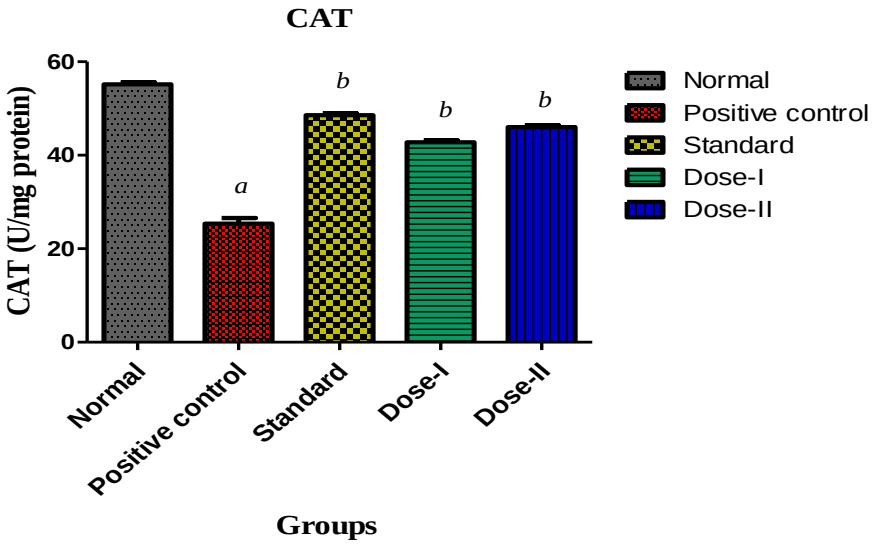
Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/mg protein)	GSH (nmoles/mg protein)
Normal	9.73±0.248	55.15±0.481	1.13±0.050	16.97±0.103
Positive control (STZ)	3.03±0.075***[a]	25.40±1.201***[a]	6.17±0.075***[a]	9.08±0.190***[a]
Standard (Metformin 5mg/kg, b.w.)+ STZ	6.21±0.127 ***[b]	48.58±0.427 ***[b]	2.04±0.051 ***[b]	14.21±0.196 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	4.95±0.048 ***[b]	42.77±0.431 ***[b]	2.97±0.071 ***[b]	12.00±0.077 ***[b]
Dose-II (400 mg/kg, b.w.)+STZ	5.25±0.039 ***[b]	46.01±0.428 ***[b]	2.19±0.034 ***[b]	13.18±0.115 ***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



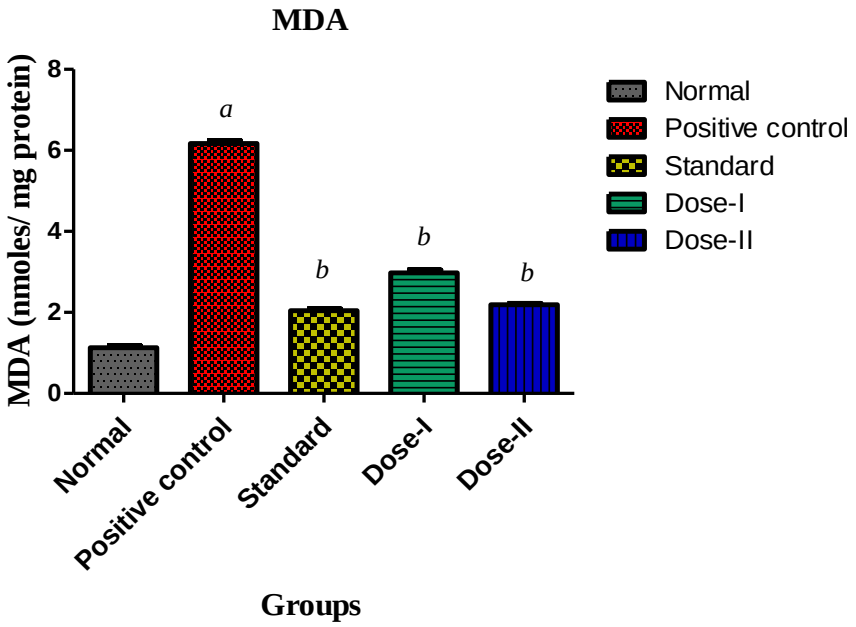
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 31.a. Effect of PHF on SOD level in Kidney in STZ induced diabetic animals



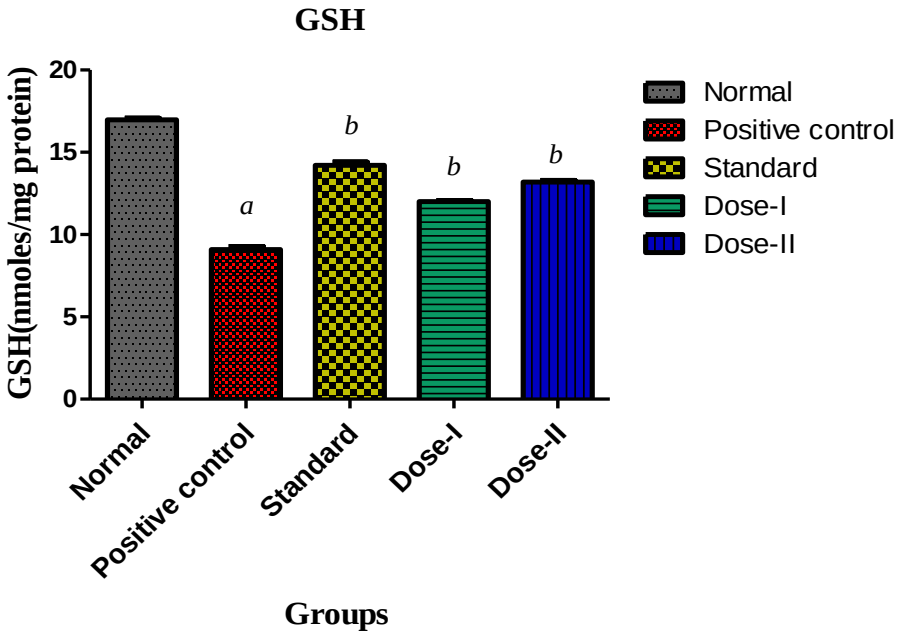
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 31.b. Effect of PHF on CAT level in Kidney in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 31.c. Effect of PHF on MDA level in Kidney in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 31.d. Effect of PHF on GSH level in Kidney in STZ induced diabetic animals

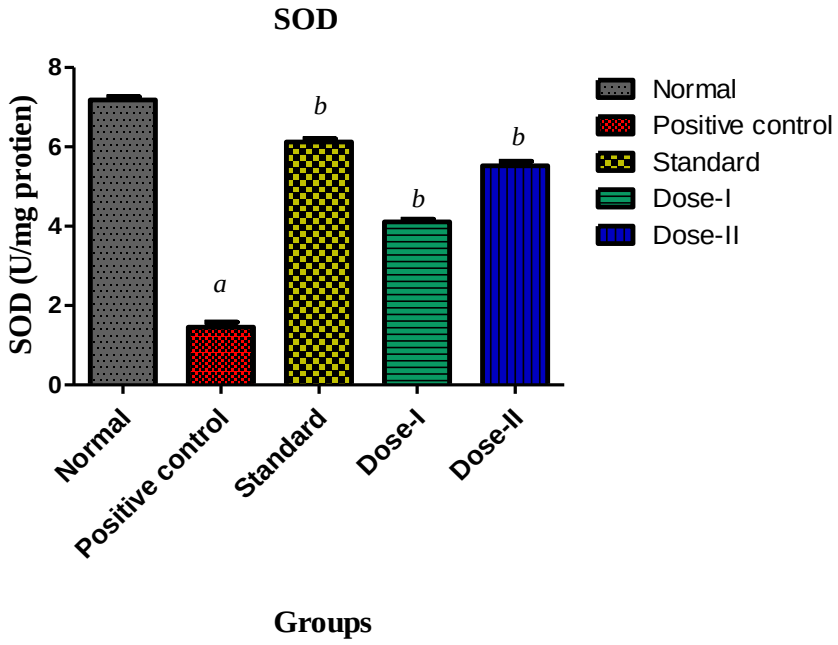
Effect of PHF on biomarker levels of oxidative stress of sciatic nerve of STZ diabetic animals

Oxidative stress profile in the sciatic nerves of untreated diabetic animals was significant compared to normal control animals. PHF treatment significantly altered the profile, in a dose dependent manner [Table-12].

Table12. Effect of PHF on biomarker levels of oxidative stress of sciatic nerve of STZ diabetic animals

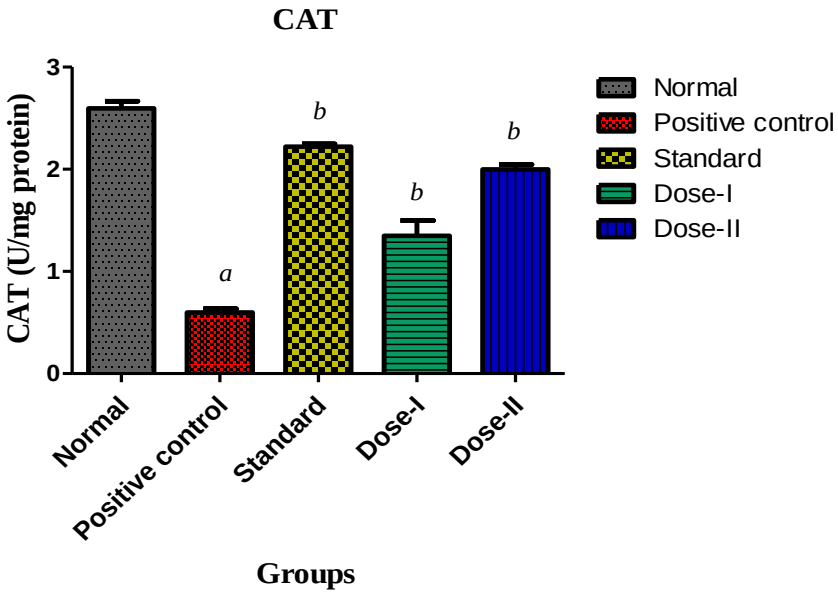
Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/mg protein)
Normal	7.18±0.080	2.59±0.070	0.87±0.062
Positive control (STZ)	1.45±0.126***[a]	0.59±0.037 ***[a]	5.98±0.082 ***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	6.12±0.079 ***[b]	2.22±0.025 ***[b]	1.39±0.064 ***[b]
Dose-I (200 mg/kg, b.w.)+STZ	4.10±0.066 ***[b]	1.34±0.151 ***[b]	2.84±0.054 ***[b]
Dose-II (400 mg/kg, b.w.)+STZ	5.52±0.109 ***[b]	1.99±0.049 ***[b]	1.94±0.065 ***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



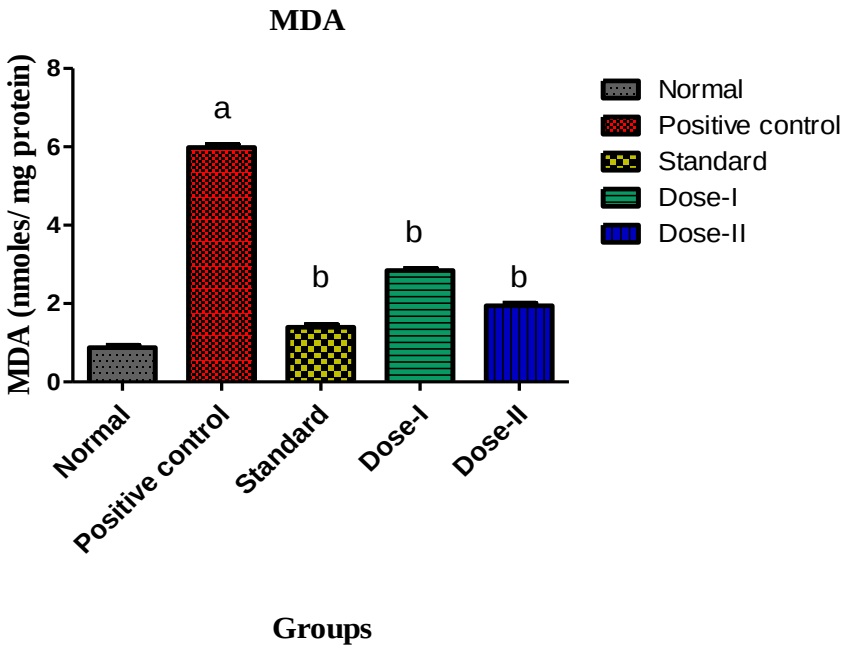
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 32.a. Effect of PHF on SOD level in sciatic nerve in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 32.b. Effect of PHF on CAT level in sciatic nerve in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 32.c. Effect of PHF on MDA level in sciatic nerve in STZ induced diabetic animals

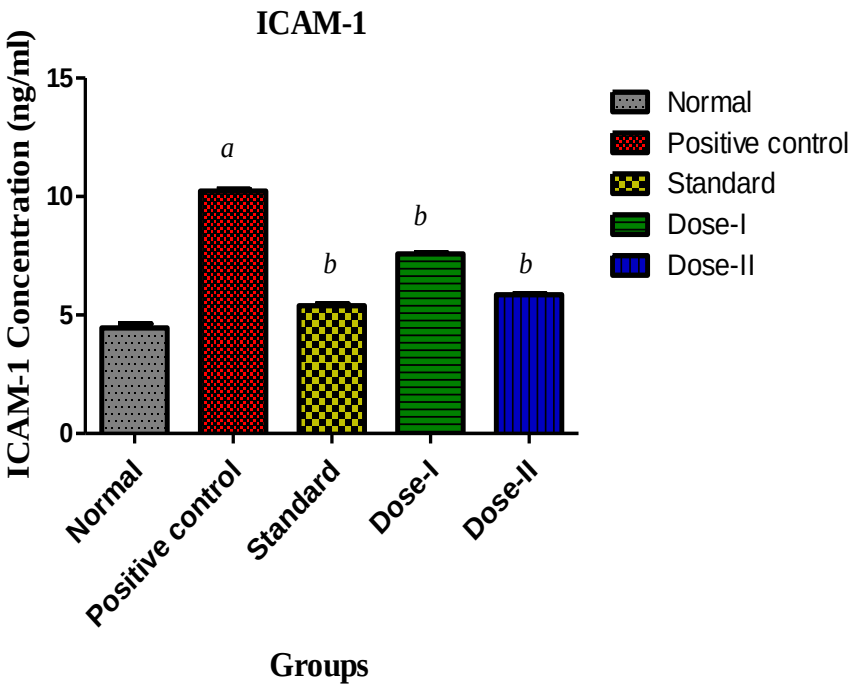
Effect of PHF on retinal ICAM-1 and VEGF Levels in STZ induced diabetic animals

VEGF and ICAM-1levels were found to be notable high in the retinas for hyperglycaemic rodents. Diabetic rats treated with PHF and metformin significantly reduces the levels of retinal VEGF and ICAM-1 contrasted with diabetic control animals ($p < 0.001$) [Table-13].

Table13. Effect of PHF on retinal ICAM-1 and VEGF levels in STZ induced diabetic animals

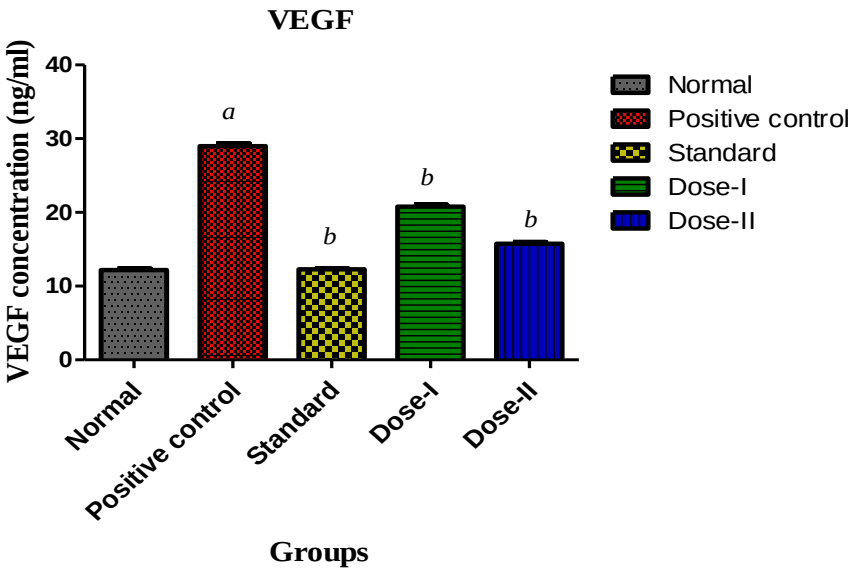
Groups	ICAM-1 (concentration in ng/ml)	VEGF (concentration in ng/ml)
Normal	4.45±0.168	12.18±0.216
Positive control (STZ)	10.23±0.077***[a]	28.99±0.365***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	5.39±0.080***[b]	12.29±0.081***[b]
Dose-I (200 mg/kg, b.w.)+STZ	7.57±0.056***[b]	20.77±0.283***[b]
Dose-II (400 mg/kg, b.w.)+STZ	5.85±0.043***[b]	15.74±0.239***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 33.a. Effect of PHF on retinal ICAM-1 level in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 33.b. Effect of PHF on retinal VEGF level in STZ induced diabetic animals

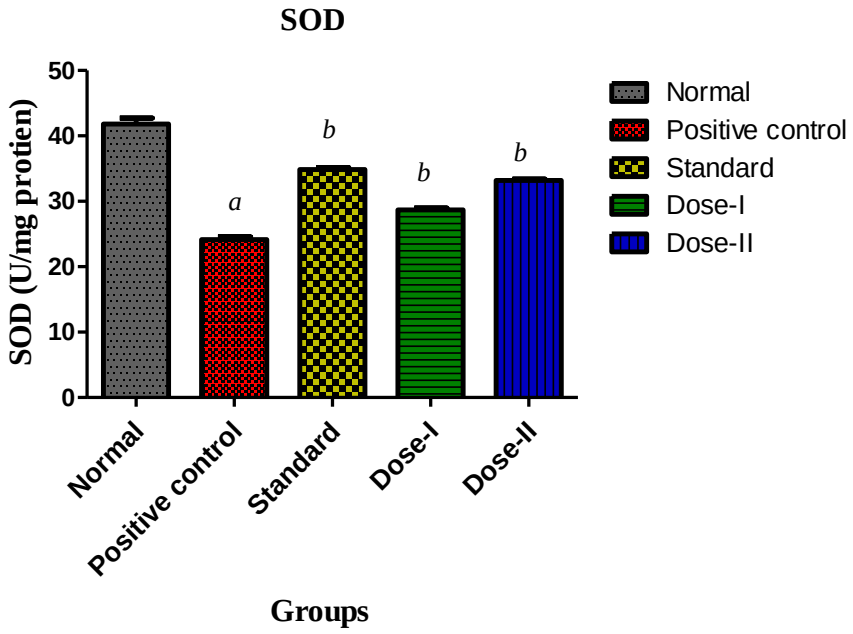
Effect of PHF on SOD and MDA levels in retina in STZ induced diabetic animals

Levels of MDA for rats administered with STZ were significantly increased, while decrease in SOD activity at the end of the study was significant. After treatment of diabetic rats with PHF and metformin exhibited notable ($P < 0.001$) decrease levels of MDA, while SOD activity recorded statistically significant increase in retina, compared to untreated diabetic animals [Table-14].

Table14. Effect of PHF on SOD and MDA levels in retina in STZ induced diabetic animals

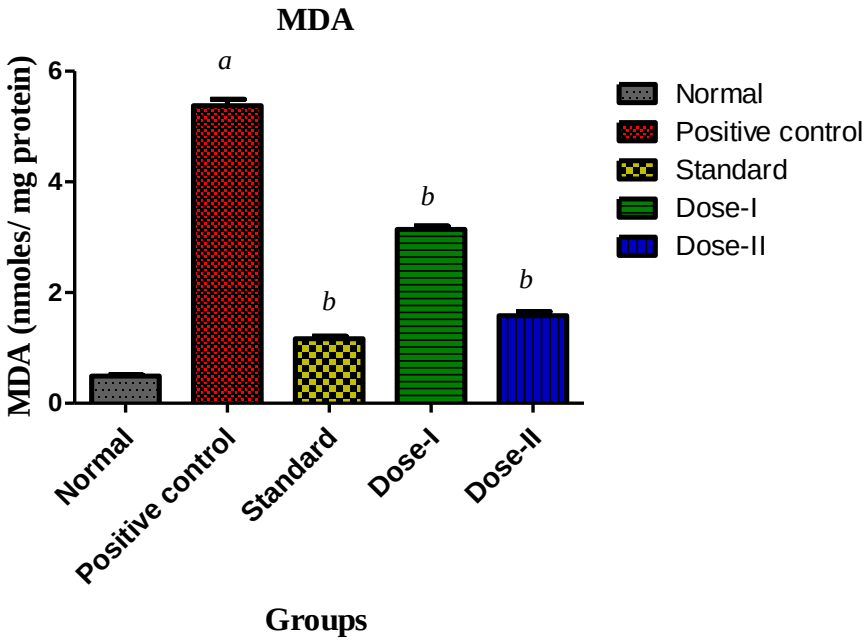
Groups	SOD (U/mg protein)	MDA (nmoles/mg protein)
Normal	41.83±0.891	0.49±0.023
Positive control (STZ)	24.14±0.420***[a]	5.37±0.115***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	34.86±0.281***[b]	1.16±0.045***[b]
Dose-I (200 mg/kg, b.w.)+STZ	28.68±0.298***[b]	3.14±0.056***[b]
Dose-II (400 mg/kg, b.w.)+STZ	33.21±0.206***[b]	1.58±0.075***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 34.a. Effect of PHF on SOD level in retina in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 34.b. Effect of PHF on MDA level in retina in STZ induced diabetic animals

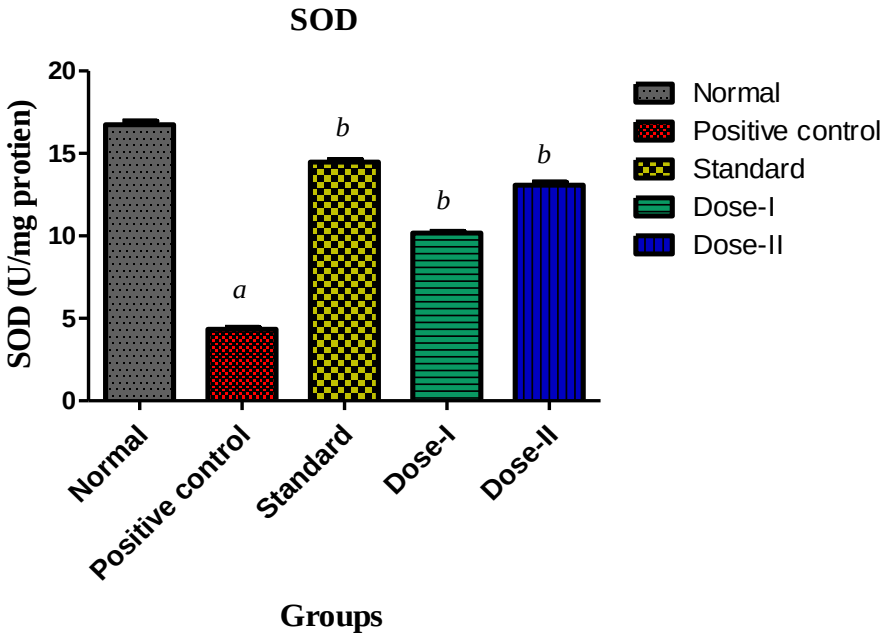
Effect of PHF on the biomarker level of thoracic aorta artery of diabetic animals

PHF and metformin treated diabetic rats recorded significantly lower levels of MDA and elevated SOD and CAT level compared to untreated diabetic animals [Table-15].

Table15. Effect of PHF on the biomarker level of thoracic aorta artery of diabetic animals

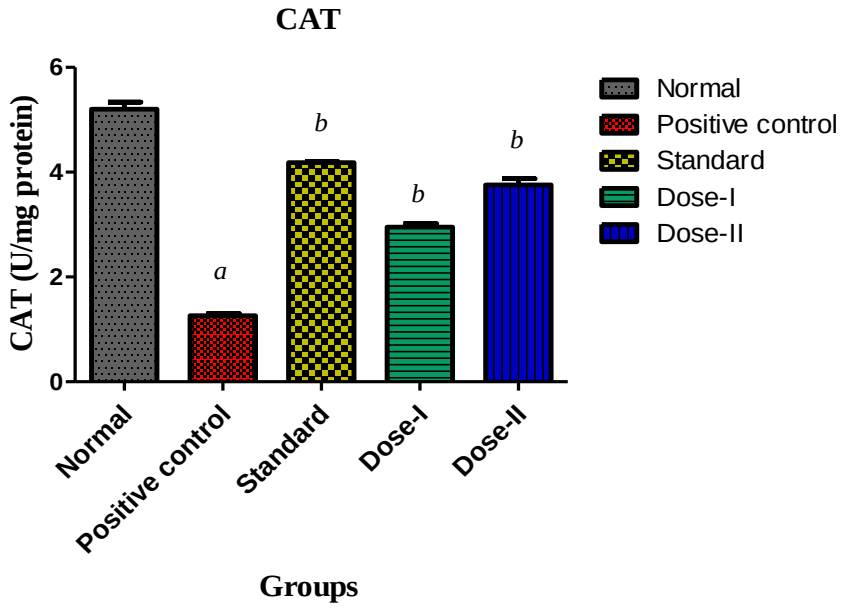
Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/mg protein)
Normal	16.74±0.228	5.20±0.135	0.67±0.035
Positive control (STZ)	4.34±0.119***[a]	1.26±0.038***[a]	5.35±0.119***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	14.47±0.146***[b]	4.18±0.018***[b]	1.43±0.115***[b]
Dose-I (200 mg/kg, b.w.)+STZ	10.18±0.101***[b]	2.95±0.063***[b]	2.80±0.073***[b]
Dose-II (400 mg/kg, b.w.)+STZ	13.08±0.177***[b]	3.76±0.117***[b]	1.89±0.077***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



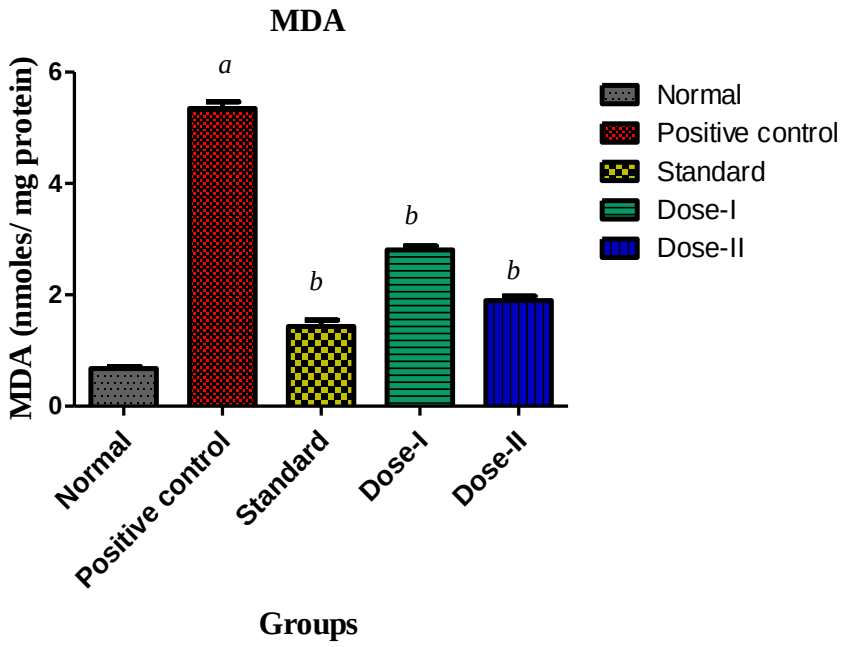
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 35.a. Effect of PHF on SOD level in thoracic aorta in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 35.b. Effect of PHF on CAT level in thoracic aorta in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 35.c. Effect of PHF on MDA level in thoracic aorta in STZ induced diabetic animals

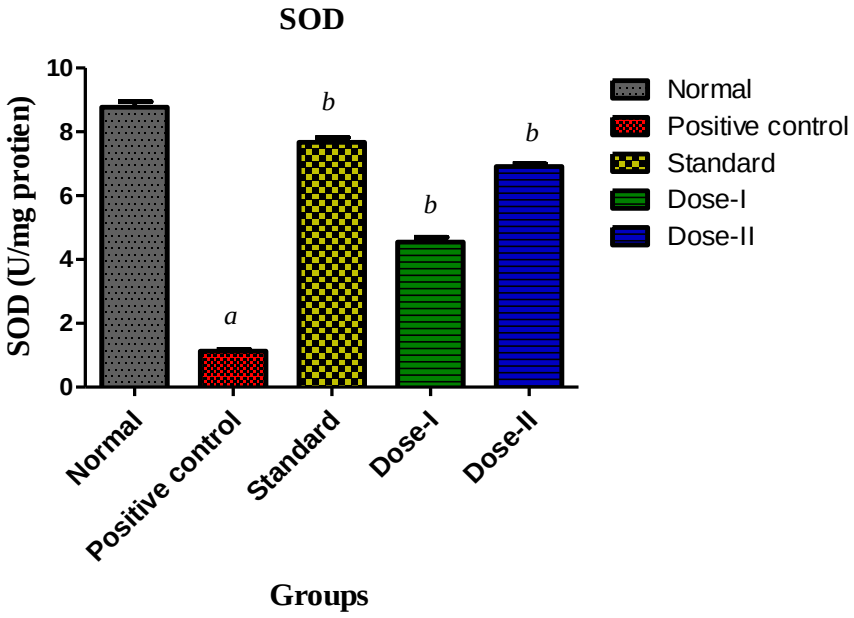
Effect of PHF on the biomarker level of liver of diabetic animals

Diabetic rats had significantly elevated level of MDA and significantly lower levels of SOD, CAT and GSH**.** Metformin and PHF treatment resulted in significantly lower levels of MDA and elevated profile of SOD, CAT and GSH, compared to untreated diabetic animals [Table-16].

Table16. Effect of PHF on the biomarker level of liver of diabetic animals

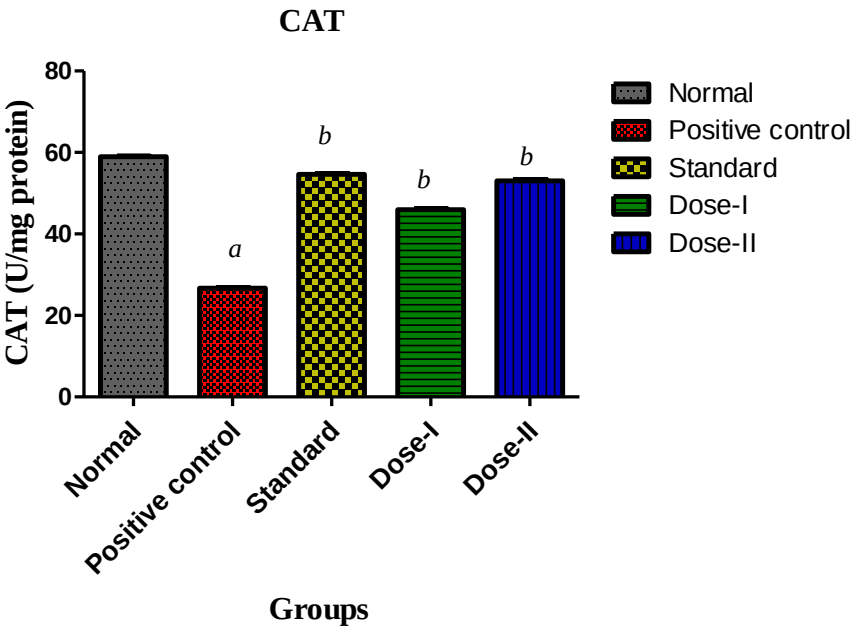
Groups	**SOD (U/mg protein)**	**CAT (U/mg protein)**	**MDA (nmoles/mg protein)**	**GSH (nmoles/mg protein)**
Normal	8.77±0.167	59.00±0.107	1.27±0.113	38.93±0.173
Positive control (STZ)	1.12±0.052***[a]	26.70±0.114***[a]	14.25±0.294***[a]	17.98±0.234***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	7.66±0.149***[b]	54.64±0.218***[b]	2.51±0.127***[b]	37.01±0.346***[b]
Dose-I (200 mg/kg, b.w.)+STZ	4.54±0.145***[b]	45.98±0.166***[b]	4.06±0.093***[b]	33.11±0.179***[b]
Dose-II (400 mg/kg, b.w.)+STZ	6.91±0.082***[b]	53.07±0.280***[b]	3.02±0.058***[b]	35.44±0.160***[b]

Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).



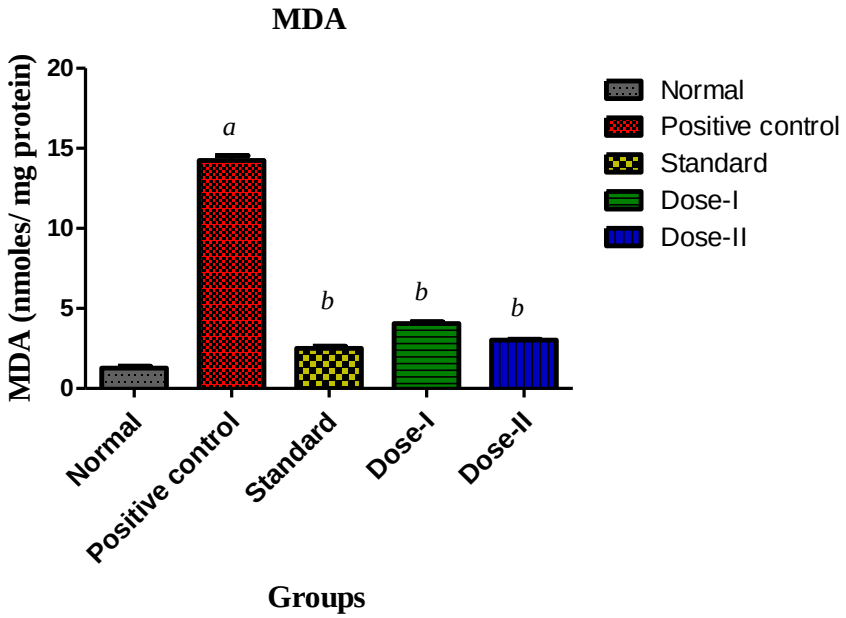
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 36.a. Effect of PHF on SOD level in liver in STZ induced diabetic animals



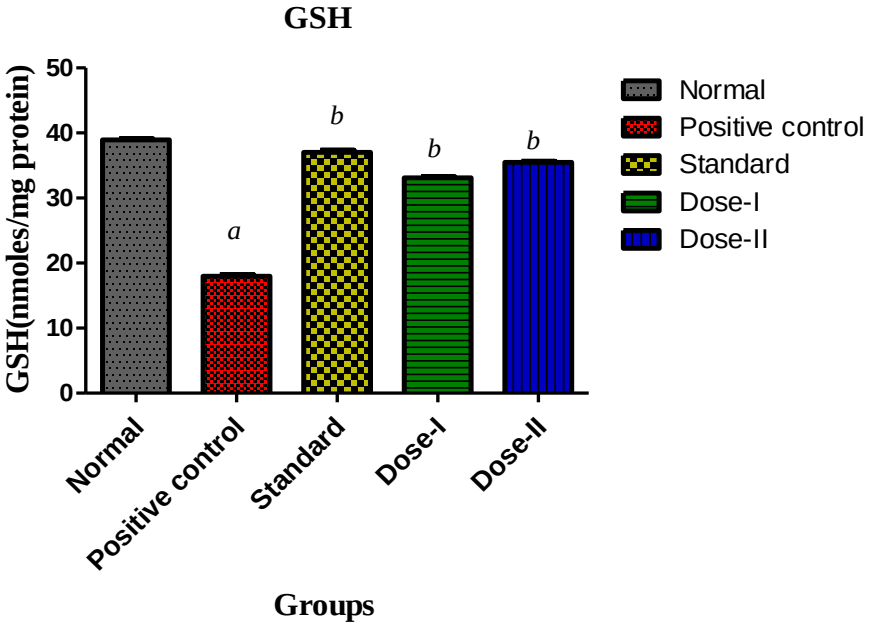
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 36.b. Effect of PHF on CAT level in liver in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 36.c. Effect of PHF on MDA level in liver in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 36.d. Effect of PHF on GSH level in liver in STZ induced diabetic animals

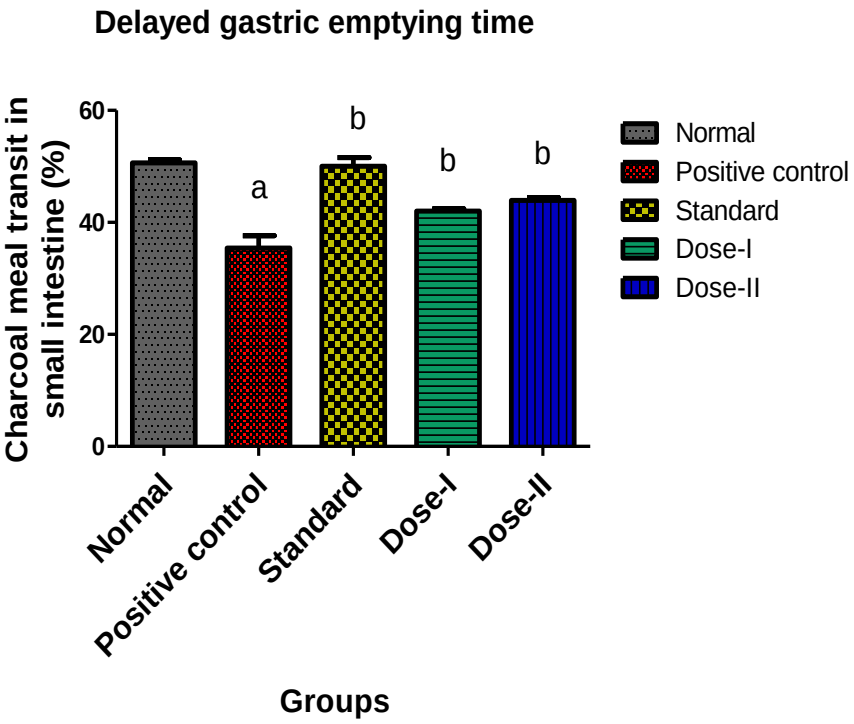
Effect of PHF on gastric emptying time in STZ diabetic animals

Rodents administered with STZ showed decrease in the small intestinal transit of charcoal meal, contrasted with normal group rodents and after treatment with PHF showed improvement in small intestinal transit when compared with diabetic control group. Significant decreases contractile response of distal colonic smooth muscle to exogenous Acetylcholine (ACh) was notified in diabetic rats. STZ diabetic animals recorded statistically significant increase in the contractile response of distal colonic smooth muscle to exogenous ACh [Table-17]

. **Table17. Effect of PHF on gastric emptying time in STZ diabetic animals**

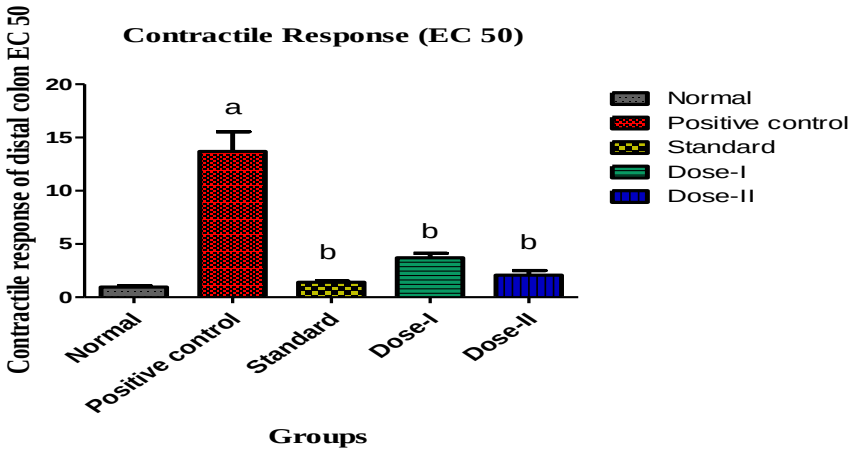
Groups	% Transit	EC50 of ACh in µg
Normal	50.63±0.551	0.95±0.13
Positive control (STZ)	35.40±2.210***[a]	13.70±1.84***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ	50.00±1.581***[b]	1.41±0.11***[b]
Dose-I (200 mg/kg, b.w.)+STZ	42.00±0.447*[b]	3.70±0.42***[b]
Dose-II (400 mg/kg, b.w.)+STZ	43.95±0.470**[b]	2.07±0.43***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, ** $p<0.01$ and * $p<0.05$when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, **p<0.01 and *p<0.05when compared to positive control group (b).

Fig. 37.a. Effect of PHF on delayed gastric emptying time in STZ induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, **p<0.01 and *p<0.05when compared to positive control group (b).

Fig. 37.b. Effect of PHF on Contractile response (EC50) in STZ induced diabetic animals

Histopathological Studies

Table 18. Histopathological Changes in the Kidney of STZ diabetic animals due to various treatment

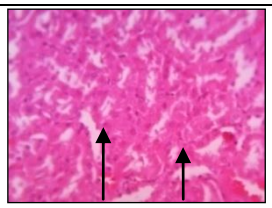
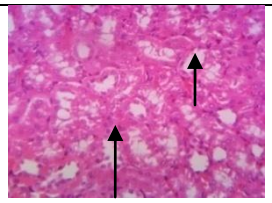
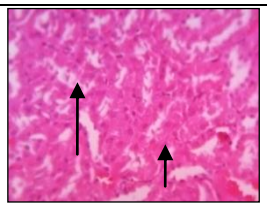
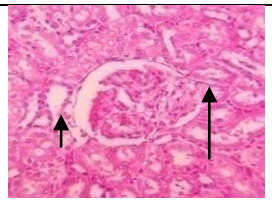
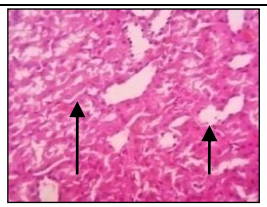
Groups	Histopathology	Observation
Normal		The tubules of the kidney demonstrated some degenerative changes and few blood vessels were clogged with diabetic control rodents when contrasted with typical auxiliary features of control rodent. In the control group, histopathological analysis for kidney tissue indicated ordinary presence of glomeruli and tubules (Long and short arrow). Treatment with Dose-I of PHF demonstrated some degenerative changes. Be that as it may, the treatment with Dose-II 400 mg/kg and metformin indicated flawless architecture when contrasted with ordinary group.
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II(400 mg/kg, b.w.)+STZ		

Table 19. Effect of PHF on histopathology of Sciatic nerve in STZ diabetic animals

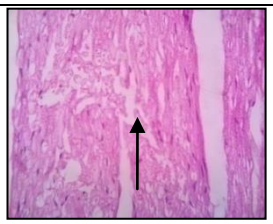
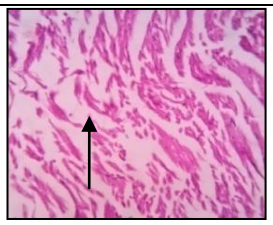
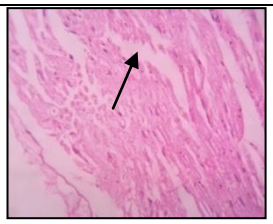
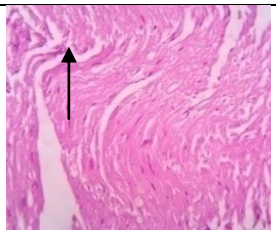
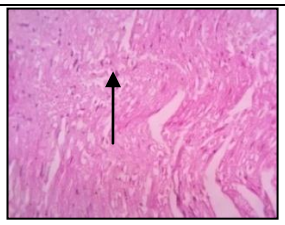
Groups	Histopathology	Observation
Normal		Histopathological examination showed loss of small myelinated fiber was more prominent than large diameter fiber loss. It was also found that Endoneurial vessel is not thickened with positive group rats as compared with normal group. Hyperglycaemic rats treated with PHF at Dose-II showed intact myelinated fiber (Arrow) density, the small myelinated fiber and large diameter fiber appear intact and the endoneurial vessel was also not thickened as compared to non diabetic animals.
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II (400 mg/kg, b.w.)+STZ		

Table 20. Histopathological Changes in the Pancreas of STZ diabetic animals due to various treatment

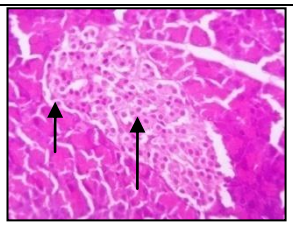
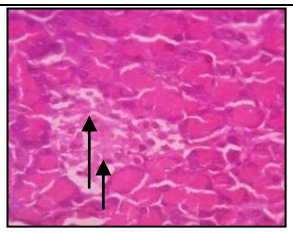
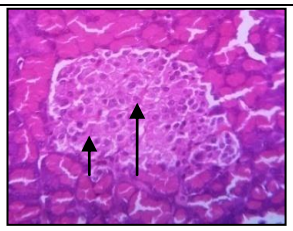
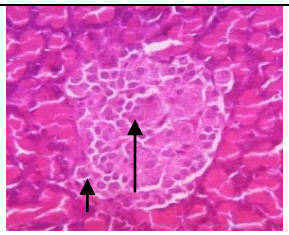
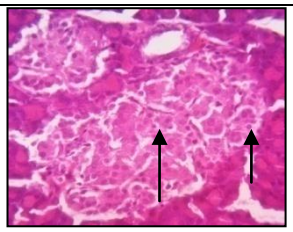
Groups	Histopathology	Observation
Normal		Histopathogical analysis showed that pancreatic lobules are separated in positive control rat. The centre of islet cells showed quantitative decrease in small β-cells (Long arrow) (30%, compared to normal control, 70%), Large α cells (Short Arrow) in the periphery (65%, compared to normal control, 25%). Treatment of hyperglycemic rats with PHF (Dose-II 400 mg/kg) showed Regenerated β cells (70%, compared to positive control group 30%), while large α cells occupying the periphery (25%, compared to positive control group 65%).
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II (400 mg/kg, b.w.)+STZ		

Table 21. Histopathological Changes in the thoracic aorta of STZ diabetic animals due to various treatment

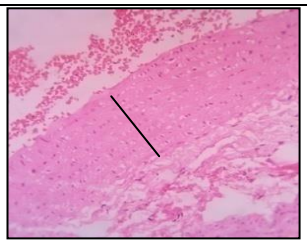
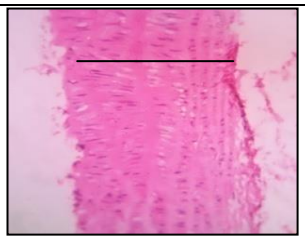
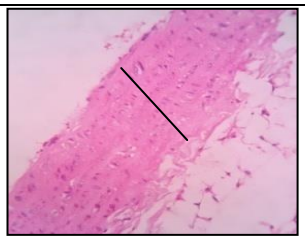
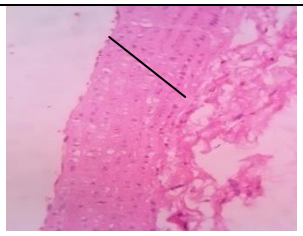
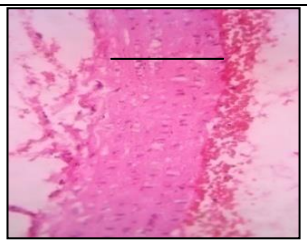
Groups	Histopathology	Observation
Normal		Section studied of diabetic control group indicated the disruption of Tunica intima and endothelium and the tunica intima media thickness was 36.4µm, as compare to normal control group features (tunica intima media thickness 24.2µm). Treatment of diabetic animals with PHF (Dose-II 400 mg/kg) showed features compare to normal control group (tunica intima media thickness 24.8µm).
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II (400 mg/kg, b.w.)+STZ		

Table 22. Histopathological Changes in the retina of STZ diabetic animals due to various treatment

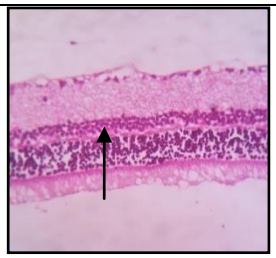
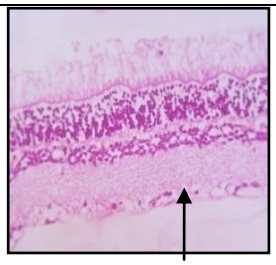
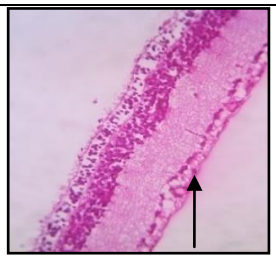
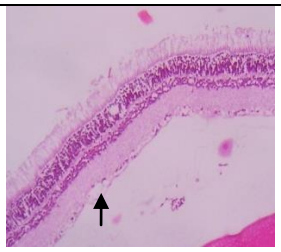
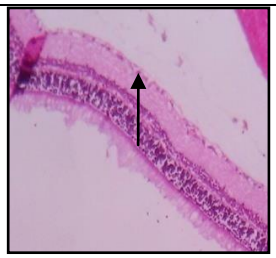
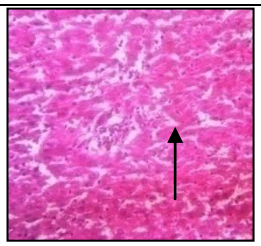
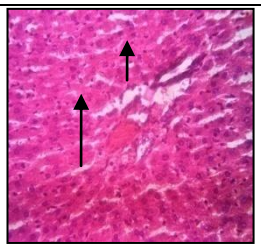
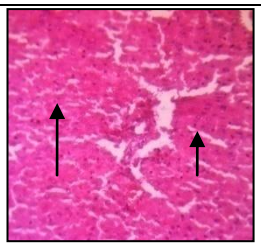
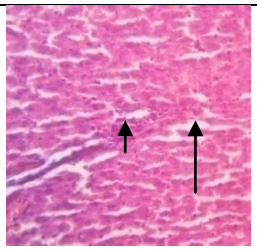
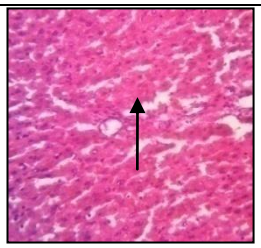
Groups	Histopathology	Observation
Normal		Histopathological analysis of positive control rats indicated extensive vacuolations in the plexiform layers and ganglion layer (arrow) as compare to normal control group. Treatment of diabetic animals with PHF (Dose-II 400 mg/kg) showed moderately reduced vacuolations [compared to positive control] in the plexiform layers and ganglion layer.
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II (400 mg/kg, b.w.)+STZ		

Table 23. Histopathological Changes in the liver in the liver architecture of diabetic animals

Groups	Histopathology	Observation
Normal		Section studied in liver of diabetic animals showed partially distorted architecture and there are seen focal areas of necrosis (Short arrow) with congested blood vessels as compare to normal control group {[shows intact architecture] (Long arrow)}. Treatment of diabetic animals with PHF (Dose-II 400 mg/kg) showed intact architecture of liver parenchyma and the perivenular, periportal and midzonal hepatocytes appear unremarkable. The central veins and sinusoids appear unremarkable as compared to untreated diabetic group of animals.
Positive control (STZ)		
Standard (Metformin 5mg/kg, b.w.) + STZ		
Dose-I (200 mg/kg, b.w.)+STZ		
Dose-II (400 mg/kg, b.w.)+STZ		

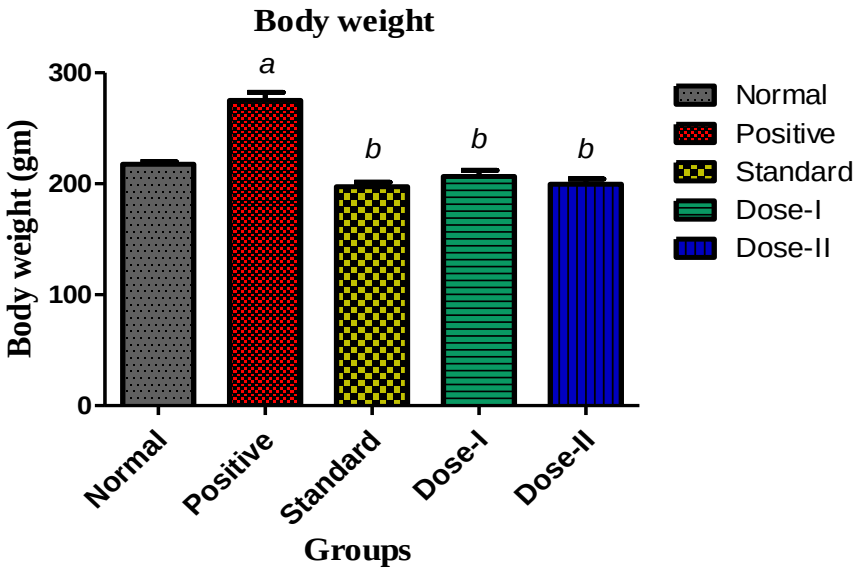
Effect of Polyherbal Formulation (PHF) on Body weight in fructose induced animals

Fructose induced diabetic animal's recorded significant increase in the body weight at the end of the study compared to non diabetic animals. Metformin and PHF treatment resulted in statistically significant reduction in body weight compared to untreated diabetic animals [Table-24].

Table 24. Effect of PHF on Body weight in Fructose induced diabetic animals

Groups	Body Weight (gm)
Normal	217.7±2.155
Positive control (Fructose 66%)	275.0±7.479***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	197±4.137***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	206.5±5.554***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	199.7±4.738***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 38. Effect of PHF on Body weight in fructose induced animals

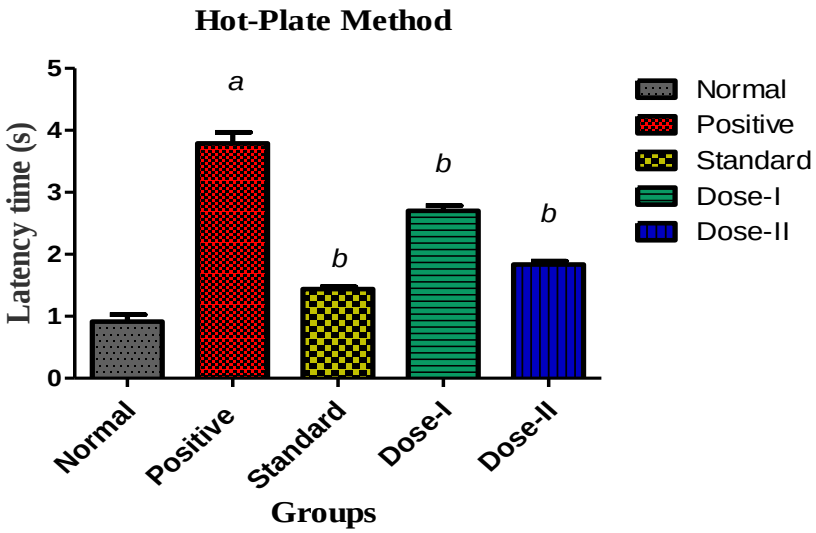
Effect of PHF on latency period in Hot-plate and Tail-flick methods in Fructose induced diabetic animals

Untreated diabetic animals demonstrated high latency time compared to normal ($P < 0.01$), non-diabetic animals in both methods employed in this study. PHF treated animals however, recorded elvation in latency time and was significantally lower than untreated diabetic animals ($P < 0.001$) [Table-25].

Table 25. Analgesic effect of PHF in fructose induced diabetic animals

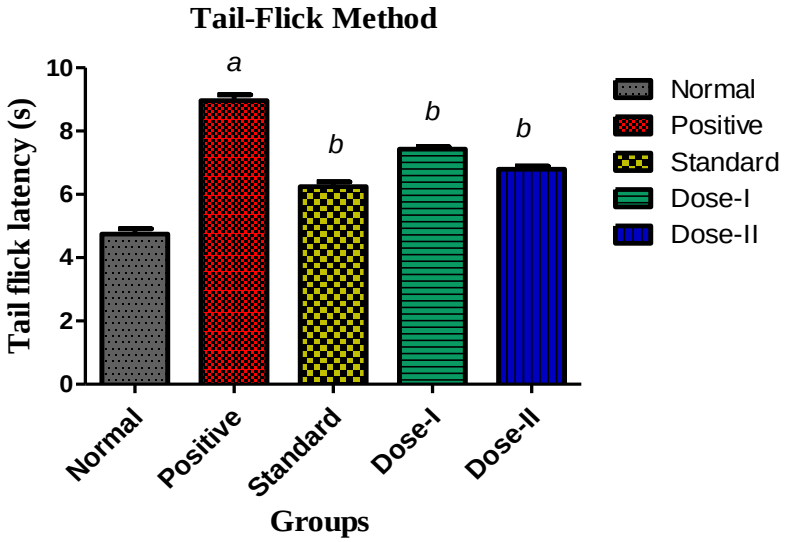
Groups	Hot-Plate Method (sec) (Latency time)	Tail-Flick Method (sec) (Latency time)
Normal	0.91±0.109	4.74±0.171
Positive control (Fructose 66%)	3.78±0.179**[a]	8.96±0.188***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	1.43±0.040***[b]	6.24±0.146***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	2.73±0.083***[b]	7.42±0.079***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	1.83±0.052***[b]	6.79±0.096***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, ** $p<0.01$when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 39.a. Effect of PHF on latency period in Hot-plate method in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 39.b. Effect of PHF on latency period in Tail-Flick method in Fructose induced diabetic animals

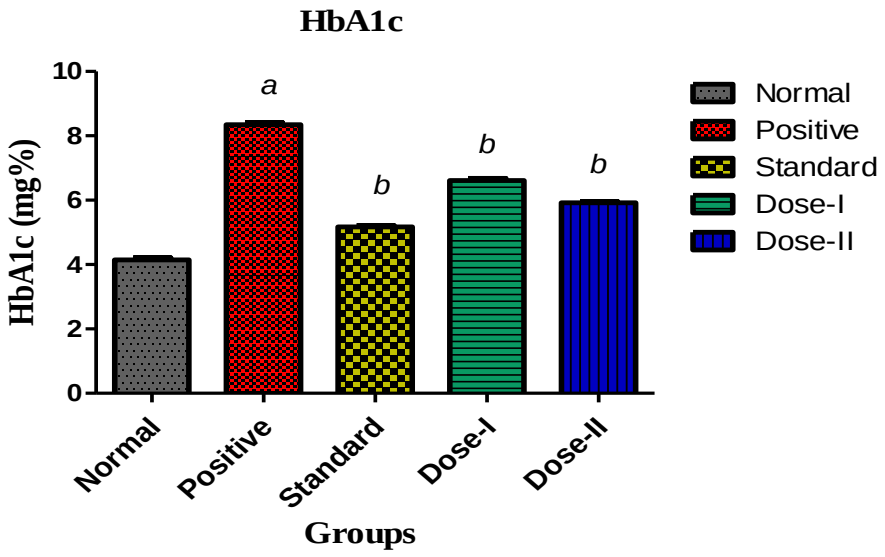
Effect of PHF on HbA1c levels in Fructose induced diabetic animals

Rodents after chronic instillation of fructose exhibited marked elevation in HbA1c levels. Treatment of rodents with PHF and metformin show significantly decreased in the HbA1c levels as contrasted with untreated diabetic control animals [Table 26].

Table 26. Effect of PHF on HbA1c levels in Fructose induced diabetic animals

Groups	HbA1c (mg%)
Normal	4.14±0.073
Positive control (Fructose 66%)	8.34±0.071***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	5.17±0.046***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	6.61±0.061***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	5.92±0.046***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 40.Effect of PHF on HbA1c levels in Fructose induced diabetic animals

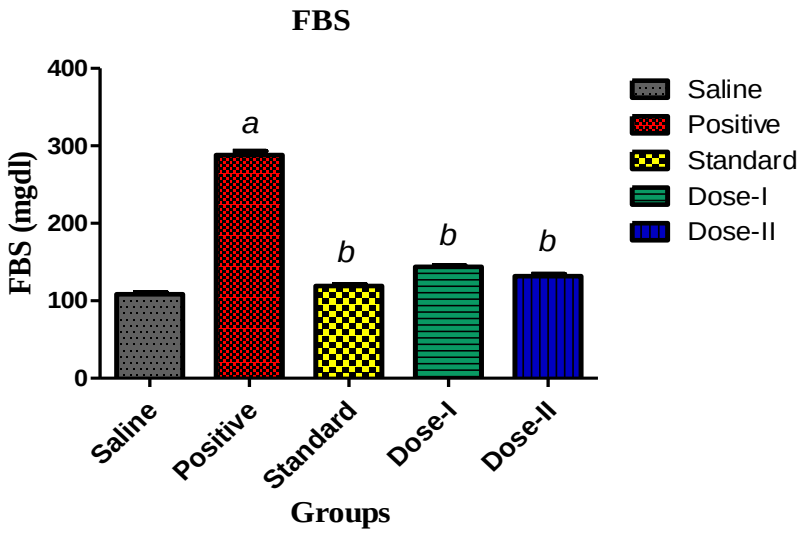
Effect of PHF on Blood glucose, Serum insulin and HOMA – IR in fructose induced diabetic rats

In, fructose induced diabetic animals, significantly elevated FBG, serum insulin and HOMA-IR compared to normal control animals. PHF treatment significantally reduced in a dose dependant manner, FBG, serum insulin and HOMA-IR, compared to untreated diabetic control animals. Dose-II of PHF resulted in changes in the above mentioned parameters similar to metformin treated diabetic animals [Table-27].

Table 27.Effect of PHF on Blood glucose, Serum insulin and HOMA – IR in fructose induced diabetic rats

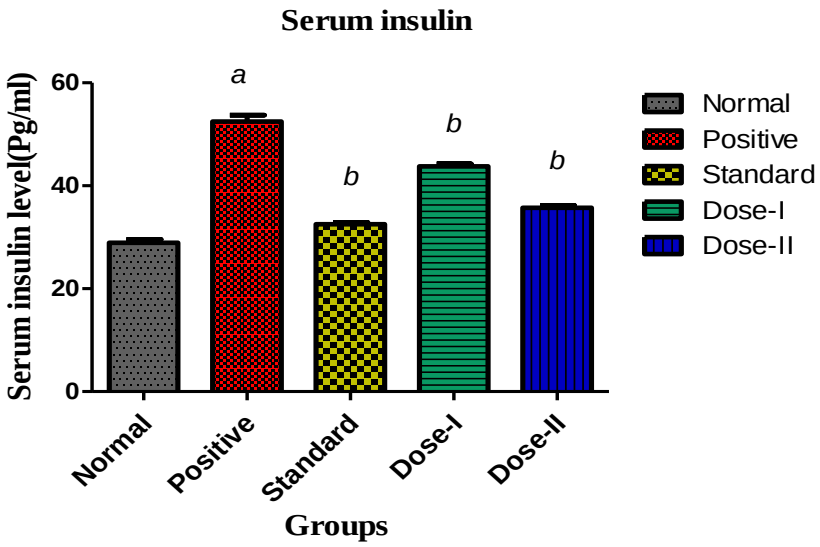
Groups	FBS (mg/dl)	Serum Insulin (µU/ml)	HOMA-IR
Normal	108.6±2.183	28.90±0.688	7.71±0.213
Positive control (Fructose 66%)	288.1±5.261***[a]	52.47±1.250***[a]	37.03±1.316***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	119.0±1.827***[b]	32.55±0.328***[b]	9.55±0.132***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	143.8±1.738***[b]	43.79±0.497***[b]	15.53±0.285***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	131.8±2.848***[b]	35.71±0.458***[b]	11.61±0.375***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



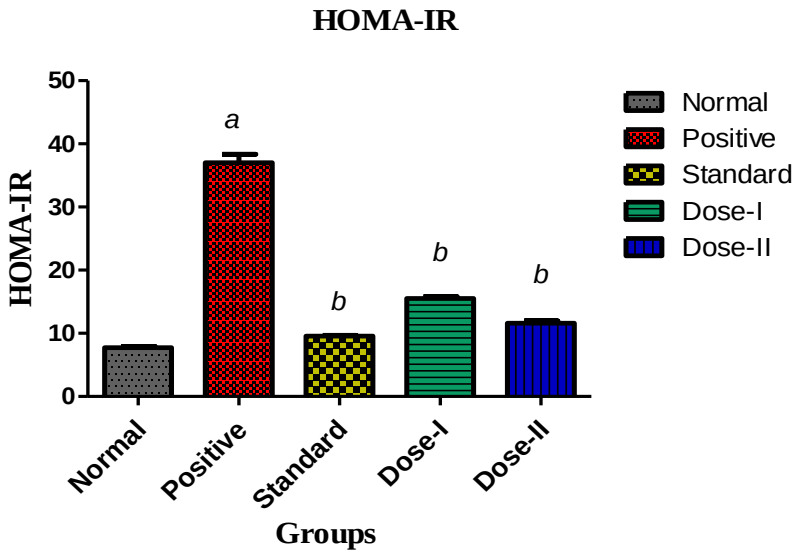
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 41.a. Effect of PHF on blood glucose in diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 41.b. Effect of PHF on Serum insulin level in fructose induced animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 41.c. Effect of PHF on HOMA-IR in fructose induced animals

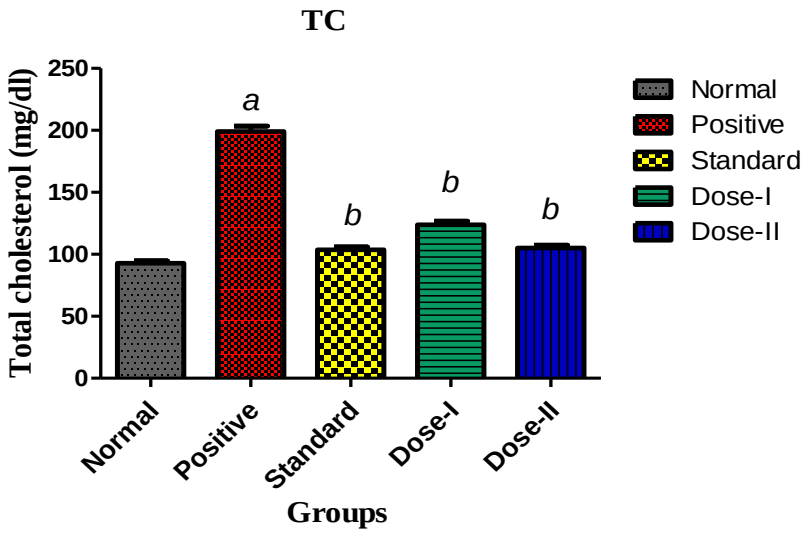
PHF effect on lipid levels in Fructose induced diabetic animals

Fructose induced diabetic animal's demonstrated statistically significant change in lipid profile compared to non diabetic group of animals. PHF and metformin treatment significantly altered lipid profile by the end of the study, compared to its untreated diabetic animals [Table-28].

Table 28.PHF effect on lipid levels in Fructose induced diabetic animals

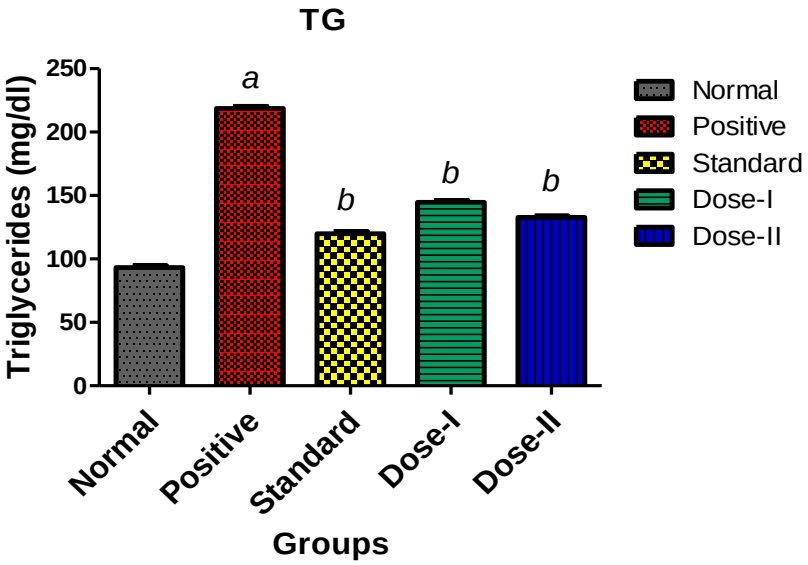
Groups	TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal	92.83±1.921	93.17±1.611	36.37±0.32	18.63±0.32	37.76±2.33
Positive control (Fructose 66%)	199.0±4.416***[a]	218.7±1.670***[a]	25.53±0.75***[a]	43.68±0.34***[a]	129.80±3.62***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	103.7±2.214***[b]	119.9±1.627***[b]	34.00±0.32***[b]	23.99±0.32***[b]	45.70±2.42***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	123.9±2.809***[b]	144.8±1.370***[b]	29.97±0.34***[b]	28.95±0.27***[b]	64.95±3.07***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	105.1±2.215***[b]	132.8±0.990***[b]	32.40±0.23***[b]	26.56±0.19***[b]	55.31±1.66***[b]

Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).



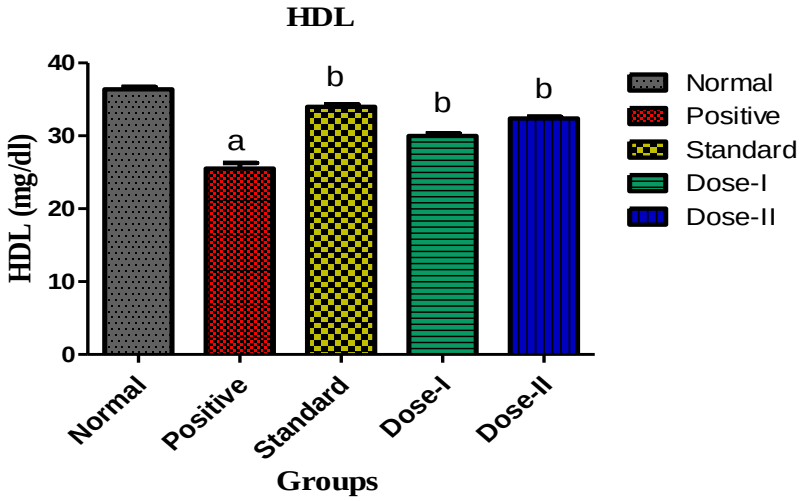
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 42.a. Effect of PHF on TC level in Fructose induced diabetic animals



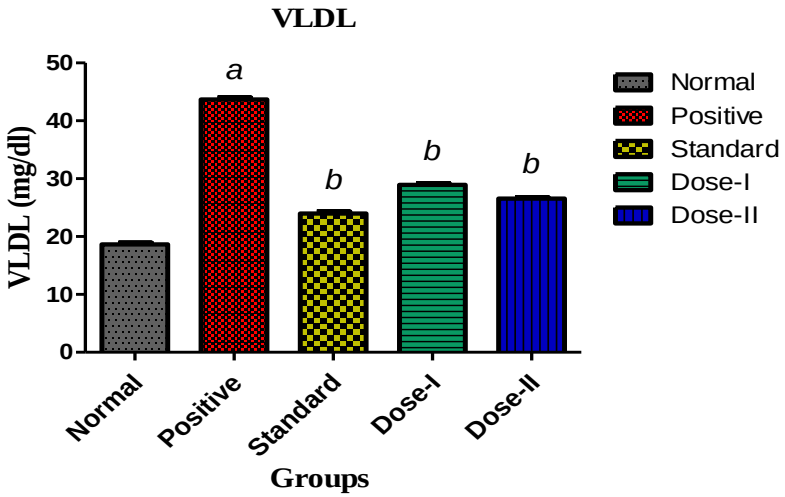
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 42.b. Effect of PHF on TG level in Fructose induced diabetic animals



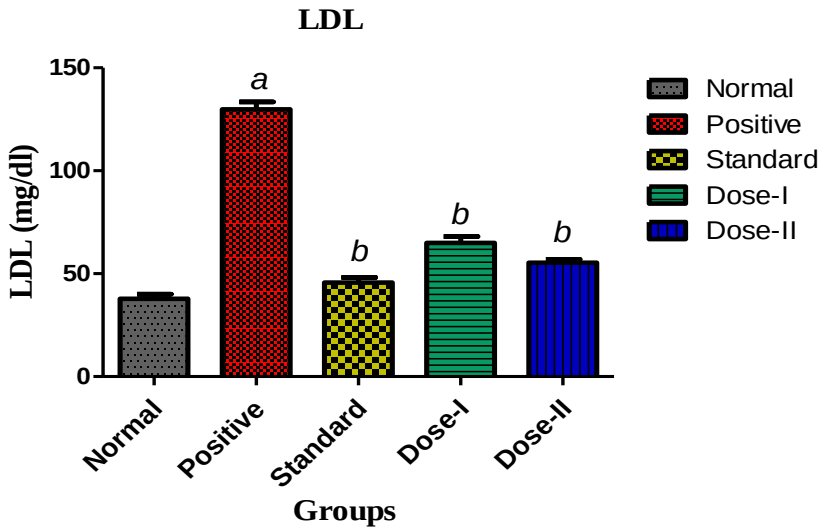
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 42.c. Effect of PHF on HDL level in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 42.d. Effect of PHF on VLDL level in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 42.e. Effect of PHF on LDL level in Fructose induced diabetic animals

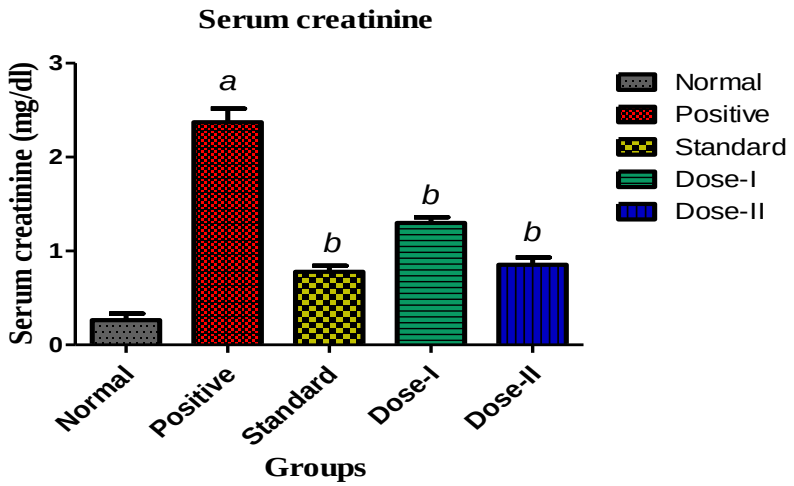
Effect of PHF on Serum creatinine, Urea, Uric acid and C –reactive protein levels in Fructose induced diabetic animals

Significantly elevated serum creatinine, urea and uric acid and C-reactive protein concentration was recorded by diabetic animals compared to its normal control group of animals. PHF treated diabetic animals recorded significantly lower of the above, compared to untreated diabetic animals [Table-29].

Table 29. Effect of PHF on Serum creatinine, Urea, Uric acid and C –reactive protein levels in Fructose induced diabetic animals

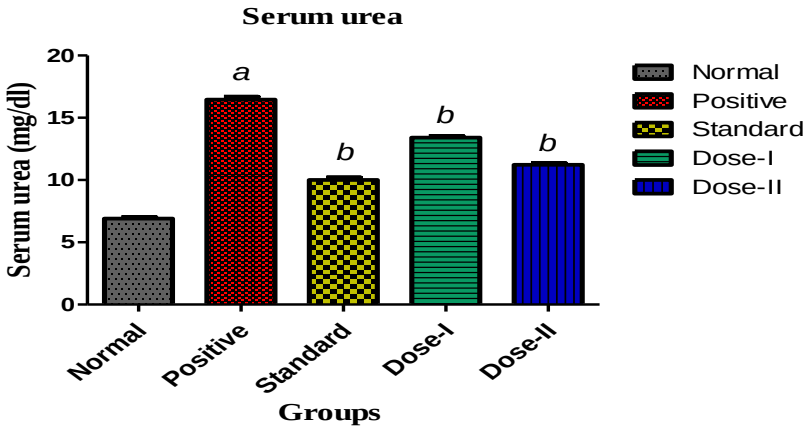
Groups	Serum creatinine(mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)	C-reactive protein mg/dl (10^{-2})
Normal	0.26±0.071	6.90±0.115	1.67±0.016	0.24±0.040
Positive control (Fructose 66%)	2.37±0.147**[a]	16.46±0.233***[a]	5.85±0.063***[a]	2.50±0.316***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	0.77±0.065***[b]	10.00±0.209***[b]	2.45±0.049***[b]	0.62±0.080***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	1.29±0.059***[b]	13.41±0.123***[b]	3.43±0.054***[b]	1.12±0.167***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	0.85±0.077***[b]	11.22±0.144***[b]	2.71±0.030***[b]	0.68±0.063***[b]

Values are expressed as mean± SEM,n=6. *** p<0.001, ** p<0.01when compared to normal control group (a) and *** p<0.001, when compared to positive control group (b).



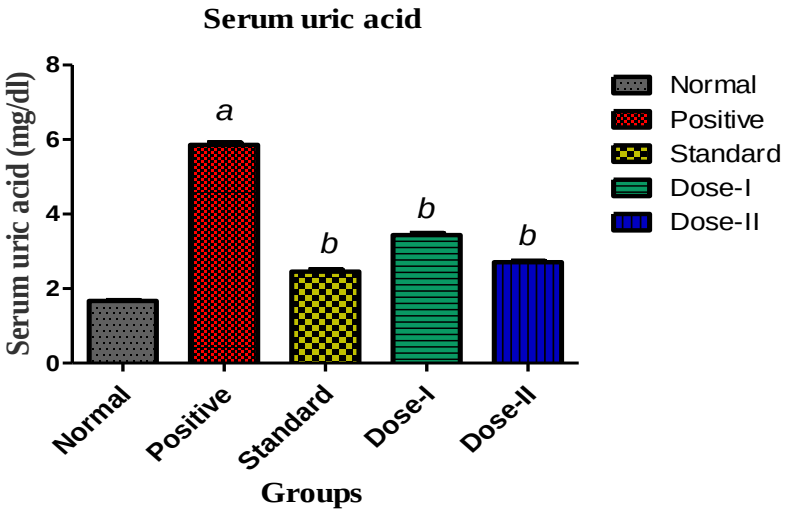
Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 43.a. Effect of PHF on Serum creatinine level in Fructose induced diabetic animals



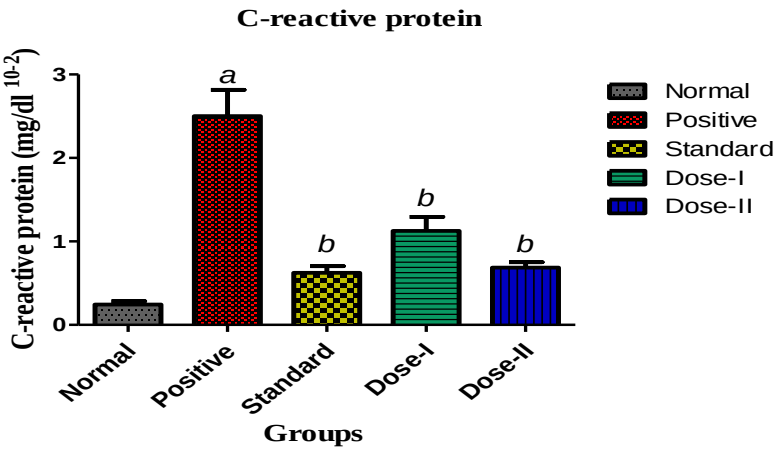
Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 43.b. Effect of PHF on Serum urea level in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 43.c. Effect of PHF on Serum uric acid level in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 43.d. Effect of PHF on C-reactive protein level in Fructose induced diabetic animals

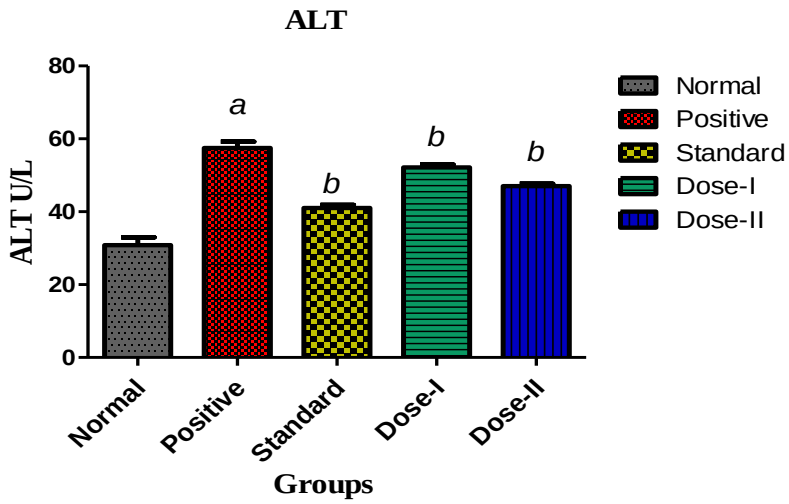
Effect of PHF on ALT and AST levels in Fructose induced diabetic animals

Untreated diabetic animals recorded statistically significant elevated level of AST and ALT concentration compared to vehicle treated non diabetic animals. Treatment of diabetic rats with PHF and metformin showed a significant ($P < 0.001$) decline in serum ALT level and Higher dose (D2) recorded a statistically significant reduction in serum AST level [Table-30].

Table 30.Effect of PHF on ALT and AST levels in Fructose induced diabetic animals

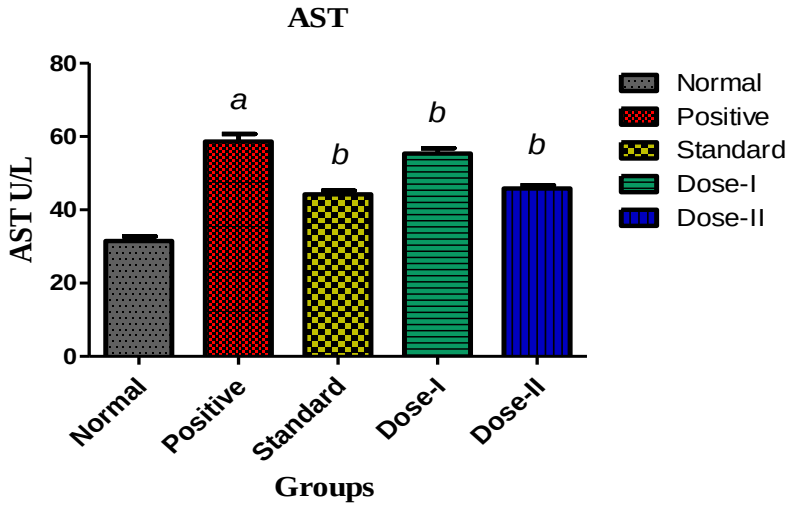
Groups	ALT(U/L)	AST (U/L)
Normal	30.82±2.13	31.50±1.28
Positive control (Fructose 66%)	57.50±1.70***[a]	58.65±2.05***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	41.00±0.88***[b]	44.20±1.00***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	52.51±0.81**[b]	55.35±1.49[ns]
Dose-II (400mg/kg, b.w.)+Fructose 66%	47.01±0.69***[b]	45.85±0.84***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, ** $p<0.01$ and ns $p<0.5$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, ** p<0.01 and ^{ns} p<0.5, when compared to positive control group (b).

Fig. 44.a. Effect of PHF on serum ALT level in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, ** p<0.01 and ^{ns} p<0.5, when compared to positive control group (b).

Fig. 44.b. Effect of PHF on serum AST level in Fructose induced diabetic animals

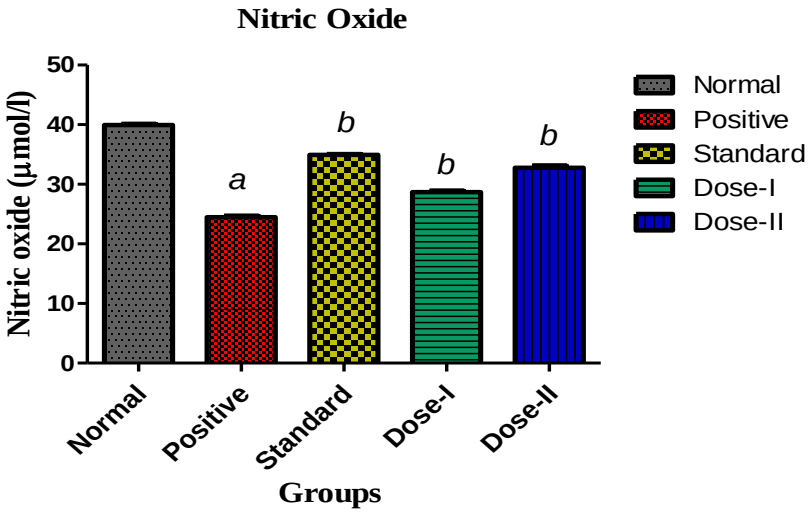
Effect of PHF on nitric oxide levels in Fructose induced diabetic animals

In renal tissue homogenate nitric oxide level was measured. T2DM caused by instillation of 66% fructose induced a noteworthy reduction in level. Treatment with PHF and metformin exhibited noteworthy increase in nitric oxide level [Table 31].

Table 31. Effect of PHF on nitric oxide level in Fructose induced diabetic animals

Groups	Nitric oxide (µmol/l)
Normal	39.95±0.158
Positive control (Fructose 66%)	24.48±0.214***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	34.93±0.119***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	28.69±0.197***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	32.78±0.327***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, ** $p<0.01$when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. ***p<0.001, ** p<0.01when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 45.Effect of PHF on nitric oxide levels in Fructose induced diabetic animals

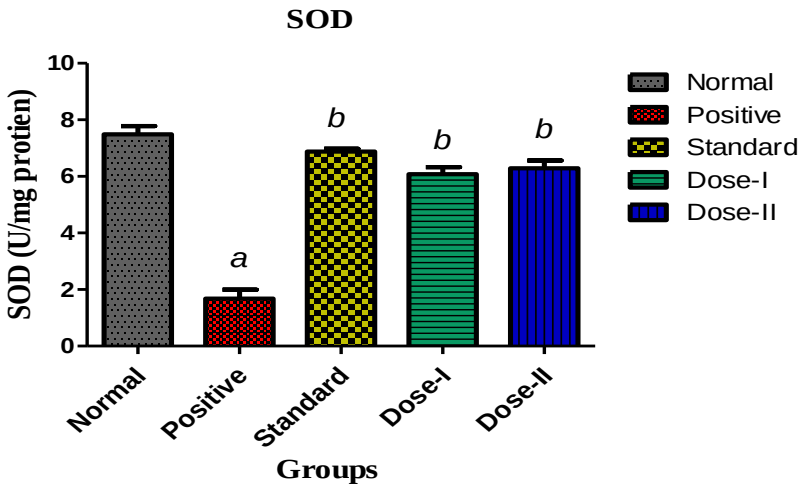
Effect of PHF on the biomarker profile of oxidative stress in the sciatic nerve in Fructose induced diabetic animals

Oxidative stress profile in the sciatic nerve profile of diabetic animals significantly altered compared to normal control group of animals. PHF treatment to diabetic animals significantly elevated SOD, CAT and decreased MDA levels. The effects are doe dependent [Table-32].

Table 32.Effect of PHF on the biomarker profile of oxidative stress in the sciatic nerve in Fructose induced diabetic animals

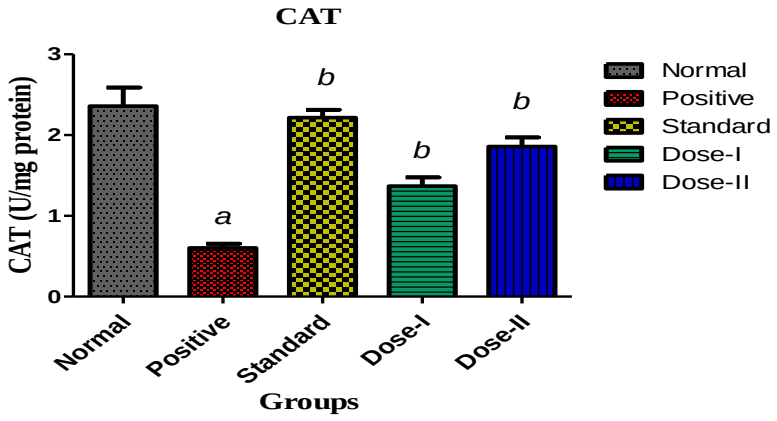
Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/mg protein)
Normal	7.48±0.297	2.36±0.229	1.28±0.032
Positive control (Fructose 66%)	1.68±0.314***[a]	0.60±0.051***[a]	7.14±0.164***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	6.87±0.099***[b]	2.21±0.096***[b]	2.02±0.022***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	6.08±0.248***[b]	1.36±0.108***[b]	3.99±0.071***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	6.28±0.277***[b]	1.85±0.113***[b]	2.41±0.078***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, when compared to positive control group (b).



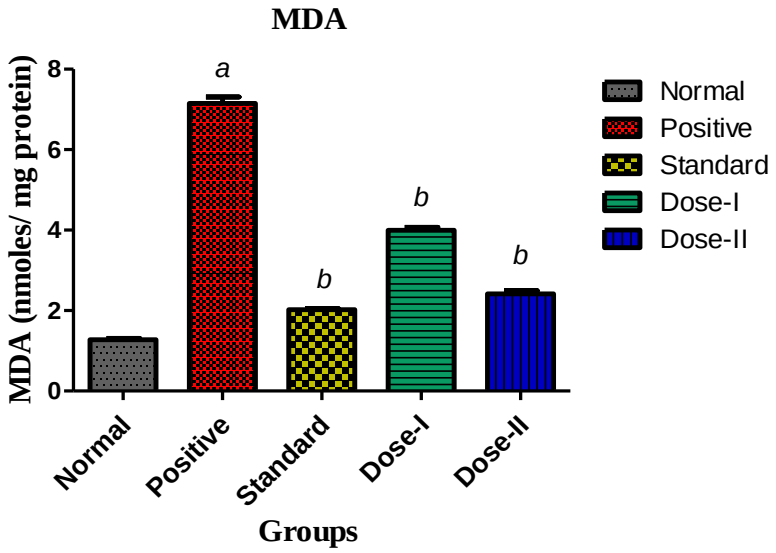
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 46.a. Effect of PHF on SOD level in sciatic nerve in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 46.b. Effect of PHF on CAT level in sciatic nerve in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, when compared to positive control group (b).

Fig. 46.c. Effect of PHF on MDA level in sciatic nerve in Fructose induced diabetic animals

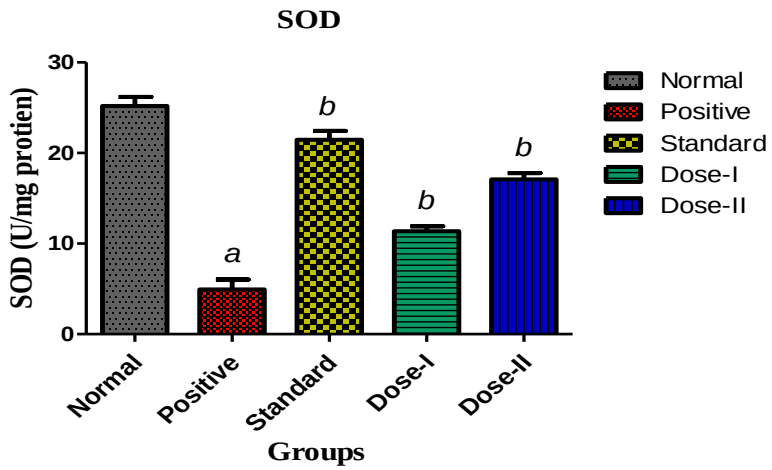
Effect of PHF treatment on biomarker profile of oxidative stress of cardiac muscles of fructose induced diabetic animals

Cardiac muscles of diabetic animals recorded significantly altered biomarker levels namely SOD, CAT and GSH compared to non-diabetic animals and PHF treatment significantly reversed the levels of them, in a dose dependent manner, compared to untreated diabetic animals [Table-33].

Table 33.Effect of PHF treatment on biomarker profile of oxidative stress of cardiac muscles of fructose induced diabetic animals

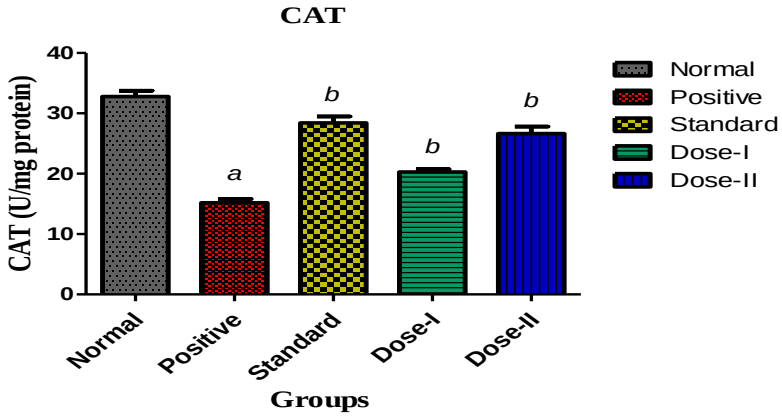
Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (nmoles/mg protein)	MDA (nmoles/mg protein)
Normal	25.18±0.99	92.83±0.97	35.21±0.97	1.42±0.04
Positive control (Fructose 66%)	4.94±1.08***[a]	15.20±0.60***[a]	8.96±0.33***[a]	5.02±0.29***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	21.46±0.98***[b]	28.43±1.05***[b]	29.07±0.39***[b]	1.86±0.05***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	11.37±0.53***[b]	20.28±0.47**[b]	17.19±0.39***[b]	3.55±0.04***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	17.08±0.71***[b]	26.64±1.17***[b]	25.21±0.58***[b]	2.66±0.06***[b]

Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$ and $^{**}p<0.01$, when compared to positive control group (b).



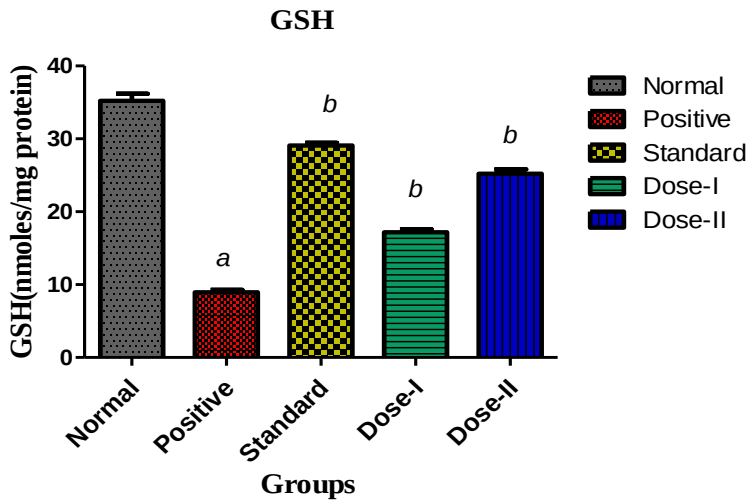
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 47.a. Effect of PHF on cardiac muscles level of SOD in Fructose induced diabetic animals



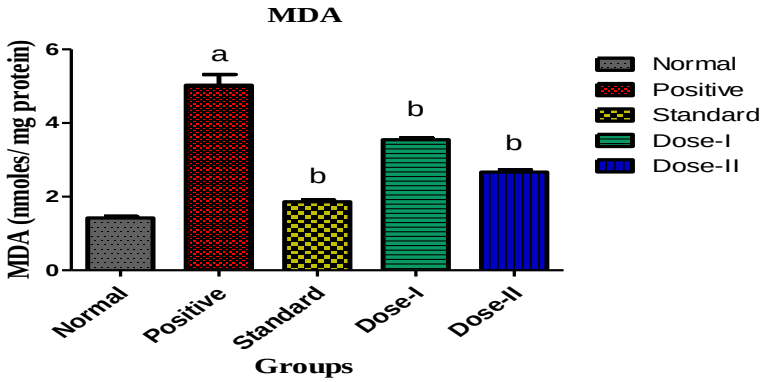
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 47.b. Effect of PHF on cardiac muscles level of CAT in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 47.c. Effect of PHF on cardiac muscles level of GSH in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 47.d. Effect of PHF on cardiac muscles level of MDA in Fructose induced diabetic animals

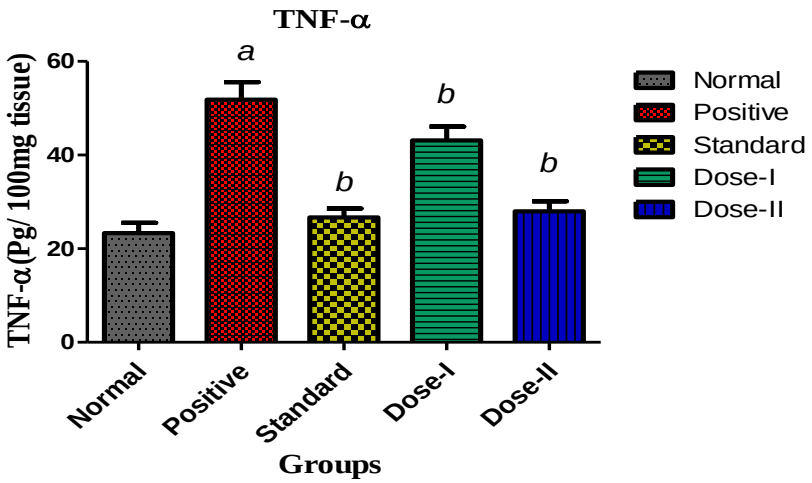
Effect of PHF on TNF-α level in fructose induced animals

Level of TNF-α exhibited marked increment in rodents with chronic administration of fructose when contrasted with normal rats, treatment of diabetic animals with PHF and metformin showed a decrease in level of TNF-α, when contrasted with positive control animals [Table-34].

Table 34.Effect of PHF on TNF-α level in fructose induced animals

Groups	**TNF-α (pg/100 mg tissue)**
Normal	23.33±2.17
Positive control (Fructose 66%)	51.81±3.77***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	26.66±1.91***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	43.12±2.95[ns]
Dose-II (400mg/kg, b.w.)+Fructose 66%	27.97±2.15***[b]

Values are expressed as mean± SEM,n=6. *** $p<0.001$, when compared to normal control group (a) and *** $p<0.001$, ns $p>0.5$when compared to positive control group (b).



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$, $^{ns}p>0.5$when compared to positive control group (b).

Fig. 48. Effect of PHF on TNF-α level in fructose induced animals

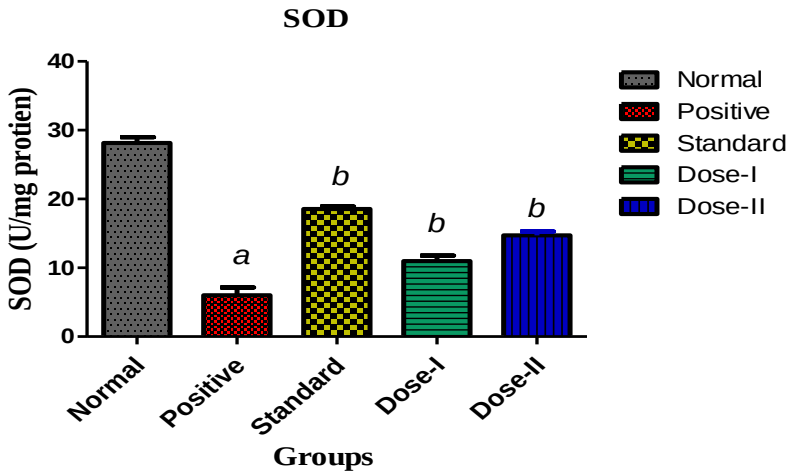
Effect of PHF treatment on biomarker profile of oxidative stress of liver of fructose induced diabetic animals

In liver tissue marked reduction in GSH level, and in antiperoxidative enzymes (SOD and CAT) when compared with normal control group. Diabetic rats treated with PHF and metformin showed noteworthy increase in SOD, CAT and GSH activities compared to diabetic control rats [Table-35].

Table 35.Effect of PHF treatment on biomarker profile of oxidative stress of liver of fructose induced diabetic animals

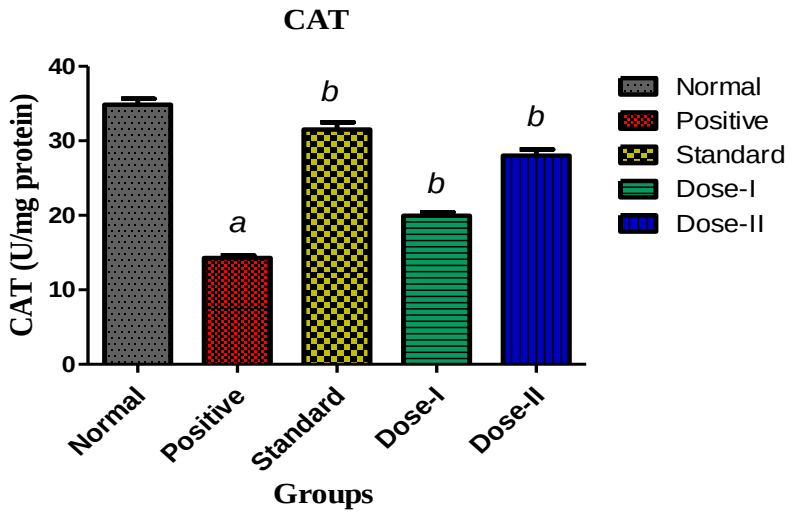
Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (nmoles/mg protein)
Normal	28.14±0.83	34.85±0.81	31.05±0.58
Positive control (Fructose 66%)	6.02±1.14***[a]	14.30±0.31***[a]	7.45±0.36***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	18.54±0.36***[b]	31.52±0.95***[b]	27.46±0.47***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	10.99±0.79***[b]	19.96±0.44***[b]	15.89±0.33***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	14.73±0.55***[b]	28.01±0.84***[b]	22.72±0.58***[b]

Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).



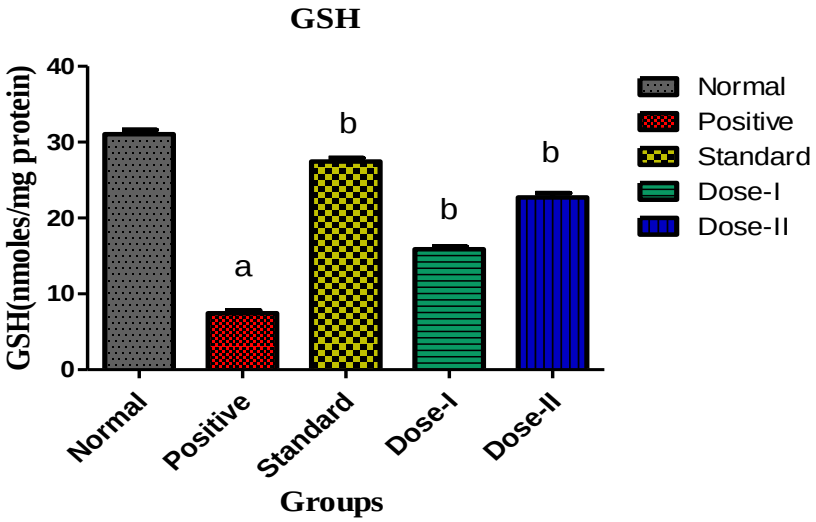
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 49.a. Effect of PHF on SOD level in liver in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 49.b. Effect of PHF on CAT level in liver in Fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Fig. 49.c. Effect of PHF on GSH level in liver in Fructose induced diabetic animals

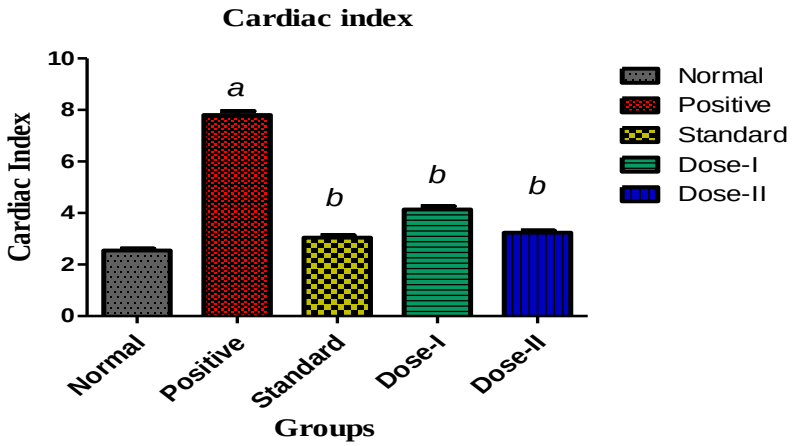
Effect of PHF on Cardiac index, Atherogenic index and Coronary Artery index in fructose induced diabetic animals

Animals after chronic treatment with fructose Recorded statistically significant, with vehicle treated control group of animals. Hyperglycaemic rats after treatment with PHF (Dose-I Dose-II) and metformin showed a significant ($P < 0.001$) decrease in Cardiac, Atherogenic and Coronary Artery index [Table-36].

Table36. Effect of PHF on Cardiac index, Atherogenic index and Coronary Artery index in fructose induced diabetic animals

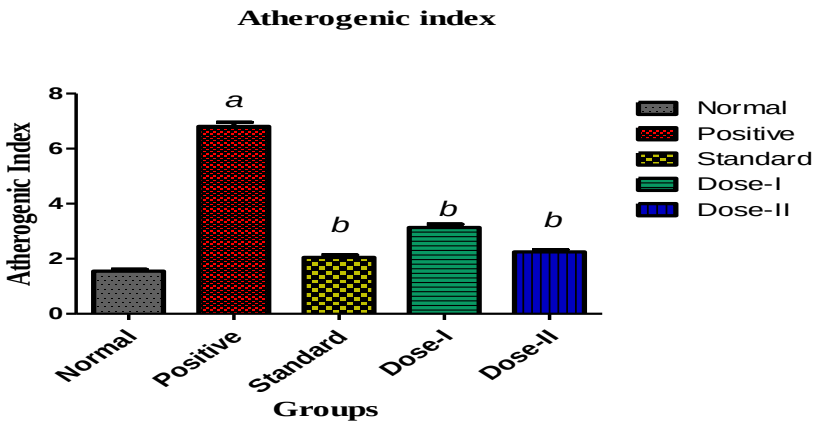
Groups	Cardiac index	Atherogenic index	Coronary Artery index
Normal	2.550± 0.06	1.550± 0.06	1.037± 0.06
Positive control (Fructose 66%)	7.807±0.14***[a]	6.807± 0.14***[a]	5.088± 0.11***[a]
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%	3.048±0.08***[b]	2.048± 0.08***[b]	1.342± 0.08***[b]
Dose-I (200 mg/kg, b.w.)+Fructose 66%	4.137±0.12***[b]	3.137± 0.12***[b]	2.167± 0.11***[b]
Dose-II (400mg/kg, b.w.)+Fructose 66%	3.240±0.08***[b]	2.250± 0.07***[b]	1.702± 0.04***[b]

Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).



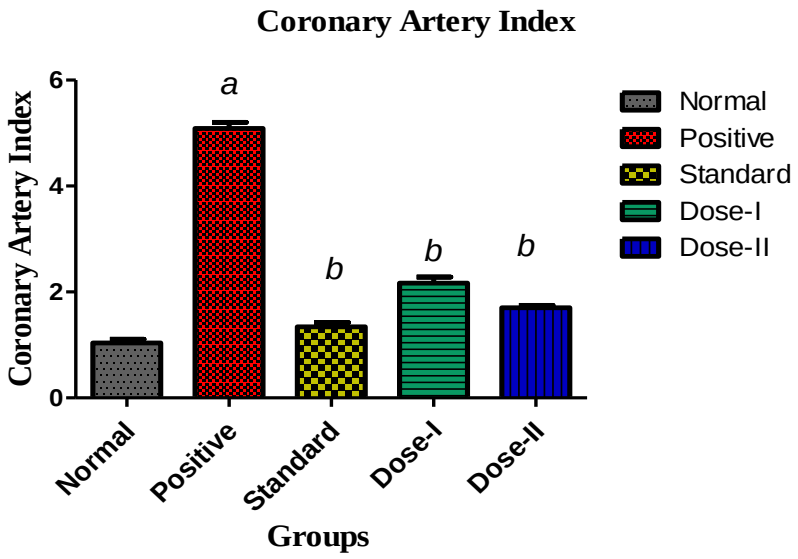
Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 50.a. Effect of PHF on Cardiac index in fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001 and **p<0.01, when compared to positive control group (b).

Fig. 50.b. Effect of PHF on Atherogenic index in fructose induced diabetic animals



Values are expressed as mean± SEM,n=6. $^{***}p<0.001$, when compared to normal control group (a) and $^{***}p<0.001$ and $^{**}p<0.01$, when compared to positive control group (b).

Fig. 50.c. Effect of PHF on Coronary artery index in fructose induced diabetic animals

Table 37.Effect of PHF on histopathology of Kidney in fructose induced diabetic animals

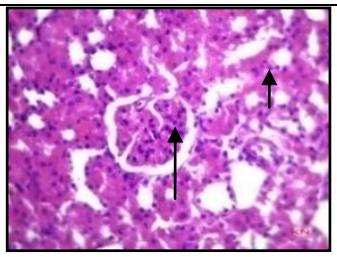
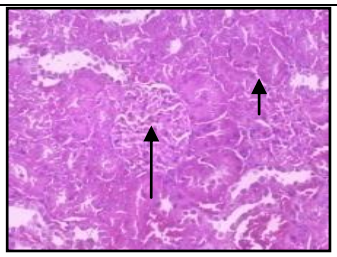
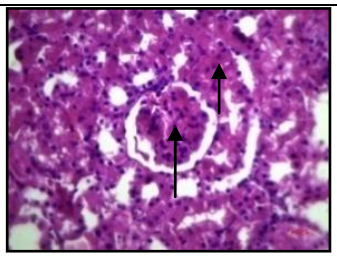
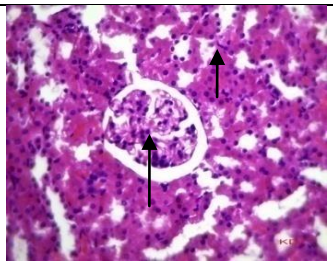
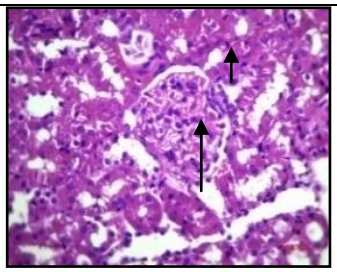
Groups	Histopathology	Observation
Normal		The tubules of the kidney indicated some degenerative changes with positive group of rodents when contrasted with normal features of animal. Histopathological analysis for normal group animals for kidney tissue indicated normal appearance of glomeruli and tubules (Long and short arrow). Treatment with Dose-I 200 mg/kg (PHF) showed degenerative changes and some blood vessels appear congested. However, the treatment with Dose-II 400 mg/kg and metformin showed intact architecture as compared to normal group.
Positive control (Fructose 66%)		
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%		
Dose-I (200 mg/kg, b.w.)+Fructose 66%		
Dose-II (400mg/kg, b.w.)+Fructose 66%		

Table 38.Effect of PHF on histopathology of Sciatic nerve in fructose induced diabetic animals

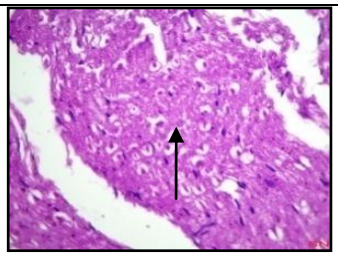
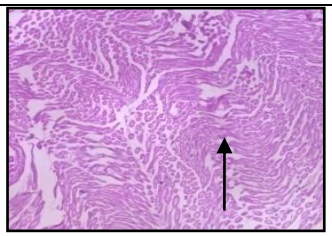
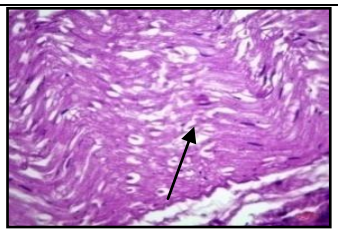
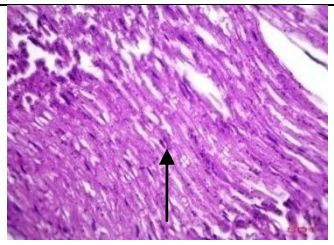
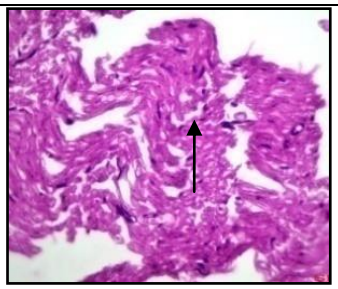
Groups	Histopathology	Observation
Normal		Histopathological analysis showed small myelinated fiber loss was more prominent than large diameter fiber loss, Endoneurial vessel was not thickened in untreated diabetic animals compared to normal animals. However, the treatment with Dose-II 400 mg/kg showed intact myelinated fiber density, the small myelinated fiber and large diameter fiber appear intact and the endoneurial vessel was also thickened as compare to normal control group.
Positive control (Fructose 66%)		
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%		
Dose-I (200 mg/kg, b.w.)+Fructose 66%		
Dose-II (400mg/kg, b.w.)+Fructose 66%		

Table 39.Effect of PHF on histopathology of Heart in fructose induced diabetic animals

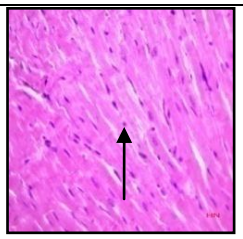
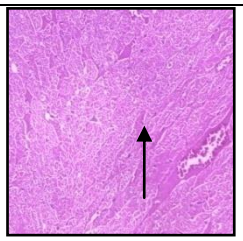
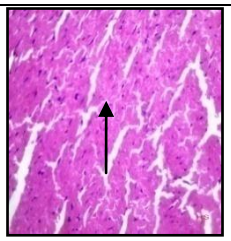
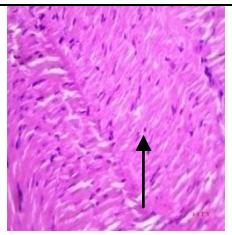
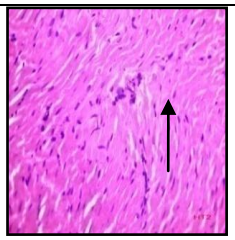
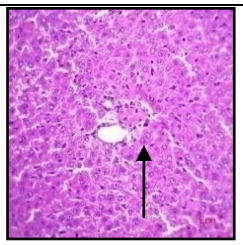
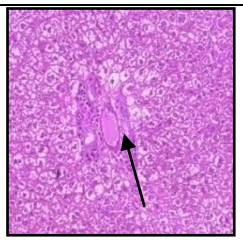
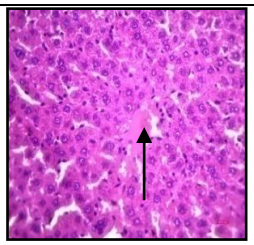
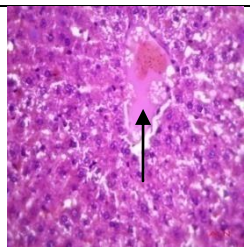
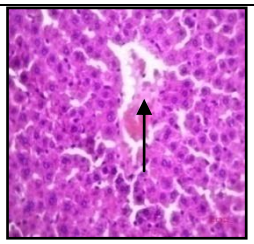
Groups	Histopathology	Observation
Normal		Histopathological analysis showed that treatment with Dose-II 400 mg/kg showed integrity of cell membrane of myocardium, myofibrillar striations and well connected to immediate myofibril as contrasted with normal group rodents.
Positive control (Fructose 66%)		
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%		
Dose-I (200 mg/kg, b.w.)+Fructose 66%		
Dose-II (400mg/kg, b.w.)+Fructose 66%		

Table 40.Effect of PHF on histopathology of Liver in fructose induced diabetic animals

Groups	Histopathology	Observation
Normal		Section studied in positive control rat showed the periportal hepatocytes , vacuolated cytoplasm and periportal region showed mild mononuclear inflammatory infiltration (Long arrow) as compare to vehicle control group of animals (showed intact architecture). Treatment with PHF (Dose-II 400 mg/kg) showed the intact architecture of liver parenchyma and the perivenular, periportal and midzonal hepatocytes appear unremarkable as compared to untreated diabetic animals.
Positive control (Fructose 66%)		
Standard Treatment (Metformin 5mg/kg, b.w.)+ Fructose 66%		
Dose-I (200 mg/kg, b.w.)+Fructose 66%		
Dose-II (400mg/kg, b.w.)+Fructose 66%		

5 DISCUSSION

- Non-insulin dependent diabetes mellitus, a common endocrine disorder with characteristic hyperglycemia, insulin resistance and insulinopenia with the progress of the disorder. Elevated hyperglycemia is associated with several secondary complications like Diabetic neuropathy, retinopathy and nephropathy to have a few. [228]

- Significant elevated level of fasting and post prandial hyperglycemia related to disturbed homeostasis of carbohydrate, lipid and protein metabolism.[229] Diabetic patients may have Relative / absolute insulin deficiency. All these factors together responsible for hyperglycemia.
- Insulin a hormone released from β-cells of pancreas responsible for glucose metabolism and peripheral mobilization into storage organs. About 176 million peoples across the world are suffering from DM and it will be double by the year 2030.
- International diabetes foundation in it's IDF diabetes Atlas (9^{th} edition in 2019) in its top 10 countries/ terriorities for number of adults (20-79 years) with diabetes, projects Indian Diabetes are projected to be 101 million by 2030,from 77 million in 2019 and 134.2 million by 2045.[230,231]
- As per epidemiological studies, India has nearly about 33 million diabetic subjects today and major contribution for this estimation is by the urban population. Most of the diabetic subjects in India and Western countries like America are linked with the shifting of people from rural to urban, moving from tradition to modern life style, changes in diets, physical inactivity and obesity.[232]

- Management of Diabetes, both type 1 and 2 are possible with insulin and oral hypoglycemic drugs, although the use is associated with hypoglycemia, fatty liver etc. Limitation of synthetic medications also includes questionable efficacy, high cost and management of adverse effects. Herbal drugs are becoming increasingly popular among diabetics due to lower incidence of adverse effects, low / affordable cost and being more efficacious. [233,234]
- T2DM is due to lack of physical activity and obesity and steadily progressive and likely to double in another15 years. Progress of T2DM is associated with development of secondary complications. [235-237]
- An imbalance between generation of reactive oxygen species and the defense mechanism results in oxidative stress. Oxidative stress and role of free radicals due to variety of stress, life style etc. is also now, being associated with pathogenesis and progresses of DM into secondary complications.[238,239]
- Healthy person is capable of overcoming oxidative stress by generating anti-oxidative enzyme, namely GST, CAT, GP *etc*. which effectively scavengers free radicals generated.[240]
- Glutathione (GSH) protective role against cellular lipid peroxidation has been already proven.[241] Low level of GSH is common in STZ diabetic animals.[242] During these investigations, Significantly lower GSH in untreated diabetic animals suggests reduced synthesis / accelerated degradation as a result of oxidative stress.

HERBAL MEDICINES AND HERBAL THERAPY

- The term ‘herbal medicines’ has various meanings, Referring to crude drugs of herbal origin /vegetable origin (natural), used in the treatment of disease, especially of chronic nature or maintain health”. The growing popularity of herbal medicines over allopathic medicine is because of the following reasons:
- They are free from side effects or less side effects
- Easy availability of drug from natural source
- Freedom from approaching various specialists
- Rising cost of medical care
- Cure from many chronic diseases
- Cure of disease influenced by life style changes and social pathology
- Possible Ethnicity.

IMPORTANCE OF TRADITIONAL MEDICINE

- Traditional medicine refers to overall knowledge and practices, For diagnosis, prevention and eliminate imbalances of physical /mental/social nature based on experience and observation from generation- verbally (Orally) or in writing. Ancient Vedic scriptures are rich source of knowledge on herbs and are indicating extensive use of herbs for management of diseases Ayurveda and Unani documents crude drugs and it’s in the treatment.
- Not just at the local level, traditional medicine is now recognized as invaluable source of medicine at the global level. Such importance can be attributed to economically less developed nature of several countries, relying heavily on traditional medicine. Traditional Indian especially Ayurvedic is a rich repository of well documented

literary legacy offering theoretical frame work for treatment. Ethno medical heritage to other Asian, African, Arab countries are equally significant.

- Plants and its parts have been used for the management of diabetes. Scientific reports points to about 343 plants with anti diabetic property, although, around 800 plants are believed to posses such property. Ascorbic acid, alpha-tocophenol can reduce glycosylated hemoglobin. All these effects are quite beneficial to the diabetic patients. However all plants believed to have anti-diabetic activity are not evaluated in this light as their nature, mode of action, potency and safety differ from each other. Whatever may be, for management of T2DM we need to find active principle responsible for reducing glucose level and elevating glycogen level.[243-246]

THE WAY HERBAL ANTI-DIABETICS WORK.

Following mechanism for lowering elevated blood glucose level has been suggested and the mechanism resembles that of currently available synthetic anti diabetic drugs:

- By improving insulin secretion (*Teucrium polium*, *Allium sativum*, *Allium cepa*,*Panax ginseng)*
- By virtue of inhibition of reabsorption of glucose from renal site *(Fraxinus excelsior)*
- influencing glycogenolysis and hepatic glycolysis *(Momordica charantia)*
- Protecting β cells in the islets of Langerhans *(Thea sinensis)*
- Reducing blood glucose and urea and improving the digestion *(Aegle marmelos)*
- Prevention of conversion of complex carbohydrates into simpler ones *(Eugenia jambolina* and *Pterocarpus marsupium)*
- Preventing conversion of b-galactocidase and a-glucocidase *(Clitoria ternata)*
- Accelerating glucose utilization by tissues and influencing α and β cell function *(Panax ginseng, Allium sativum, Allium cepa)*

- Being acting in synergistic manner of exogenously administered insulin.
- By reducing cortisol level *(Inula racemosa, Boerhaavia diffusa and Ocimum sanctum).*

Most of the potent drugs which are used presently in the form of synthetics are extracted from plants and made from lead molecules which are extracted from plants. In developing countries, medicinal plants consider to be the main source of medication. Among the drugs that are currently used in different countries, 77% of them are being obtained from plants in traditional medicine.

The preliminary phytochemical screening of aqueous extract of PHF tested positive for

alkaloid, glycoside, carbohydrates, flavonoid, protein, saponins and sterol.

- Streptozotocin (STZ) generates free radicals.[247]
- Such free radicals destroy pancreatic beta cells- thus, a diabetogenic in nature and is widely used to generate or produce diabetes in lab animals.[248,249]
- STZ found its usefulness in the cancer chemotherapy as cytotoxic to β cells of pancreas. Cytotoxicity can be attributed to its ability to alkylate macromolecules especially DNA, which in turn activates various phosphorylation (ADP). Superoxide radicals are generated by virtue of depletion of NAD and ATP, accelerated dephosphorylation of ATP, promoting Xanthine oxidase.. Further, toxic amounts of NO produced by STZ also participates in DNA damage. Beta-cells now undergo necrosis. Relative toxicity of STZ to beta-cells can be attributed to relatively higher levels of GLUT2. Interestingly, STZ also induces insulin deficiency and produces diabetic ketoacidosis. In our investigation, dose of STZ was chosen in such concentration to produce mild insulinopenia without ketoacidosis.[250,251]

- In the present investigation, oral administration of PHF to diabetic rats produced anti-hyperglycemic and antioxidant activity. Decrease in body weight and polyuria, similar to diabetes and these are results of metabolic changes which occur due to deficiency of insulin, because of damage to β-cells. Due to destruction of structural proteins in diabetic rats it results in significantly reduced body weight. Treatment of diabetic animals with PHF showed improvement in body weight, which showed the protection to muscle wasting. Administration of PHF decreases the blood glucose levels such animals, which resembles action of metformin. Increase level of serum insulin which is attributing to insulin secretion from remaining β-cell indicates PHF anti-diabetic potency as evidenced by insulin estimation. Further these results were supported by hisopathology that PHF has potential ability to regenerate β-cells and to prevent pancreatic β-cells loss. Increase in urine volume after the administration of STZ indicate early sign of diabetic nephropathy, it may be attribute to increase in activity of Na^+K^+ATPase.[252] Diabetic animals treated with PHF showed reduced polyuria in diabetic animals, thus correlate with significantly reduced Na^+K^+ATPase activity and renoprotective.
- Raised levels of HbA1c in diabetic animals could be due to indefatigable hyperglycemia. Since concentration of HbA1c connects with neuropathy, nephropathy and retinopathy[253], the attenuated levels of HbA1c by PHF indicates its ability to stop polygenic disease associated complications. In our study, increase levels of HbA1c in diabetic animals were switched towards typical level after treatment with PHF, suggesting potential of the PHF to prevent diabetic associated complications.
- Neuropathy, a common associated complication of T2DM, is due to result in duration and severity of hyperglycaemia and apart from this is also associated with releasing of torment go betweens like algogenic substance E2 prostaglandins which, for their

strength and communication with different substances as from least focuses, have fringe sharpening impact and trigger nociceptors centripetous. Cytolysis offers cell films for corruption, which is one of wellspring of star provocative and algogenic substances. Its metabolites incorporate prostaglandins and prostacyclins, leukotriens, tromboxanes, among others, which will take an interest in the purported "provocative soup". Extraordinary algogenic substances (ace fiery) substance discharged when nociceptors collaborate with when they are energized. These substances which are discharged in the interstitium are likewise spread without really trying and act at a separation like other algogenic and calming substances, additionally diminishing nociceptors limits. Excruciating receptors have likewise their very own receptors, for example, those distinguishing the nearness of proteolytic compounds, particularly triptases. In diabetes probably nerve damage results in loss of pain perception and induction of diabetic peripheral neuropathy. PHF treatment resulted in statistically significant hypoalgesia in conventional model frequently employed for evaluating analgesic drugs. Therefore, agents or compounds with pleiotropic activity, such as antioxidant, antidiabetic, aldose reductase inhibitory and antiglycation properties, are likely to be more effective than agents with a single action.[254] PHF treatment significantly increased latency of pain threshold, reduced sensitivity to pain as compared to untreated diabetic group of animals, consequence to improved antioxidant defence system controlling the sciatic nerve oxidative damage from the ROS that's why increased in SOD activity. The improvement of diabetic neurological difficulties is due to age of oxygen-determined free radical in diabetes and neuropathic torment. Lipid peroxidation–interceded tissue harm has been watched in the improvement of types, I and T2D. PHF treatment significantly increased latency of pain threshold, reduced sensitivity to pain as compared to untreated diabetic group

of animals. [255] These data suggests that PHF may have potent neuronal antioxidant activity, which may facilitate neuroprotective action. Histopathological analysis supported that treatment with PHF showed intact myelinated fiber density, the small myelinated fiber and large diameter fiber appear intact when compared to diabetic control group.

- Delayed gastrointestinal transit is a one of common clinical condition of DM, which involves an impaired cholinergic neurotransmission and reduced smooth muscle response to neurotransmitters. These breakdowns might be attributed to the oxidative harm of autonomic neurons and down guideline of muscarinic receptors in colonic smooth muscles, individually. Diminished intestinal travel of charcoal feast in STZ-actuated diabetic rodents was seen in the present investigation. Expanded oxidative stresses in ceaseless diabetic state were accounted for in STZ-actuated diabetic rodents. Treatment with PHF improved the reaction of colonic smooth muscle to acetylcholine (Ach) and altogether upgraded the diminished motility recommending a defensive impact of PHF in STZ actuated diabetic rodents that might be because of the anti-oxidant property of PHF.[256]
- Cardiomyopathy is an associated and independent complication of DM which can occurs in the absence of other heart diseases also. Pathogenesis of diabetic cardiomyopathy (DCM) involved oxidative stress, inflammation and apoptosis. [257] During this investigation, we established ameliorative effect of PHF in DCM in an animal model of diabetes (STZ-NA). Functional impairment of organs in diabetes can be related to elevate lipid profile and low HDL.[258] However data of present study indicates that PHF significantly reduced lipid profile in diabetic animals normalizes HDL level. These suggest that PHF could alter the hepatic glycogenolysis and gluconeogenesis process.

- Role of TNF-α in the development of insulin resistance is well established. Apart from exceedingly kind of human diseases, as well as T2D dysregulation of TNF-α production is seen.[259] Diabetic animals treated with 200 mg/kg of PHF produced insignificant fall TNF-α level and higher dose significantly reduced TNF-α level, possibly due to relatively lesser dose or shorter duration to produce a significant change that is comparable to standard drug treated animals. Vascular dysfunction in thoracic aorta could be due to increase production of MDA, which is substantiating the hypothesis of elevated levels could leads to generation of ROS in DM. The present study suggests that significantly reduced levels of biomarkers Viz., MDA, SOD and CAT in the post PHF treatment with PHF, contributes for the protection of thoracic aorta by oxidative stress. Antioxidant therapy results in reversing these oxidative damages affecting various vital organs and macromolecules, subsequently altering cell function. Section studied in positive control group showed the tunica intima and endothelium appears disrupted and the tunica intima media thickness was 36.4µm, as compare to normal control group features (tunica intima media thickness 24.2µm). Whereas treatment with PHF resulted in reversing the damage to layers of thoracic artery as indicated in transverse section (tunica intima media thickness 24.8µm).
- Nephropathy in TD2M leads to end stage renal disease which is recognized as leading cause of morbidity and mortality in diabetic patients. Cytotoxic STZ significantly affected renal function as suggested by elevated renal profile compared to normal levels.[260] An increased urea concentration in diabetes indicates greater protein catabolism. Earlier studies have demonstrated the relationship between hyperglycemia and DN. We observed that single dose of STZ impaired kidney functions. Diabetic animals treated with PHF and Metformin resulted in statistically significant fall

kidney markers compared to untreated positive control group of animals suggesting impaired renal function. This could be due to the treatment with PHF may protect diabetic animals from renal damage. Since diabetic hyperglycemia led to renal dysfunction.

- Several studies and reports are pinpointing the definitive role of oxidative stress playing on important initiation and / progress of the diabetes related damages / injuries. Free radicals, lipid peroxidation can initiate cell and tissue damage. Antioxidants and antiperoxidative like, CAT, SOD, GSH ensure that these endogenous antioxidant enzymes protects the cells and tissues, therefore the organs against oxidative stress.[261] Our investigations also demonstrated that oxidative stress played a pivotal role in the STZ induced diabetic animals. It has been additionally appeared that lipid peroxidation was fundamentally diminished after the treatment with PHF and metformin. Histopathological examination indicated that the tubules, blood vessels, interstitium and glomeruli of the kidney appear in normal cellularity after treatment with PHF when compared to positive control group.
- Diabetic retinopathy (DR) is also a one of noteworthy inconvenience of T2DM which continue from nonproliferative irregularities to proliferative DR, is distinguished by retinal edema, haemorrhage, expanded neovascularisation and neuronal degeneration in the retina. Although different initiators of this complication have been proposed, expanded oxidative pressure incited by hyperglycaemia is by all accounts the bringing Complications of diabetes influencing polyol pathway, which could enact the polyol pathway, increment advanced glycosylation end product (AGE) arrangement, actuate protein kinase C (PKC), hexosamine pathways all leads to improvement of DR. Among all cytokines which are associated with DR, vascular endothelial growth factor (VEGF), as an essential initiator of proliferative DR. In the retina, VEGF is one

of the cytokine which can prompt intracellual adhesion molecule-1(ICAM-1) articulation and leucocytes attachment, which together lead to the blood-retina breakdown (BRB), can be accounted for observed VEGF and ICAM -1 level in the retina and related revascularization in diabetic patients with retinopathy. This study demonstrated that the retinal degrees of VEGF and ICAM-1 were essentially diminished after treatment with PHF contrasted with diabetic group. Further PHF showed protective effect against oxidative stress by decreasing MDA level and increased in SOD level, which proposes PHF may avert the advancement of diabetic retinopathy by means of the counter angiogenic, anti-inflammatory and antioxidative consequences in rodent.[262]

- Section of retina studied indicated that positive control rats showed extensive vacuolations in the plexiform layers and ganglion layer as compare to normal control group (without any vacuolations in the plexiform layers and ganglion layer). Treatment with PHF showed moderately reduced vacuolations [compared to positive control] in the plexiform layers and ganglion layer, which was rendered in histopathological studies.
- Production of free radicals and subsequent oxidative stress are well established factors in the development and progress of secondary complication of DM.[263] Catalase and Glutathione are scavengers of free radicals and protective in nature against oxidative stress. In addition malondialdehyde reflects the degree of the organic lipid peroxide and cell damage. Our data found that PHF can reverse the biomarker profile, suggesting attenuated oxidative stress in diabetic animals. Section studied analysis showed that in positive control animals the liver parenchyma partially distorted architecture and there are seen focal areas of necrosis with congested blood vessels as compare to normal control group (showed intact architecture). Histopathological

studies of the liver from PHF treated diabetic animals revealed features like intact nature of parenchyma and hepatocytes from peripheral, periportal and mid zonal region appears normal, so also the central veins and sinusoids.

- Fructose is an improving substitute (fructose corn syrup) for glucose or sucrose which utilized economically. It has been as of late affirmed that the utilization of high measures of refined sugars in nourishment and refreshment expands the danger of dyslipidaemia, and other medical conditions. Recently a relationship between diabetes commonness and sugar accessibility was found in ongoing epidemiological study in people. Besides, perpetual utilization of a Western eating regimen, described by nourishments wealthy in sugar and plentiful altogether and immersed fat, has been proposed to assume a job in the advancement of T2D.[264]
- Oral administration of 66% fructose is accounted for to create cardinal signs and attributes of metabolic disorder, i.e., hyperglycemia, dyslipidemia and hyperinsulinemia, In the present investigation, it has been discovered that the qualities and cardinal signs delivered by oral organization of 66% fructose are comparable and reliable with those revealed before.[265]
- In the present study, another model fructose induced diabetes, indicated that body weight of the animals were increased in fructose-bolstered rodents that might be because of an expansion in the adiposity. PHF showed critical weight decrease in fructose-nourished rodents. Notable fall in body weight, probably due to reduced feed intake. It s also possible that this observed effect is likely to be due to combined effect appetite suppression, hypoglycaemia and hypolipidemia.
- We found that in fructose-sustained group, blood glucose levels were essentially expanded. PHF treatment demonstrated a statistically significant reduction in blood glucose profile when compared to untreated fructose induced diabetic animals.

- It was also observed in the study that the insulin levels in fructose-administered group had significantly high levels of insulin. Due to defect in insulin signal transduction mechanism insulin resistance occur in T2D, Which result in failure of circulating insulin to induce uptake of glucose into the cells and the hyperglycaemia develops. For the compensate of the hyperglycemia, pancreas secretes more insulin leading to hyperinsulinemia.[266] PHF altogether decreased the hyperinsulinemia in fructose induced diabetic animals. Results of our investigation indicate that PHF significantly reduced the hyperinsulinemia in fructose-treated group. The HOMA model is the most broadly utilized surrogate measure for evaluating insulin opposition and β-cell work in clinical and epidemiologic investigations. HOMA-IR showed an increasingly steady capacity to anticipate T2D contrasted and other insulin obstruction indexes.[267] In accordance with these discoveries, our examination has affirmed that HOMA-IR was a vigorous surrogate contrasted and fasting glucose and insulin levels in fructose-bolstered rodents. Insulin opposition which is estimated by HOMA-IR, was enhance fundamentally with the PHF.
- Constant aggravation is additionally firmly connected with diabetes and IR. Aggravation causes IR through hindering the flagging downstream of insulin receptor. Phosphorylation decreases consequent tyrosine phosphorylation of IRS-1 because of insulin and its capacity to connect with the insulin receptor, accordingly repressing downstream insulin flagging.[268] C-reactive protein (CRP), which predicts the risk of CVD. In this study, the administration of PHF lowered the concentration of CRP levels, and therefore lowered the risk of CVD in high fed fructose rats. The critical decrease of plasma CRP levels in diabetic rodents following PHF treatment shows that restraint of endless irritation is another contributory factor, notwithstanding the

reduction of free unsaturated fats, to the observed improved fasting blood glucose levels in PHF treated animals.

- Our investigations points to altered level TNF- α are an important factor in the regulation of insulin resistance. In view of reports pointing to confirmed role played by TNF- α leading to insulin resistance, and paucity of reports of insulin resistance in non-obese model, confirming significance of obesity in the development of insulin resistance. Hindrance to the movement TNF α affects development of insulin resistance. The examination confirms that PHF can improve irritation by diminishing TNF-α level. The outcomes acquired can be impacts of the PHF which enhanced insulin affectability and glycemic control.[269]
- The high pervasiveness and seriousness of cardiovascular sicknesses in DM require new screening device for better assessment. Atherogenic index, coronary risk index can be solid markers for anticipating the danger of atherosclerosis and coronary illness. For prediction of atherosclerosis atherogenic index of plasma has been used by the researchers and consider as an independent cardiovascular risk factor.[270] Regarding the effects of PHF on lipid profiles, findings from our study showed significant reduction in serum lipid levels with concomitant increase in HDL. In T2D, adipocytes are impervious to the activity of insulin, and lipolysis proceeds with unchecked. VLDL, which adds to the atherosclerotic disease, has been added by arrival of expanded free unsaturated fats from adipocytes.[271] In this investigation, serum triglyceride and cholesterol levels in fructose-treated group were expanded significantly. PHF fundamentally decreased the serum triglyceride and cholesterol levels in fructose-treated group. This impact might be a direct result of the counter steatosis activity of PHF. Histopathological examination reveals that in PHF treated

diabetic animals- integrity of myofibrillar structure, myocardial membrane and continuity with other myofibrils is restored.

- It is realized that despite the fact that fructose likely leads to increments in the creation of uric acid. Late epidemiological proof showed that hyperuricemia may be consider as a one of hazard factor for dysfunction of renal. Weakened renal capacity showed by increment in serum creatinine and urea after chronic administration of fructose to rodents which may showed hyperuricemia. Then again, PHF brought about progress of kidney work, likely due to hypouricemic properties and expanded nitric oxide generation individually.
- Uric acid has capability to diminish endothelial nitric oxide (NO) bioavailability in exploratory animals. Insulin resistance may also leads to decreasing endothelial NO dimensions. Therefore endothelial NOS-lacking rodent display the highlights of metabolic disorder.[272] Histopathology study indicated that tubules of the kidney showed some degenerative changes with diabetic control rats as contrasted with normal animal. Treatment with PHF showed intact architecture, normal appearance of tubules and blood vessels as compared to positive control group.
- Diabetic control rodents demonstrated a factually critical increment in the latency time and a measurably huge abatement in the reaction time, compared to vehicle treated. Diabetic animals treated with PHF and Metformin demonstrated a factually huge increment in latency time and a measurably noteworthy lessening in the reaction time, contrasted with positive control group. PHF has decreased MDA action which has avoided lipid peroxidation role in the advancement of oxidative stress. Additionally, increment in the SOD and CAT levels, which have decreased superoxide radical generation due to synergistic impact of blend.[273] Histopathological analysis showed small myelinated fiber loss was more prominent than large diameter

fiber loss Endoneurial vessel was also not thickened with positive rodents as contrasted with normal control group. However, the treatment PHF showed intact myelinated fiber density,the small myelinated fiber and large diameter fiber appear intact and the endoneurial vessel was also thickened as compare to normal control group.

- Liver likewise controls the synthesis (glycogenesis) furthermore, degradation of glycogen (glycogenolysis) as a part of their glucose homeostasis system. During T2DM because of absence of or protection from insulin, increment in hepatic gluconeogenesis, glycogenolysis and decline glycolytic, glycogenic action were observed.[274]
- Oxidative stress or free radicals are one of the causative variables in the improvement results of diabetes. In the present investigation, the exercises of hepatic oxidant/ antioxidant status were estimated to find out the job of PHF in the easing of modified antioxidant safeguard framework. The dimension of GSH was diminished in T2D rodents when looked at to normoglycemic animals. PHF treated animals have ensured their exercises essentially. On the other hand, the expanded dimensions of MDA in T2D rodents were weakened essentially in PHF treated group.
- Liver proteins, for example, alkaline transaminase (ALT) and aspartate transaminase (AST) are marker proteins for liver capacity. Hyperglycemia is typically joined by an expansion in the exercises of the catalysts of the liver.[275] Organization of PHF has prompted a noteworthy abatement in ALT and AST levels. The improvement in the hepatic creator compound levels by PHF treatment shows that it does not cause hepatotoxicity that is likely brought about by few classes of antidiabetic drugs. PHF appears to force hepatoprotective potential alongside antidiabetic movement. Improvement in the exercises of liver marker catalysts level by PHF treatment

demonstrated that PHF has hepatoprotective activity alongside antidiabetic action. Section of liver studied in positive control rat showed the periportal hepatocytes, vacuolated cytoplasm and periportal region showed mild mononuclear inflammatory infiltration as compare to normal control group (shows intact architecture). In diabetic animals treated with PHF, histological features of liver Viz., parenchyma, hepatocytes at perivenular, periportal and at mid zones seems to be restored.

- It is reported that PHF under study contain *Coccinia indica* W.&A, *Momordica charantia* Linn., *Lagerstroemia speciosa* Linn. showed presence of various phytoconstituents like, flavonoid, , alkaloids, amino acids, , triterpenoid, anthranoids, glycoside, minerals, glycosides, carbohydrates, flavanoids, cephalandrol, vitamins and inorganic compounds, amines, carboxylic acid derivatives, peptidoglycans, polyphenol and its derivatives, saponins, β-sitosterol alkaloids, heptacosane.[276] The antidiabetic property of PHF may be attributed to mainly presence of cephalandrine a, cephalandrine b[277], charantin, vicine, glycosides, karavilosides along with polypeptide-p[278], Ellagic acid and corosolic acid.[279] Preliminary phytochemical analysis of PHF under study confirmed flavonoids and tannins, the natural antioxidants, scavenging free radicals.[280,281] Thus the antioxidant potential of PHF can be related to phenolic compounds. Further, significant antioxidant activity coupled with antihyperlipidemic activity. In the management of non-insulin dependent DM and prevention of its secondary complications.

6 SUMMARY

Elevated blood glucose level -a hall mark of diabetes of both types results from insulinopenia. Diabetic patients identified by elevated blood glucose level as liver and skeletal muscle are not able to store glycogen and tissues are unable to utilize glucose. Any of type of DM if it will not manage, it may leads to chronic hyperglycaemia, which will result in damage of various organ and leads to diabetes and its associated complications. T2D is the most common disorder around the world and it will accounts for 95% of total diabetic population. Effective glucose control is necessary for quality life in diabetic patients. As herbal medicines are relatively safer with fewer adverse effects, there is more interest on use of herbal drugs compared to synthetic ones.

Phytochemical screening and evaluation of its efficacy were carried out in two animal models of diabetes for antidiabetic activity, as well it's potential to reverse secondary complications of diabetes mellitus.

PHF is a rich mixture of alkaloids, flavonoids, carbohydrates, proteins, glycosides, steroids and saponins which is confirmed by detailed chemical analysis performed as a part of our investigations.

Acute (Oral) toxicity study done as per OECD TG 425 confirmed the safety of PHF to be up to 2000mg/kg.

Role of PHF in secondary complications of T2DM have been assessed in the present examination by utilizing rodents in two animal models i.e.,

1. Streptozotocin induced diabetes
2. Fructose induced diabetes

In Streptozotocin (STZ) induced diabetic animals were fasted for 12 hours and after that imbued with Streptozotocin with single intraperitoneal (i.p.) mixture. STZ in citrate buffer (4.5 pH). Nicotinamide (110mg/kg) in normal saline was administered fifteen minutes prior to STZ injection. STZ, being a cytotoxic to β cells of the pancreas induced diabetes within 3 days of its administration. Rats showing fasting blood glucose above 200mg/kg body weight were selected for our investigations.

Body weight, urine volume and various physiological and biochemical parameters pertaining to diabetes and its associated complications such as Hot-plate and Tail-flick method, HbA1C level in blood, Serum FBG, insulin level, lipid markers, kidney markers, Inflammatory markers like TNF-alpha, ICAM-1 and VEGF levels were assessed. Enzymatic oxidative stress in different organs was assessed and to support the above study histopathological evaluation has been done for vital organs.

When PHF were tested at two doses for its antidiabetic potential associated with complications in STZ induced diabetes, there was a dose-dependent antidiabetic effect offered by PHF, as evidenced by histopathological studies. However, the PHF exhibited a significant anti-diabetic action compared to Metformin. The antioxidant potential of PHF may be the possible mechanism for observed anti-diabetic activity. PHF demonstrated a significant improvement in physiological parameters, body weight, serum insulin level and showed significant reduction in biochemical and inflammatory markers level, elevated levels of endogenous antioxidant and histopathology study revealed protective effect of PHF to all vital organs against STZ induced injury.

In fructose induced diabetes model animals were instigated by chronic administration of fructose (66% solution orally for about a month and a half) with the exception of in normal control group. After the 6 week ingestion of fructose blood glucose level was observed and

those rodents whose blood glucose level was in excess of 180 mg/dl were consider in the study.

Body weight, physiological parameters associated with secondary complications like Hot-plate and Tail-flick method were assessed and various biochemical parameters like HbA1C level, serum FBG, insulin level, lipid markers, kidney markers and liver function test and inflammatory marker like TNF-alpha were carried out. Enzymatic oxidative stress in different organs was assessed and to support the above study histopathological evaluation has been done for vital organs.

PHF showed significant improvement in physiological parameters and significant reduction in weight of body and biochemical parameters in fructose induced diabetes. PHF also demonstrated significant improvement in oxidative levels which is supported by histopathological analysis, demonstrated that protective effect of PHF against diabetes, oxidative stress and its associated complications.

PHF showed protective effect in STZ and fructose induced diabetes model in rodents and its associated secondary complications and also showed potent antioxidant activity. Future work will be focused on isolation of bioactive guided molecules and molecular docking.

7 CONCLUSION

- In conclusion results suggest that PHF has beneficial role in controlling the FBG and weight of body restores, serum enzymes, prevent lipid peroxidation associated complications with STZ and fructose- induced experimental diabetic rats. The antioxidant property to prevent diabetes and its associated complications (as evidenced by histopathological findings) of PHF are the basis of its protective mechanism. Further results provided a pharmacological evidence of PHF as anti-diabetic and offering protection against few of the associated complications of DM in both the models testes. These may attribute to mainly presence of cephalandrine a, cephalandrine b, charantin, vicine, glycosides, karavilosides along with polypeptide-p, Ellagic acid and corosolic acid. These results are comparable with metformin a prototype of anti-diabetic drug. Histopathological changes in diabetic animals were increased due to PHF treatment, especially of β cell mass and its islets numbers. This attributed to various phytoconstituents having multiple targets operating in diabetes mellitus (DM). These results indicate PHF could be associated with decreases in oxidative stress and it is a potential free radical scavenger. Active constituent associated with its efficacy as antidiabetic drug and its likely mechanism of action. This PHF can be taken as the source for the development of new chemical entity for diabetes research. Our present investigation supports traditional use of all the 3 components of PHF (*Coccinia indica* W.&A, *Momordica charantia* Linn., *Lagerstroemia speciosa* Linn.) in treatment of DM and its associated complications.

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9 ANNEXURE

निस्केयर
NISCAIR

राष्ट्रीय विज्ञान संचार एवं सूचना स्रोत संस्थान
NATIONAL INSTITUTE OF SCIENCE COMMUNICATION AND INFORMATION RESOURCES
(वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद्)
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14, सत्संग विहार मार्ग, नई दिल्ली 110 067
14, SATSANG VIHAR MARG, NEW DELHI 110 067

Ref. NISCAIR/RHMD/Consult/2011-12/1879/179/03 October 9, 2011

Dr. H.B. Singh
Chief Scientist & Head
Raw Materials Herbarium & Museum
Phone: 25841143
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CERTIFICATE FOR CRUDE DRUG IDENTIFICATION

This is to certify that a crude drug sample No.MACF /11003 received as fruits of Momordica charantia from M/s Green Chem, Bangalore vide their letter No nil Dated 2nd November 2011. After macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with the authentic samples deposited in the RHMD, the sample was identified as fruits of *Momordica charantia* L.

Yours sincerely

(Dr. H. B. Singh)

Mr. Rajendran R
Green Chem
Lakshmi, 5 BDA, 2nd Stage, 3rd phase,
Domlur
BANGALORE-560 071

दूरभाष/Phone: डा. के. एस. कृष्णन् मार्ग/Dr. K. S. Krishnan Marg: 91-11-25846301, 25842303, 25846304-07, 25842990, 25840602, 25847544, 25847566 फैक्स/Fax: 91-11-25847062, 25849949
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निस्केयर
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CERTIFICATE FOR CRUDE DRUG IDENTIFICATION

This is to certify that a crude drug sample No.CCH /11010 received asleaves & fruits of Coccinia cordifolia syn. Coccinia indica from M/s Green Chem, Bangalore, vide their letter No nil Dated 8 August 2011. After macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with the authentic samples deposited in the RHMD, the sample was identified as fruits & leaves of *Coccinia grandis* (L.) Voigt syn. *Coccinia cordifolia* Cogn.

Yours sincerely

(Dr. H. B. Singh)

Mr. Rajendran R
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RAW MATERIAL HERBARIUM AND MUSEUM, DELHI (RHMD)

Ref.No. NISCAIR/RHMD/Consult/2016/ 2982-09-25 10/10/2016

CERTIFICATE FOR CRUDE DRUG SAMPLE AUTHENTICATION

This is to certify that the leaves sample of *Lagerstroemia speciosa*, BATCH No. LAG/002, received from M/S Green Chem, Bangalore vide their letter No. nil dated 16th Sept. 2016 for authentication has been found correct as dried leaves of ***Lagerstroemia speciosa* Pers.** which is commonly known as **Jarul**. The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD). Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD.

Identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic sample deposited in the Raw Materials Herbarium and Museum, Delhi (RHMD). Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD. This certificate is not issued for any judicial purpose.

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INSTITUTIONAL ANIMAL ETHICS COMMITTEE

This is to certify that the project proposal No. **01/PA/2015** entitled **Role of - nutraceuticals and their relevance in secondary complications of Diabetes mellitus using different animal models** has been approved by the IAEC.

Name of the Chairperson: **Prof [Dr] Purnima Ashok**

Name of the CPCSEA Nominee: **Dr Ramachandra SG**

Signature
7th November 2015
Chairperson, IAEC

Signature
7th July 2015
CPCSEA Nominee, IAEC

NOV

POSTER PRESENTATIONS AND PUBLICATIONS

POSTER PRESENTATIONS

1. Surana YS, Ashok P, Rajendran R. Antidiabetic Activity of Polyherbal Formulation in Streptozotocin- Nicotinamide Induced Type-2 Diabetes in Rats. Golden Jubilee International Conference of Indian Pharmacological Society, Southern Region, July 4-5, 2017. Puducherry

2 . Surana YS, Ashok P, Rajendran R. Evaluation of Polyherbal Formulation on Experimental Diabetic Neuropathy in Rodents. 69th IPC, December 22-24, 2017. Chandigarh.

PUBLICATIONS

1.Yuvraj Singh Surana, Purnima Ashok, Rajendran R. Evaluation of Antidiabetic, Hypolipidemic and Antioxidant Activity of Polyherbal Formulation in Streptozotocin-Nicotinamide Induced Diabetes in Rats. Int J Pharma Pharmaceutical Sci 2017: 9(10); 105-110.

2.Yuvraj Singh Surana, Purnima Ashok, Rajendran R and Krishna Rajendran. Role of Polyherbal Formulation in Secondary Complications Like Neuropathy and Retinopathy in Streptozotocin-Nicotinamide Induced Diabetes in Rats. The Pharma Innova J 2018; 7(10): 43-48.

CERTIFICATE

GOLDEN JUBILEE INTERNATIONAL CONFERENCE OF INDIAN PHARMACOLOGICAL SOCIETY

SOUTHERN REGION 2017

Theme: "Systems Pharmacology in Health Care"

(Sponsored by DBT, SERB, NAMS, MCI & ICMR)

This is to Certify that Dr. Mr. Mrs. Ms. Yuvraj Singh Surana Participated as Delegate at GOLDEN JUBILEE INTERNATIONAL CONFERENCE OF INDIAN PHARMACOLOGICAL SOCIETY - SOUTHERN REGION, Organized by the Department of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Puducherry held on 04th and 05th July 2017 and presented a Paper / Poster Titled

Antidiabetic activity of Polyherbal formulation in Streptozotocin - Nicotinamide induced Type-2 diabetes in rats.

This Conference has been Granted Four Credit Hours by Tamil Nadu Medical Council.

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CHANDIGARH
22nd - 24th December, 2017

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has presented a paper entitled EVALUATION OF POLYHERBAL FORMULATION ON EXPERIMENTAL DIABETIC NEUROPATHY IN RODENTS.

in the Scientific Poster Session of 69th IPC held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

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Original Article

EVALUATION OF ANTIDIABETIC, HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF POLYHERBAL FORMULATION IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETES IN RATS

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ABSTRACT

Objective: To evaluate antidiabetic, hypolipidemic and antioxidant activity of polyherbalformulation (PHF) aqueous extract in streptozotocin-nicotinamide induced diabetes in rats.

Methods: Fasting blood glucose, lipid profiles, serum insulin and glycosylate haemoglobin (HbA1C) were determined in normal and streptozotocin-nicotinamide induced diabetic rats after oral administration of the PHF for 45 d. Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) levels were evaluated in kidney and liver tissue. Histopathological changes in diabetic rat vital organs were also observed after PHF treatment.

Results: Daily oral administration of PHF (200 and 400 mg/kg, b.w.) and metformin (5 mg/kg, b.w.) showed beneficial effects on blood glucose level ($P<0.001$) and hyperlipidaemia due to diabetes. The PHF treatment also enhances serum insulin level and body weight of diabetic rats as compared to diabetic control group. Furthermore, the PHF has favourable effects on histopathological studies, in streptozotocin-nicotinamide induced diabetes. Antioxidant enzymes and GSH levels were found to be significantly increased and levels of MDA were decreased in treated diabetic animals.

Conclusion: PHF possesses antidiabetic, hypolipidemic and antioxidant properties. PHF has also showed favourable effect on histopathological changes in streptozotocin-nicotinamide induced diabetic animals.

Keywords: Streptozotocin, Nicotinamide, Metformin, Polyherbal formulation

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INTRODUCTION

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia with disturbance in carbohydrate, lipid and protein metabolism resulting from impaired insulin secretion or insulin action [1]. DM is a major challenge in worldwide healthcare systems and strongly associated with several major health risk factors [2]. It can be seen as burden of diabetes increasing worldwide and estimation suggest that their number will be 366 million by 2030. Chronic hyperglycaemia is consider as a major risk factor in the development of secondary complications like cardiopathy, nephropathy, retinopathy and neuropathy [3]. Four prominent theories have been proposed to explain that how hyperglycaemia might lead to chronic complications of DM and these are; advanced glycosylation end products (AGEs), increase glucose metabolism via sorbitol pathway, increase the formation of diacylglycerol leading to activation of protein kinase (PKC) and elevated levels of blood glucose increase the flux through the hexosamine pathway, which produces fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production [4]. In diabetes there is a state of increased free radical production, which results from an imbalance between the radical generating and radical scavenging systems leading to an increased production of reactive oxygen species (ROS) including superoxide radical (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (OH-). It may include not only the increased non-enzymatic and auto-oxidation glycosylation but also involved in metabolic stress which results from change in energy metabolism, levels of inflammatory mediators and the status of antioxidant defences [5]. In spite of the availabilities of insulin and oral hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major burden on world population [6]. Many Indian medicinal plants are reported to be useful in diabetes, which might provide new anti-diabetic drug and can counter the costlier and availability of present day drugs in the rural market [7]. India is a rich source of medicinal plants and in Ayurveda and Siddha system of medicines, number of plant extracts have been found to be useful to manage diabetes. The advantage of a traditional medicinal plant is fewer side effects with multiple therapeutic action due to the presence of different bioactive compounds. Following world health organisation (WHO) has recommended that the research on the beneficial uses of medicinal plants in the treatment of DM have also gained momentum [8]. Moreover diet and spice therapies become the major approaches recently for the management of diabetes; and a significant amount of work has been carried out with *Momordica charantia* Linn., *Coccinia indica* W. andA. and *Lagerstroemia speciosa* Linn. All of these herbs possess significant antidiabetic activity [9-11]; however their antioxidant activity in DM has not been thoroughly studied. In this view point, the present study has been taken up to determine the synergistic antidiabetic activity of the combination of these plants and compare its potential in streptozotocin-nicotinamide induced diabetic rats [12].

MATERIALS AND METHODS

Chemicals

Streptozotocin and nicotinamide (Sigma-Aldrich, USA), serum insulin kit (Mercodia, Swedan), HbA1C kit (Accurex Biomedical PVT. LTD. Maharashtra, India) and biochemical reagents for fasting blood sugar, lipid profile and for kidney function markers were purchased from Agapee diagnostics, India.

Animals

Healthy albino Wistar rats (200-250g) were procured from, a registered breeder. Animals were housed at institutes animal house facility in polypropylene cages and maintained under standard conditions (12 h light/dark cycles, 22±2 °C and 55±5% relative humidity). They were fed

with standard rat pellet diet and water ad libitum. The animals were kept in accordance with committee for the purpose of control and supervision of experimental animals (CPCSEA) guidelines for the care and use of laboratory animals. The study protocol was approved by institutional animal ethics committee (IAEC), KLE University's College of Pharmacy, Bengaluru (01/PA/2015).

Preparation of solutions

Test drug and metformin were dissolved in distilled water and administered orally for experimental purpose. All the drugs were freshly prepared each time before use.

Determination of acute oral toxicity

Acute oral toxicity of PHF was carried out according to organization for economic cooperation and development (OECD) guidelines 425 by using female albino Wistar rats (150-200g), which were maintained under standard conditions. Animals were kept under fasting 12 h prior to the experiment, water given ad libitum. Test drug was given to all animals in a single dose of 2000 mg/kg by using a stomach tube and all the animals were observed individually for signs of toxicity [13].

Animals observed for first four hours and thereafter for a total of 14 d.

Induction of diabetes and experimental design

Diabetes was induced in overnight fasted animals (deprived of food 16h but had been allow to free access to water) by a single intraperitoneal (i. p.) injection of streptozotocin (STZ) dissolved in citrate buffer (65 mg/kg, b.w.) 15 min after the i. p. administration of 110 mg/kg, b.w. of nicotinamide dissolved in normal saline. Hyperglycaemia was confirmed by elevated glucose levels in plasma, determined after 72 h injections of STZ. Animals with blood glucose concentration more than 200 mg/dl were used for the study. Diabetic animals were randomly divided into five groups containing twelve in each group. All groups receive STZ except normal control and the treatment protocol is as follows:

Group I–Normal control (saline treatment)

Group II–Positive control (STZ treatment)

Group III–Aqueous PHF (STZ+Dose I, 200 mg/kg, b.w.)

Group IV–Aqueous PHF (STZ+Dose II, 400 mg/kg, b.w.)

Group V–Standard group (STZ+Metformin, 5 mg/kg, b.w.)

The drugs were administered orally using an intragastric tube once daily for 45 d, continuously. Body weight of animals was measured throughout the experiment. At the end of the experiment, the animals were fasted overnight and blood collected for various biochemical estimations. The animals were then sacrificed (under the influence of overdosed isoflurane anaesthesia). The kidney, liver and pancreas were quickly excised, immediately rinsed in ice-cold saline; a portion of the organs were fixed in 10% neutral buffered formalin for histopathological study and the remaining portion were stored for further biochemical estimations.

Biochemical parameters

Fasting blood glucose analysis was done using a commercially available kit (Agapee Diagnostic, India). Serum insulin levels were measured by the Elisa kit from Mercodia, Swedan. Glycosylated haemoglobin (HbA1C %) was determined in EDTA-blood samples using a commercial assay kit (Accurex Biomedical PVT. LTD. Maharashtra, India). Blood samples were centrifuged at 7000 rpm for 15 min at 4• C to separate the serum. Serum creatinine, uric acid and urea levels measured using the respective assay kits (Agapee Diagnostic, India) using a semi-automatic biochemical analyzer (RMS Biochemical Analyzer, Chandigarh, India).

Lipid profiles

The serum total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride (TG) were measured using the respective assay kits (Agapee Diagnostic, India) using a semi-automatic biochemical analyzer. The fraction of very low-density lipoproteins (VLDL-C) and low-density lipoproteins (LDL-C) in the serum were calculated as follows-

VLDL =TG/5

LDL=Total cholesterol-TG/5-HDL

***In vivo* antioxidants**

A 10% (w/v) homogenate of liver and kidney were prepared by using Remi homogenizer at a speed of 10,000 rpm. The homogenized tissue preparation was used to measure the levels of antioxidant enzymes in liver and kidney tissues. GSH was estimated according the method described by Pompella *et al.*, CAT, SOD and lipid peroxidation (MDA content) were measured according to the method described by Sinha, Kakkar *et al.* and Ohkawa *et al.* [14-17].

Histopathological studies

At the end of the treatment, blood samples were collected (retro orbital plexus) from all the animals of different groups and then animals were sacrificed using mild anaesthesia (Isoflurane). The pancreas, kidney and liver tissues were collected and fixed in neutral formalin solution for 48 h, dehydrated by passing through graded series of alcohol embedded in paraffin blocks and 4 µm thick sections were prepared using a semi-automated rotator microtome.

Statistical analysis

All values are taken from mean±SEM. Graphpad prism version 5 was used for statistical analysis. Results of this study were compared by ANOVA, followed by Dunnett's Multiple Comparison Test.

RESULTS

Acute oral toxicity test

Acute oral toxicity study of PHF was done according to OECD guidelines for 425 and revealed the non-toxic nature of PHF at the limit test dose of 2000 mg/kg b.w. p. o till the end of the study.

Effect of PHF and metformin on body weight in STZ induced animals

The body weight of the diabetic rats showed a significant (P<0.001) decrease after the administration of STZ-nicotinamide. The treatment with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin shows mild reduction in b.w. compared with diabetic control rats [fig. 1].

Effect of PHF and metformin on blood glucose level, serum insulin level and glycosylated haemoglobin level in STZ induced animals

In the diabetic control rats, HbA1C level was significantly (P<0.001) increased when compared to normal control rats. Diabetic rats treated with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed significant (P<0.001) reduction in HbA1C levels as compared to diabetic control rats. In the diabetic control rats, fasting blood glucose level was significantly (P<0.001) increased when compared to normal control rats. Diabetic rats treated with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed significant (P<0.001) reduction in fasting blood glucose levels as compared to diabetic control rats. In addition, the positive control group animals showed significantly lower insulin levels. At the end of 45 d of treatment, there was a decrease in blood glucose treated with standard (metformin 5 mg/kg, b.w.) and PHF at two different doses (200 and 400 mg/kg, b.w.) showed 42.78%, 49.75% and 41.23% respectively decrease of glucose level [fig. 2] [table 1].

Effect of PHF on urine volume, serum creatinine, urea and uric acid levels in STZ induced diabetic animals

In the diabetic control group, urine volume was significantly (P<0.001) increased when compared to the normal control rats. When diabetic rats treated with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed significant (P<0.001) reduction in urine volume as compared to diabetic control rats. Streptozotocin-nicotinamide injection caused a marked reduction in renal function, as characterized by significant (P<0.001) increase in serum creatinine, urea, and uric acid levels as compared to normal control rats. Thus, these data suggest that a single i. p. injection of STZ-nicotinamide impairs kidney functions. Treatment with PHF

(Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed a significant (P<0.001) reduction in serum creatinine, uric acid and urea levels as compared to diabetic control rats [table 2].

Effect of PHF on TC, TG, HDL, VLDL and LDL levels in STZ induced diabetic animals

Diabetic control rats showed a significant (P<0.001) increase in the levels of triglycerides, cholesterol, VLDL, LDL and a decrease in HDL when compared with normal control group. The treatment of diabetic rats with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed (P<0.001) decrease in the levels of triglycerides, cholesterol, VLDL, LDL and increase in HDL when compared with diabetic control rats [table 3].

Effect of PHF on SOD, CAT, MDA, GSH levels in Kidney in STZ induced diabetic animals

The content of MDA, the end product of lipid peroxidation and a marker of oxidative stress was significantly (P<0.001) increased in renal tissue of diabetic control rats as compared to non-diabetic rats. There was a significant (P<0.001) decrease in the levels of GSH, an endogenous antioxidant and antiperoxidative enzymes (SOD and CAT) in renal tissue as compared to normal control group. The treatment of diabetic rats with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed a significant (P<0.001) decrease in the levels of MDA as compared to diabetic control rats and showed a significant (P<0.001) increase in SOD, CAT and GSH activities [table 4].

Effect of PHF on SOD, CAT, MDA and GSH levels in liver in STZ induced diabetic animals

The results showed that compared with rats in the normal control group, MDA levels in diabetic rats were significantly increased, while SOD, CAT and GSH activity was significantly decreased at the end of the study (P<0.001). The treatment of diabetic rats with PHF (Dose-I, 200 mg/kg, Dose-II, 400 mg/kg) and metformin showed a significant (P<0.001) decrease in the levels of MDA as compared to diabetic control rats and showed a significant (P<0.001) increase in SOD, CAT and GSH activities [table 4].

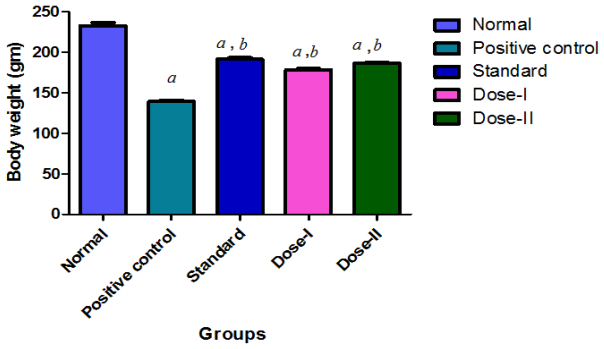

Fig. 1: Effect of PHF and metformin on body weight in STZ induced diabetic animals, values are expressed as mean±SEM, n=6, a= ***P<0.001when compared to normal control group, b= ***P<0.001when compared to positive control group

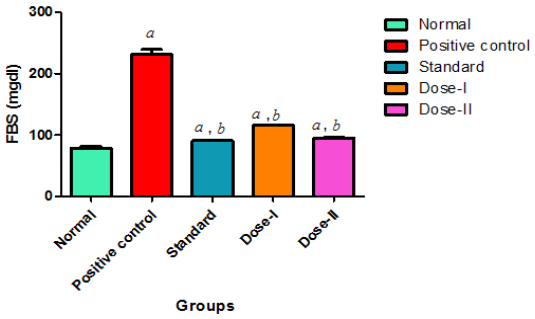

Fig. 2: Effect of PHF and metformin on fasting blood glucose levels in STZ induced diabetic animals, Values are expressed as mean±SEM, n=6, a= ***P<0.001when compared to normal control group, b= ***P<0.001when compared to positive control group

Table 1: Effect of PHF on serum insulin and HbA1c levels in STZ induced diabetic animals

Groups	Serum insulin (µU/ml)	HbA1c (mg%)
Normal	31.06±0.514	4.275±0.049
Positive control (STZ)	12.02±0.506***a	8.375±0.096***a
Standard Treatment (Metformin 5 mg/kg, b.w.)+STZ	26.72±0.381***b	6.105±0.080***b
Dose-I (200 mg/kg, b.w.)+STZ	21.22±0.125***b	7.325±0.056***b
Dose-II (400 mg/kg, b.w.)+STZ	25.17±0.319***b	6.613±0.064***b

Where, HbA1c-Glycosylate haemoglobin, values are expressed as mean±SEM, n=6. ***P<0.001, when compared to normal control group (a) and ***P<0.001, when compared to positive control group (b).

Table 2: Effect of PHF on serum creatinine, urea and uric acid levels in STZ induced diabetic animals

Groups	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)
Normal	0.465±0.921	23.11±0.539	1.715±0.015
Positive control (STZ)	1.917±0.092***a	58.47±0.521***a	4.108±0.021***a
Standard treatment (Metformin 5 mg/kg, b.w.)+STZ	0.870±0.023***b	44.72±0.644***b	2.427±0.022***b
Dose-I (200 mg/kg, b.w.)+STZ	1.357±0.046***b	47.26±0.293***b	3.277±0.018***b
Dose-II (400 mg/kg, b.w.)+STZ	1.247±0.061***b	37.56±0.389***b	2.735±0.012***b

Values are expressed as mean±SEM, n=6. ***P<0.001, when compared to normal control group (a) and ***P<0.001, when compared to positive control group (b).

Table 3: Effect of PHF on TC, TG, HDL, VLDL and LDL levels in STZ induced diabetic animals

Groups	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
Normal	91.66±1.457	111.90±2.098	35.79±0.337	22.37±0.419	33.68±1.224
Positive control (STZ)	182.20±1.572***a	182.60±1.629***a	25.78±0.859***a	36.52±0.325***a	119.90±1.918***a
Standard (Metformin 5 mg/kg, b.w.) +STZ	122.80±1.771***b	130.40±1.185***b	33.02±0.485***b	26.07±0.236***b	63.74±2.066***b
Dose-I (200 mg/kg, b.w.)+STZ	147.10±1.389***b	155.10±0.853***b	29.76±0.400***b	31.02±0.170***b	86.33±1.404***b
Dose-II (400 mg/kg, b.w.)+STZ	132.40±0.779***b	142.90±0.923***b	31.93±0.366***b	28.57±0.184***b	71.93±1.014***b

Where, TC-total cholesterol, TG-triglyceride, HDL-high density lipoprotein, VLDL-very low-density lipoprotein, LDL-low density lipoprotein, values are expressed as mean±SEM, n=6. ***P<0.001, when compared to normal control group (a) and ***P<0.001, when compared to positive control group (b).

Table 4: Effect of PHF on SOD, CAT, MDA, GSH levels in kidney and Liver in STZ induced diabetic animals

Groups	Organ	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/mg protein)	GSH (nmoles/mg protein)
Normal	Kidney	9.73±0.248	55.15±0.481	1.13±0.050	16.97±0.103
	Liver	8.77±0.167	59.00±0.107	1.27±0.113	38.93±0.173
Positive control (STZ)	Kidney	3.03±0.075***a	25.40±1.201***a	6.17±0.075***a	9.08±0.190***a
	Liver	1.12±0.052***a	26.70±0.114***a	14.25±0.294***a	17.98±0.234***a
Standard (Metformin 5 mg/kg, b.w.)+STZ	Kidney	6.21±0.127***b	48.58±0.427***b	2.04±0.051***b	14.21±0.196***b
	Liver	7.66±0.149***b	54.64±0.218***b	2.51±0.127***b	37.01±0.346***b
Dose-I (200 mg/kg, b.w.)+STZ	Kidney	4.95±0.048***b	42.77±0.431***b	2.97±0.071***b	12.00±0.077***b
	Liver	4.54±0.145***b	45.98±0.166***b	4.06±0.093***b	33.11±0.179***b
Dose-II (400 mg/kg, b.w.)+STZ	Kidney	5.25±0.039***b	46.01±0.428***b	2.19±0.034***b	13.18±0.115***b
	Liver	6.91±0.082***b	53.07±0.280***b	3.02±0.058***b	35.44±0.160***b

Where, SOD-superoxide dismutase, CAT-catalase, MDA-malondialdehyde, GSH-glutathione, values are expressed as mean±SEM, n=6. ***P<0.001, when compared to normal control group (a) and ***P<0.001, when compared to positive control group (b).

Histopathological studies of kidney, liver and pancreas

The tubules of the kidney showed some degenerative changes and few blood vessels were congested with diabetic control rats as compared to normal structural features of a control animal. In the normal control group, the histopathological examination of kidney tissue showed the normal appearance of glomeruli and tubules (long arrow). Treatment with Dose-I 200 mg/kg (PHF) showed some degenerative changes. However, treatment with Dose-II 400 mg/kg and metformin showed intact architecture as compared to normal group [fig. 3].

Section studied in positive control rats shows the liver parenchyma having partially distorted architecture and there are seen focal areas of necrosis (short arrow) with congested blood vessels as compared to normal control group {[shows intact architecture] (long arrow)}. Treatment with PHF (Dose-II, 400 mg/kg) shows the liver parenchyma having intact architecture and the perivenular, periportal and midzonal hepatocytes appear unremarkable. The central veins and sinusoids appear unremarkable as compared to positive control group [fig. 4].

Section studied shows in positive control rat that pancreatic lobules separated by connective tissue septa. The centre of islet cells consists of a quantitative decrease in small β-cells (long arrow) (30%, compared to normal control, 70%), while the periphery comprises of large α-cells (short arrow) (65%, compared to normal control, 25%). Treatment with PHF (Dose-II, 400 mg/kg) shows regeneration of β-cells (70%, compared to positive control group 30%), while the periphery comprises of large α-cells (25%, compared to positive control group 65%) [fig. 5].

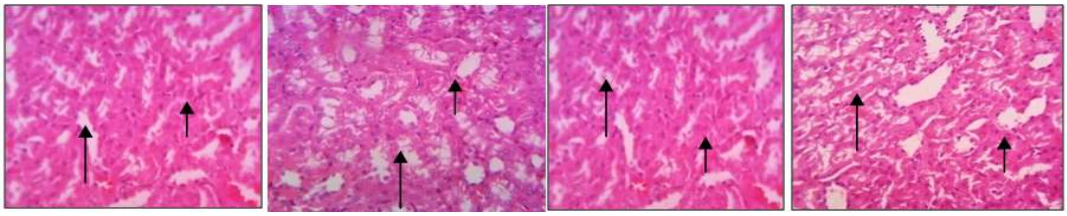

Fig. 3: Histopathology of kidney tissues from rats. (a) Normal control group, (b) Positive control group, (c) Standard (d) Dose-II 400 mg/kg (H and E 400×), Where, H and E-Hematoxylin and eosin

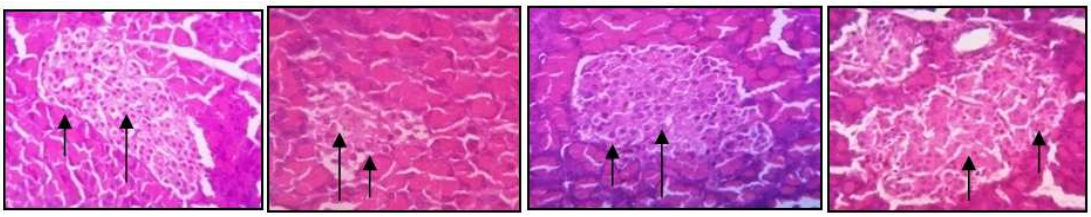

Fig. 4: Histopathology of pancreas from rats. (a) Normal control group, (b) Positive control group, (c) Standard (d) Dose-II 400 mg/kg (HandE 400×), Where, H and E-Hematoxylin and eosin

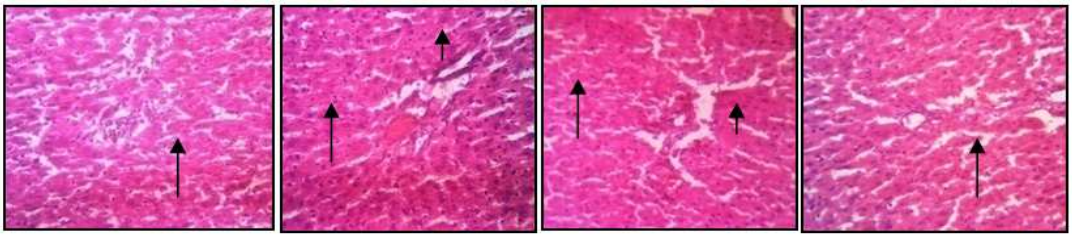

Fig. 5: Histopathology of liver from rats. (a) Normal control group, (b) Positive control group, (c) Standard (d) Dose-II 400 mg/kg (H and E 400×), Where, H and E-Hematoxylin and eosin

DISCUSSION

DM is a long-term disorder characterized by elevated blood glucose level due to absolute or relative insulin deficiency [18]. The present study was undertaken to evaluate antidiabetic, hypolipidemic and antioxidant activities of PHF in normal rats, STZ-induced untreated rats and treated diabetes rats with PHF and metformin.

STZ-induced hyperglycemia is a widely applied experimental model because of the ability of STZ to selectively target and destroy insulin-producing pancreatic islet β-cells. The intraperitoneal administration of STZ (65 mg/kg) partially damages the insulin-secreting pancreatic β-cells by breaking the DNA strand, which results in increased blood glucose levels and decreased in endogenous insulin release. Oral administration of PHF (200 and 400 mg/kg) resulted in significant reduction in fasting blood glucose levels. The increased serum insulin levels in PHF treated STZ-diabetic rats could be due to the protection of function β-cells from further deterioration. Increased levels of insulin might help in improving glycemic control in STZ-diabetic rats.

In our study, the body weight of STZ-induced untreated diabetic group showed a significant decrease. Oral administration of PHF at a dose of 400 mg/kg for 45 d showed an improvement in body weight in comparison to diabetic control and rats treated with metformin. The higher body weight of PHF treated rats might be due to their improved glycemic control.

HbA1C levels are monitors as a consistent index of glycemic control in diabetes [19]. Administration of PHF results in decreased fasting blood glucose levels, further leading to significant reduction in HbA1C levels in diabetic rats.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [20]. The excess of fatty acids in the plasma may support the hepatic conversion of fatty acids into phospholipids and cholesterol. These changes may usually lead to secondary complications of diabetes such as atherosclerosis and increased coronary heart disease. Our results indicate lipid profiles viz cholesterol, triglyceride, VLDL and LDL were reduced significantly by PHF administration. HDL plays a key role in protecting against heart disease because of its role in the transportation of excess cholesterol out of the body and is known as "good cholesterol". In the present study, PHF significantly increased the HDL level in treated diabetic animals.

Oxidative stress plays a crucial role in the development of hyperglycaemia, which generates reactive oxygen species (ROS) causing cellular injury and several deleterious effects on cellular physiology and these have a key role in the development of secondary complications of diabetes. An elevated level of MDA in diabetic put forward for consideration that peroxide injury may be involved in diabetic complications. In the present study, a marked increase in the levels of tissue malondialdehyde (MDA) content in STZ-diabetic rats leading to tissue injury and failure of antioxidant defence mechanism. The diabetic rats treated with PHF significantly decreased the levels of MDA in kidney and liver [21]. Several studies indicated that there is a generation of oxygen free radicals in STZ-treated β-cells and that the overexpression of antioxidant enzymes, such as SOD, CAT. Reduced activities of SOD and Cat in liver and kidney have been observed during diabetes. SOD is vital defence enzyme which catalyses the dismutation of superoxide radicals. CAT is a heme protein which catalyses the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [22]. Low activity of catalase which has been reported with schizophrenia and atherosclerosis [23] are the same way with the assumption that long-term oxidative stress may lead to development to type 2 diabetes. Our results indicate that treatment with PHF significantly increased the SOD and CAT levels in kidney and liver. PHF treated diabetic rats also significantly increase the levels of GSH in vital organs of rats.

Histopathology studies of the pancreas showed that degeneration of β-cells of the islets with a reduction in mass of islet cells in positive control rat. Groups treated with PHF showed regeneration of β-cells and increase in mass of islets as compared to positive control rats. Metformin (5 mg/kg) treated diabetic rats also showed regeneration of β-cells. Histopathology of kidney revealed that PHF significantly enhanced the normal appearance of glomeruli and tubules and also showed intact architecture when compare to positive control rats which showed degenerative changes and few blood vessels congestion.

Histopathology studies of liver also revealed the protective effect of PHF. Treatment with PHF showed the intact architecture of liver parenchyma and the perivenular, periportal and midzonal hepatocytes. The central veins and sinusoids appear unremarkably normal compared to the positive control group which showed the liver parenchyma partially distorted.

CONCLUSION

In conclusion, data from the present study states that the PHF has potent antidiabetic, hypolipidemic and antioxidant activities. Biochemical and histopathological results of study also revealed the degree of protection offered by PHF to diabetic animals. Further studies are required for bioactivity guided drug discovery to isolate lead compounds, which may be responsible for these claimed activities.

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AUTHOR CONTRIBUTION

Dr. Purnima Ashok-The present work was initiated by the author.

Yuvraj Singh Surana-Author has contributed the major experiment part.

Rajendran R.-Author has helped in the statistical analysis.

CONFLICT OF INTERESTS

The authors state that they have no conflicts of interest.

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Role of Polyherbal formulation in secondary complications like neuropathy and retinopathy in streptozotocin-nicotinamide induced diabetes in rats

Yuvraj Singh Surana, Purnima Ashok, Rajendran R and Krishna Rajendran

Abstract
Objective: To evaluate neuropathy, retinopathy and antioxidant properties of a polyherbal formulation (PHF) aqueous extract in Streptozotocin- nicotinamide induced diabetic rats.
Methods: Fasting blood glucose (FBG), serum insulin and glycated haemoglobin (HbA1C) levels were determined in normal and Streptozotocin- nicotinamide induced diabetic rats after oral administration of the PHF for 45 days. Neuropathic analgesia was assessed by tail-flick and hot-plate methods and to assess retinopathy, increase levels of vascular endothelial growth factor (VEGF) and intracellular adhesion molecule (ICAM-1) were measured. Antioxidant property was evaluated by estimating superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels in sciatic nerve and retina. Histopathological changes in sciatic nerve and retina were also observed after PHF treatment.
Results: Daily oral administration of PHF (200 and 400 mg/kg, b.w.) and metformin (5mg/kg, b.w.) showed beneficial effect on blood glucose levels ($P < 0.001$) of diabetic animals. The PHF treatment enhances serum insulin levels and body weight of diabetic rats as compared to diabetic control group. PHF treated animals showed decrease in tail immersion latency time, increase in pain sensitivity and significant decrease in levels of VEGF and ICAM-1, when compared to diabetic group. Furthermore, the PHF has a favourable effect on histopathological studies, in Streptozotocin- nicotinamide induced diabetic animals. Antioxidant enzyme levels were found to be significantly increased and that of MDA were decreased in PHF treated diabetic animals.
Conclusion: PHF showed potent anti-diabetic and protection against associated secondary complications of diabetes like neuropathy and retinopathy and also possesses antioxidant properties. Histopathological studies support these claims of PHF in Streptozotocin- nicotinamide induced diabetic animals.

Keywords: Streptozotocin, Nicotinamide, Metformin, Polyherbal formulation

1. Introduction
Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production [1]. The worldwide prevalence of DM has risen dramatically over the past two decades. The prevalence of type 2 DM is expected to rise more rapidly in future because of increasing obesity and reduce activity levels [2].
The chronic complications of DM effect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Neuropathy and retinopathy are common complications of DM and are related to duration and severity of hyperglycaemia [3, 4].
Usually in neuropathy it will take two or more than two decades for the appearance of symptoms in 50% of the affected population. Diabetic neuropathy affects all peripheral nerves including pain fibers, motor neurons, and the autonomic nervous system [5].
More than 60% of Type 2 DM patients develop retinopathy after 20 years. It progresses from nonproliferative abnormalities to proliferative diabetic retinopathy and is characterized by retinal edema, haemorrhage, increased neovascularisation and neuronal degeneration in the retina. The major factors responsible for the development of diabetic retinopathy are hyperglycaemia and poor diabetic control. Apart from hyperglycaemia, other factors which are responsible for the development of diabetic retinopathy are increased activity of aldose reductase (AR) and protein kinase C (PKC), as well as promoting nonenzymatic glycation and glycooxidation of proteins like advanced glycation end products (AGEs).

It has been reported that the up-regulation of proinflammatory factors and angiogenic parameters, such as tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1) and interleukin-1β (IL-1β), contribute to the blood–retinal barrier (BRB) breakdown, which directly leads to macular edema in diabetic retinopathy (DR) [6].

Ethno-botanical information reports around more than 800 plants that may possess antidiabetic activity when assessed using the presently available experimental techniques [7]. India is a rich source of medicinal plants and in Ayurveda and Siddha system of medicines, number of plant extracts are available to manage diabetes. The advantage of traditional medicinal plant is fewer side effects with multiple therapeutic actions due to presence of different bioactive compounds. World Health Organisation (WHO) has also recommended that the research on the beneficial uses of medicinal plants in the treatment of DM have also gained momentum [8]. Moreover diet and spice therapies become the major approaches recently for the management of diabetes. A significant amount of work has been carried out with *Momordica charantia* Linn [9], *Coccinia indica* W. & A [10]. and *Lagerstroemia speciosa* Linn [11]. and all these herbs individually possess significant antidiabetic activity. This is an attempt to expose the possibility of these herbs in combination as antidiabetic and also explore their protective effect in secondary complications of DM in Streptozotocin-nicotinamide induced diabetic animals.

2. Materials and Methods

2.1. Chemicals

Streptozotocin and nicotinamide (Sigma-Aldrich, USA), serum insulin kit (Mercodia, Swedan), HbA1C kit (Accurex Biomedical PVT. LTD. Maharasthra, India) Intracellular adhesion molecule-1 Elisa kit (YH Biosearch laboratory, Shanghai, China), Vascular endothelial growth factor Elisa kit (RayBio, USA) and biochemical reagent for Fasting blood sugar Agapee diagnostics, India, were purchased.

2.2. Animals

Healthy albino Wistar rats of approximately same age group (200-250 g) were procured from, a registered breeder. Animals were housed at Institutes animal house facility in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, 22 ± 2^0 C and 55 ± 5% relative humidity). They were fed with standard rat pellet diet and water *ad libitum*. The animals were maintained in accordance with Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) guidelines for the care and use of laboratory animals. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), KLE University's College of Pharmacy, Bengaluru (01/PA/2015).

2.3. Preparation of solutions

Test drug and metformin were dissolved in distilled water and administered orally for experimental purpose. All the test drugs were freshly prepared each time before use.

2.4. Determination of acute oral toxicity [12]

The acute oral toxicity of Polyherbal formulation (PHF) was carried out according to Organization of Economic Cooperation and Development (OECD) guidelines 425 by using female albino Wistar rats (150-200g), which were maintained under standard conditions. Animals were kept under fasting 12 h prior to the experiment, water given *ad libitum*. Test drug was administered to all animals in a single dose of 2000 mg/kg by using a stomach tube and all the animals were observed individually for signs of toxicity.

Animals observed for first four hours and thereafter for total of 14 days.

2.5. Induction of diabetes and experimental design [13]

Diabetes was induced in overnight fasted animals (deprived of food 16 h but had been allow to free access to water) by a single intraperitoneal (i.p.) injection of freshly prepared Streptozotocin (STZ) dissolved in citrate buffer (65 mg/kg, b.w.) 15 min after the i.p. administration of 110 mg/kg, b.w. of nicotinamide (NA) dissolved in normal saline. Hyperglycaemia was confirmed by elevated glucose levels in plasma, determined after 72 h injection of STZ. Animals with blood glucose concentration more than 200 mg/dL were used for the study. The diabetic animals were randomly divided into five groups containing twelve in each group. All groups receive STZ - Nicotinamide except normal control and the treatment protocol is as follows:

Group I – Normal control (saline treatment)
Group II – Positive control (STZ -Nicotinamide treatment)
Group III – Aqueous PHF (STZ -Nicotinamide + Dose I, 200 mg/kg, b.w.)
Group IV – Aqueous PHF (STZ -Nicotinamide + Dose II, 400 mg/kg, b.w.)
Group V – Standard group (STZ -Nicotinamide + Metformin, 5mg/ kg, b.w.)

The test drugs were administered orally using an intragastric tube once daily for 45 days, continuously. Body weight of animals was measured throughout the experiment. After 2 h of Standard and PHF treatment on the last day, the animals were subjected to Eddy's hot-plate test and tail-flick method to assess the development of neuropathy [14]. At the end of the experiment, animals were fasted overnight and blood collected by retro-orbital puncture under light anaesthesia for various biochemical estimations.

The animals were then sacrificed (under the influence of overdosed isoflurane anaesthesia), after that rat eyes were collected and the left one was used for the measurements of vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1 (ICAM-1) using respective ELISA kits [6]. The sciatic nerve and retina were quickly excised, immediately rinsed in ice cold saline; a portion of the nerve was fixed in 10% neutral buffered formalin for histopathological study and remaining portion was stored for further biochemical estimations.

Estimation of Biomarkers of Oxidative Stress in Nerve: Sciatic nerve was placed in 10% w/v potassium chloride (KCl) solution, homogenize and centrifuge at 5000 rpm for 10 min. The supernatant obtain was used for the following assay:

- SOD [15]
- CAT [16]
- MDA [17]

Measurements of SOD and MDA levels in Retina: At the end of the experiment, left eye was collected and from that retina was isolate and centrifuge at a speed of 3,500 rpm for 10 min, the supernatant obtain was used for the assay of SOD and MDA.

3. Results

Acute oral toxicity study

Acute oral toxicity study of polyherbal formulation (PHF) was performed as per the OECD guideline 425 and it showed the non-toxic nature of PHF at the limit test dose of 2000 mg/kg, b.w.p.o.

Effect of PHF and metformin on body weight, blood glucose level, serum insulin level and glycated haemoglobin level in STZ-nicotinamide induced diabetic animals

The body weight of the diabetic rats showed a significant ($P < 0.001$) decrease after the administration of STZ-nicotinamide. The treatment with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed mild reduction in the body weight as compared with diabetic control rats.

In the diabetic control rats, FBS level was significantly ($P < 0.001$) increased when compared to normal control rats. The diabetic rats treated with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed more significant ($P < 0.001$) reduction in FBS levels as compared to diabetic control rats. The STZ-nicotinamide injection significantly decreases insulin levels.

In the diabetic control rats, HbA1C level was significantly ($P < 0.001$) increased when compared to normal control group. The diabetic rats treated with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed significant ($P < 0.001$) reduction in HbA1C levels as compared to diabetic control rats [18].

Effect of PHF on latency period in Hot-plate and Tail-flick methods in STZ-nicotinamide induced diabetic animals

Diabetic control rats showed a statistically significant ($P < 0.001$) increase in the tail-flick latency time and significant ($P < 0.001$) decrease in the response time with hot-plate method when compared with normal control group. The treatment of diabetic rats with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed a statistically significant ($P < 0.001$) increase in the tail-flick latency time and significant ($P < 0.001$) decrease in the response time with hot-plate method when compared with the positive control group [Table-1].

Table 1: Effect of PHF on latency period in Hot-plate and Tail-flick methods in STZ- nicotinamide induced diabetic animals

Groups	Hot-Plate Method (sec.)	Tail-Flick Method (sec.)
Normal	0.61±0.036	4.99±0.066
Positive control (STZ- Nicotinamide)	3.93±0.109***a	8.25±0.088***a
Standard (Metformin 5mg/kg, b.w.)+ STZ Nicotinamide-	1.11±0.048 ***b	5.95±0.052 ***b
Dose-I (200 mg/kg, b.w.)+STZ -Nicotinamide	2.07±0.034 ***b	7.17±0.039 ***b
Dose-II (400 mg/kg, b.w.)+STZ- Nicotinamide	1.56±0.071 ***b	6.12±0.048 ***b

Values are expressed as mean± SEM, n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Effect of PHF on SOD, CAT and MDA levels in sciatic nerve in STZ-nicotinamide induced diabetic animals

The content of MDA, end product of lipid peroxidation and marker of oxidative stress was significantly ($P < 0.001$) increased in sciatic nerve of diabetic control rats as compared to non-diabetic rats. There was a significant ($P < 0.001$) decrease in the levels of anti-peroxidative enzymes (SOD and CAT) in sciatic nerve as compared to normal control group (Fig.1). The treatment of diabetic rats with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed a significant ($P < 0.001$) decrease in the levels of MDA as compared to diabetic control rats (Fig.2) and showed a significant ($P < 0.001$) increase in SOD and CAT activities.

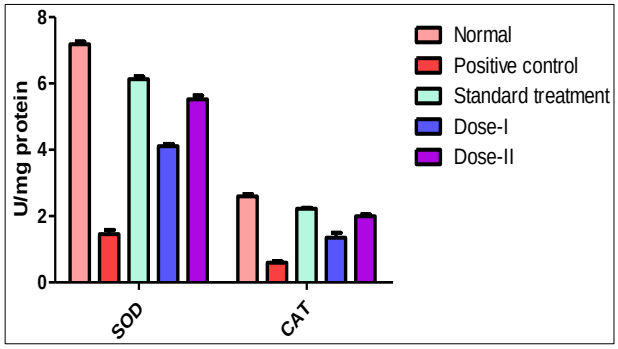

Fig 1: Effect of PHF on SOD and CAT levels in sciatic nerve in STZ-nicotinamide induced diabetic animals

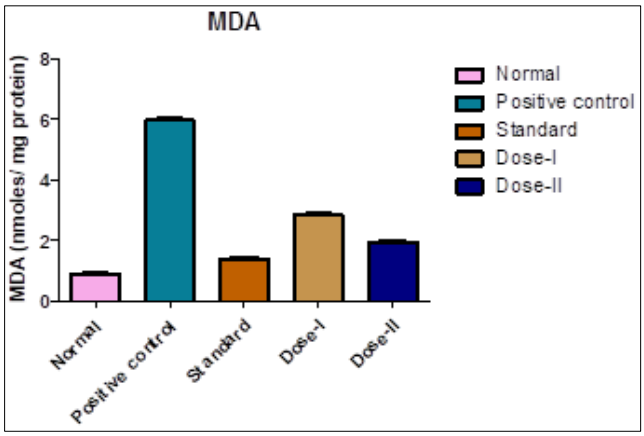

Fig 2: Effect of PHF on MDA levels in sciatic nerve in STZ-nicotinamide induced diabetic animals

Effect of PHF on retinal VEGF and ICAM-1 Levels in STZ-nicotinamide induced diabetic animals

Significantly increased levels of VEGF and ICAM-1 were observed in retina of diabetic rats at the end of the study. Treatment with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin significantly reduced the levels of retinal VEGF and ICAM-1 compared with diabetic control group ($p < 0.001$) and the effect of PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) was comparable to that of standard metformin group [Table-2].

Table 2: Effect of PHF on retinal VEGF and ICAM-1 levels in STZ-nicotinamide induced diabetic animals

Groups	ICAM-1 (concentration in ng/ml)	VEGF (concentration in ng/ml)
Normal	4.45±0.168	12.18±0.216
Positive control (STZ)	10.23±0.077***[a]	28.99±0.365***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ -Nicotinamide	5.39±0.080***[b]	12.29±0.081***[b]
Dose-I (200 mg/kg, b.w.)+STZ- Nicotinamide	7.57±0.056***[b]	20.77±0.283***[b]
Dose-II (400 mg/kg, b.w.)+STZ- Nicotinamide	5.85±0.043***[b]	15.74±0.239***[b]

Values are expressed as mean± SEM,n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Effect of PHF on SOD and MDA levels in retina in STZ-nicotinamide induced diabetic animals

The results showed that compared with rats in the normal control group, MDA levels in diabetic rats were significantly increased, while SOD activity was significantly decreased at the end of the study ($p < 0.001$). The treatment of diabetic rats with PHF (Dose-I 200 mg/kg, Dose-II 400 mg/kg) and metformin showed a significant ($P < 0.001$) decrease in the levels of MDA as compared to diabetic control rats and showed a significant ($P < 0.001$) increase in SOD activities [Table-3].

Table 3: Effect of PHF on SOD and MDA levels in retina in STZ-nicotinamide induced diabetic animals

Groups	SOD (U/mg protein)	MDA (nmoles/mg protein)
Normal	41.83±0.891	0.49±0.023
Positive control (STZ- Nicotinamide)	24.14±0.420***[a]	5.37±0.115***[a]
Standard (Metformin 5mg/kg, b.w.) + STZ -Nicotinamide	34.86±0.281***[b]	1.16±0.045***[b]
Dose-I (200 mg/kg, b.w.)+STZ- Nicotinamide	28.68±0.298***[b]	3.14±0.056***[b]
Dose-II (400 mg/kg, b.w.)+STZ- Nicotinamide	33.21±0.206***[b]	1.58±0.075***[b]

Values are expressed as mean± SEM, n=6. ***p<0.001, when compared to normal control group (a) and ***p<0.001, when compared to positive control group (b).

Histopathological studies of sciatic nerve and retina

Histopathological analysis showed small myelinated fiber loss which was more prominent than large diameter fiber loss. Endoneurial vessel was also not thickened with diabetic control rats as compared to normal control group. However, the treatment with Dose-II 400 mg/kg showed intact myelinated fiber (arrow) density, the small myelinated fiber and large diameter fiber appear intact and the endoneurial vessel was also not thickened as compare to normal control group [Fig-3].

Section studied in positive control rat shows extensive vacuolations in the plexiform layers and ganglion layer (arrow) as compare to normal control group (without any vacuolations in the plexiform layers and ganglion layer). Treatment with PHF (Dose-II 400 mg/kg) showed moderately reduced vacuolations [compared to positive control] in the plexiform layers and ganglion layer [Fig-4].

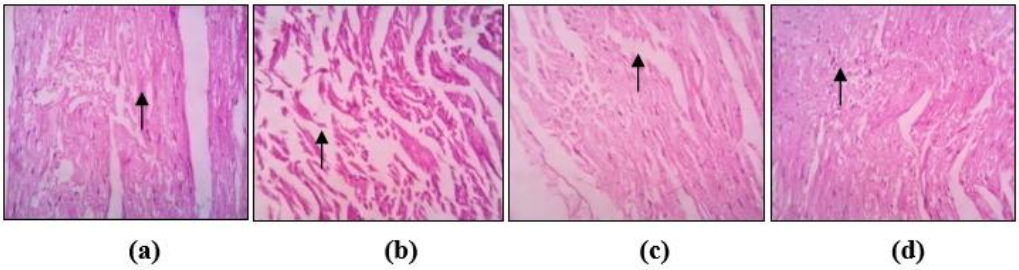

Fig 3: Histopathology of sciatic nerve from rats. (a) Normal control group, (b) Positive control group, (c) Standard (d) Dose-II 400 mg/kg (H&E 400×)

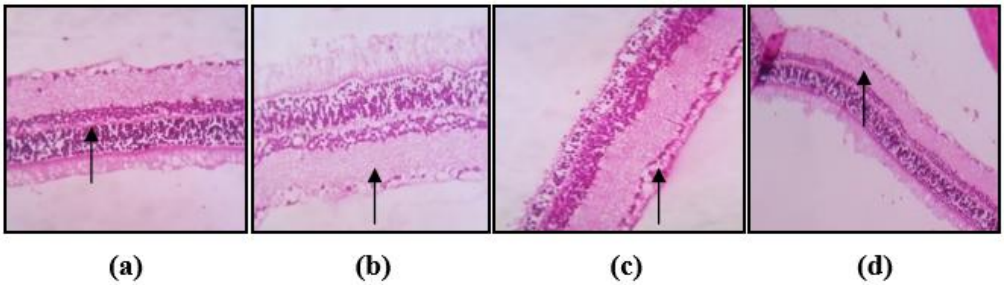

Fig 4: Histopathology of retina from rats. (a) Normal control group, (b) Positive control group, (c) Standard (d) Dose-II 400 mg/kg (H&E 400×)

4. Discussion

This study explored the protective effect of PHF on Streptozotocin-nicotinamide induced diabetes in Wistar albino rats.

STZ is a broad-spectrum antibiotic that is toxic to insulin-producing pancreatic islet β-cells, and it is a widely used experimental model to induce hyperglycemia. The intraperitoneal administration of STZ (65 mg/kg) partially

damage the insulin secreting pancreatic β-cells by breaking the DNA strand, which results in increased blood glucose levels and decreased in endogenous insulin release [19]. Oral administration of PHF for 45 days (200 and 400 mg/kg) resulted in significant reduction in fasting blood glucose levels. The increased serum insulin levels in PHF treated STZ- nicotinamide diabetic rats could be due to protection of functional β-cells from further deterioration. Increased levels of insulin might help in improving glycemic control in STZ-diabetic rats.

In this study, the body weight of STZ-nicotinamide induced untreated diabetic group showed significant decrease in body weight. Per Oral administration of PHF at dose of 400 mg/kg for 45 days showed an improvement in body weight in comparison to diabetic control and rats treated with metformin. The increased in body weight of PHF treated rats might be due to their improved glycemic control.

HbA1C levels are monitored as a consistent index of glycemic control in diabetes [20]. In this study administration of PHF decreased fasting blood glucose levels, further leading to significant reduction in HbA1C levels in diabetic rats.

The pain perception is significantly low in diabetic animals when compared to normal control animals may be due to nerve damage and induction of peripheral neuropathy [21, 22]. The PHF untreated animals showed a significant decrease in paw withdrawal magnitude, which indicates the development of hyperalgesia. This study revealed that treatment with PHF decreases the neuropathic pain in animals.

There are various theories of diabetic retinopathy have been proposed, increased oxidative stress induced by hyperglycemia seems to be the one of the mechanism of diabetic complications, which can lead to activate the polyol pathway, increase AGE formation, activate PKC and hexosamine pathways and all leading to the development of diabetic retinopathy (DR). Among all the cytokines involved in DR, VEGF, has been identified as a primary initiator of proliferative DR and as a potential mediator of nonproliferative retinopathy [23]. In the retina, VEGF can induce ICAM-1 expression and leucocyte adhesion, which together with VEGF lead to the BRB breakdown, and it has also been reported that retinal VEGF and ICAM-1 levels are strongly correlated with neovascularization in patients with DR [24]. In our present study, significantly increased levels of VEGF and ICAM-1 were observed in retina of diabetic rats at the end of the study. Treatment with PHF significantly reduced the levels of retinal VEGF and ICAM-1 compared with diabetic control group and this effect of PHF was comparable to that of standard metformin.

Oxidative stress plays an important role in the development of hyperglycaemia, which may result in generation of reactive oxygen species (ROS) causing cellular injury and several deleterious effects on the cellular physiology and these have important role in the development of secondary complications associated with diabetes. Increased level of MDA in diabetic put forward for consideration that peroxide injury may be involved in the diabetic complications.

In the present study, a significant increase in the levels of tissue malondialdehyde (MDA) content in STZ- nicotinamide induced diabetic rats leading to tissue injury and failure of antioxidant defence mechanism has been observed. The diabetic rats treated with PHF significantly decreased the levels of MDA in sciatic nerve and retina. Several studies showed that there is generation of oxygen free radicals in STZ-treated β-cells, and that the over expression of antioxidant enzymes, such as SOD, CAT [25]. Reduced activities of SOD and CAT in sciatic nerve and retina have been observed during diabetes. SOD is vital defence enzyme which catalyses the dismutation of superoxide radicals. CAT is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals [26]. In schizophrenia and atherosclerosis low activity of catalase has been reported [27], with same assumption that long-term oxidative stress may lead to development of type 2 diabetes mellitus. Our results correlates with these findings as treatment with PHF significantly increased the SOD, CAT levels in kidney and liver and also significantly increased the levels of GSH in vital organs of diabetic rats which is of vital importance in the treatment of DM.

Histopathological study of sciatic nerve showed that a degenerative change like small myelinated fiber loss was more prominent than large diameter fiber loss and endoneurial vessel was also not thickened with diabetic control rats as compared to normal control group. Treatment with PHF showed intact myelinated fiber density, the small myelinated fiber and large diameter fiber appear intact which reveals the protective effect of PHF in secondary complications DM.

Histopathological study of sciatic nerve also showed protection from extensive vacuolations in the plexiform layers and ganglion layer when treated with PHF, which can be considered as a promising effect. This lead to state that may be PHF can be considered in the treatment of secondary complications of diabetes mellitus specially in neuropathy and retinopathic complications.

5. Conclusion

In conclusion, data from the present study states that the PHF has potent antidiabetic activity and also shown protective effect in neuropathy and retinopathy, secondary complications associated with DM. The biochemical and histopathological results of present study also revealed the degree of protection offered by PHF to diabetic animals. Further studies are required for bioactivity guided drug discovery to isolate lead compounds, which may be responsible for these claimed activities.

6. Conflict of interest

The authors declare that they have no conflicts of interest.

7. Acknowledgements

The authors are thankful to Green Chem Herbal Extracts and Formulations, Domlur, Bangalore.

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