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A Polyherbal extract treats Streptozotocin induced Diabetes mellitus: Pharmacognostic study, HPLC analysis and *in vivo* antidiabetic activity

Research Article

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Abstract

The plants used in study have been used traditionally for various purposes skin problems, diabetes, astringent, anti-inflammatory, antioxidant, hepatoprotective activities, anthelmintic, antipyretic, antidysentric, anti-hyperlipidemic, anticancer properties. The present study involves the evaluation of pharmacognostic parameters of the four herbal drugs (*Azadirachta indica* leaves, *Pterocarpus marsupium* heartwood, *Picrorhiza kurroa* rhizomes, and *Withania coagulans* berries and fruit coat), High performance Liquid chromatography (HPLC) as well as antidiabetic activity of polyherbal extract. The qualitative as well as quantitative analysis was done. The drug was given to Streptozotocin induced diabetic rats in doses 250 mg/kg and 500 mg/kg body weight for 21 days. In HPLC analysis it was found that major component in all the extracts was beta carotene along with hesperidin, tannic acid, catechin, ferulic acid, caffeic acid along with other polyphenolic components responsible for the antioxidant activity as well as antidiabetic activity of polyherbal extract. Rats were found to show an increase in body weight, improved blood sugar levels and hemoglobin. The liver glycogen, total proteins, serum lipid profile were restored back to normal range after using the polyherbal extract in diabetic rats. The histopathological study also shows an improvement in diabetic rats at cellular level. The results were found to be dose dependent.

Keywords: Polyherbal, HPLC, Morphology, In vivo antidiabetic, Beta carotene.

Introduction

According to a report by WHO, approx. 422 million adults are suffering from diabetes in the year 2014. There have been 3.7 million deaths due to diabetes and its complications in the year 2012. It is one of the diseases growing at a tremendous speed that is supposed to affect 693 million adults by 2045 (1). Diabetes is a serious and enduring disease resulting into cardiovascular diseases, liver disease, obesity, kidney problems. Diabetes occurs because of either insufficient production of insulin or because of insufficient utilisation of insulin (2).

For the treatment of Diabetes mellitus generally synthetic Oral antidiabetic drug therapy is used which contains drugs belonging to various classes. These drugs act through different mechanisms for temporary relieve of hyperglycemia. The oral antidiabetic like Sulphonylureas, Biguanides, α-glucosidase inhibitors, Thiazolidinedione may lead to hypoglycemia, lactic acidosis, obstructive jaundice, hypernatremia, gastrointestinal intolerance, anaemia, congestive heart failure, hematological-dermatological reactions, pulmonary oedema and weight gain. The drugs due to all above mentioned adverse effects are contraindicated in patients having the problems of liver, kidney, heart or lungs diseases, Irritable Bowel Syndrome (IBS) (3), (4).

The oral antidiabetic drugs with such a profile of adverse effects can't be considered as a safe choice of treatment. This therapy could be replaced with a safe alternate i.e. Herbal drug therapy. The drugs from natural sources provide broad healing window, high performance, cost efficacy and negligible side effects (5), (6).

The plants contain a large number of phyto-constituents like alkaloids, glycosides, phenols, flavonoids, sterols, volatile oils, tannins, and resins (7). The phytoconstituents simultaneously acting on multiple targets like (8) blocking the pancreatic β-cell K+ channel, stimulates cAMP (2nd messenger) and re-absorb glucose in kidney, arousing insulin exudation from β-islets cells and or prohibition of insulin degradation, decreasing the cortisol levels, insulin resistance and the β-islets cells damage. They also provide Ca, Zn, Mg, Mn and Cu for the β-islets cells along with other required nutrients which help in regeneration and repair of pancreatic β-islets cells, enhancing the size and quantity of β-islets cells of Langerhans, stimulating the glycogen synthesis and

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hepatic glucose breakdown along with decreasing blood sugar and urea and thereby improve the digestion. They inhibit β-galactocidase, α-glucosidase and alpha amylase-6 along with conversion of starch to glucose (9).

In combination i.e. polyherbal formulations, when compared to the individual extract meanderingly leads to enhanced patient compliance and therapeutic outcome (10). Therefore, in present study we are taking 4 different herbal medicines like *Azadirachta indica* leaves, *Picrorhiza kurroa* roots and rhizomes, *Pterocarpus marsupium* heartwood and *Withania coagulans* fruit which act in treatment of diabetes mellitus through various mechanism of actions (11). The plants above mentioned have been used traditionally for various purposes like *Azadirachta indica* (*Neem*) has been used for skin problems, diabetes, astringent, anti-inflammatory, antioxidant and hepatoprotective activities along with many more actions (12). *Pterocarpus marsupium* (*Rasna*) has been used for its antidiabetic, antioxidant, anthelmintic, antipyretic, anti-dysentric, antihyperlipidemic properties (13). *Picrorhiza kurroa* traditionally known as *karu* have been used for its *deepan, pachan, lekhan and bhedan* properties. It also has been used as hypoglycemic, anticancer, antiviral, purgative, hypolipdemic properties (19). *Withania coagulans* traditionally known as *paneer dodi* or *"tukhm e hayat"* have been used for hypoglycemic, hepatoprotective, anti-flatulant, wound healing, digestive, and cheese making properties (15). The study involves the pharmacognostic evaluation, phytochemical evaluation and pharmacological evaluation of antidiabetic activity in Streptozotocin induced diabetic rats.

Materials and methods

Collection as well as certification of the plant material

The drug samples were collected from the local areas of Ropar and validated at NIPER's Natural Product Field Laboratory and Nursery, SAS Nagar, Pb. Neem leaves, Vijay Sar wood, Kutki rhizomes and Paneer dodi fruit, were authenticated and their respective voucher specimens were preserved there at A.S.B.A.S.J.S. College of Pharmacy in Bela, Ropar, and Pb.

Pharmacognostic evaluation

Organoleptic evaluation

Different sensory organs were used to examine the macroscopic evaluation of the selected plant material. It relates to the examination of a medication using the sense organs, which includes the look, smell, taste, and colour. The length, breadth, and thickness of unrefined material may all be measured using a graduated ruler in mm (16), (17).

The microscopic characteristics of the powdered medication were studied under microscope. The powdered drugs were tested with several chemicals and its behavior was observed in daylight.

Preparation of extracts

All the four authenticated drugs were dried, powdered and their individual ethanolic extract were prepared using cold maceration method and were concentrated below 60°C until dry and were kept at a cool temperature.

In order to prepare a polyherbal extract all the above mentioned four extracts were mixed in ratio 1:1:1:1 (18). So that a polyherbal extract (PHE) can be obtained and further used for *in vivo* studies.

Phytochemical analysis

Specific tests were used to assess the occurrence of alkaloids, tannins, glycosides, saponins, phenolics, terpenes, flavonoids and sterols in the prepared extracts (19).

HPLC analysis

The individual ethanolic plant extracts were investigated using Water's HPLC 2489 equipped with C18 column (Phenomix; 100 mm × 4.6 mm, 5 μM), at rate of 1 mL/min. The solvents were used in different gradients consisting of (A) Acetonitrile and formic acid (99.8:0.2 v/v) and (B) water, acetonitrile, and formic acid (96:3.8:0.2 v/v). The gradient of elution comprised of A:B (95:5) at 5min, 85:15 at 25 min, 80:20 at 30 min, 75:25 at 39 min, 55:45 at 43 min, 5:95 at 48 min to 55 min, 80:20 at 55 min and 100:0 at 60 min, using an injection volume of 10 μL. A total of fifteen commercial standards like Chlorogenic acid, Gallic acid, Caffeic acid, Catechin HYD, Ellagic acid, Rutoside, Ferulic acid, Rutin, Benzoic acid, Tannic acid, Hesperidin, Quercetin, beta carotene were used for identification. The results were expressed as ug/g of Dry Plant extract (20).

Physical evaluation (21)

Foreign matter

To determine the amount of foreign matter in a drug sample, the drug was distributed in a fine layer and inspected with the naked eye for foreign matter. Any foreign stuff acquired in this manner was weighed and a %age calculated.

pH value

Using a Digital pH meter, the pH of one % of the medication solution was calculated.

Microbial load analysis

The microbiological content of the formulation was evaluated using the WHO Quality control procedures for herbal materials as a guideline. This aids in determining whether or not microorganism growth in the formulation is below acceptable limits (22).

Ash value

Total ash was estimated by heating 2 g of powdered medication in a silicon crucible to 600°C until the powder became white or was carbon free. Weighed the amount of ash left in the crucible after it had cooled for 30 min. Total ash was dissolved in weak HCl, boiled it for 5 min., filtered it and washed using

ash-free filter paper to determine acid-insoluble ash. The ash-free filter paper containing ash content was moved to the crucible and burnt to consistent weight. In the event of ash that is water soluble, the whole ash obtained in the first stage was transferred to 25 mL water, after 5 min heating sieved it in ash-free filter paper. The whole content was burned again at a temperature below 450°C to quantify water-soluble ash in mg/g of air-dried product.

Extractive value

The water and alcohol soluble extraction values were calculated using the cold maceration technique. 4 g of medication was accurately weighted and placed in a stoppered conical flask. To begin the maceration, 100 mL of water and absolute ethanol were put into the conical flask according to the method. Maceration was carried out for a total of 24 h, with the flask being stirred infrequently for the first 6 h, subsequently left undisturbed for next 18 h. The material was sieved through filter paper and concentrated for 6 h at 105°C. For each medication, the % extractive value was determined in mg/g of air-dried drug.

In vivo antidiabetic activity

Healthy adult Wistar male rats of 180-220 g weight were bought from a licensed breeder. Rats were acclimatised according to the rules marked by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in New Delhi, India, and was authorised by the Institutional Animal Ethics Committee (IAEC) (Reg. No.: ASCB/IAEC/ 14/20/148).

Acute Oral Toxicity

The experiment used healthy male Wistar rats (3 animals per dosage) to assess the acute oral toxicity. Overnight fasted rats were given plant extracts at respective dosages of 5, 50, 300, 500, and 2000 mg/kg body weight. The behavioural as well as neurological characteristics of the animals were studied. For 24 h, researchers monitored touch, pain, and gait, as well as autonomic (defecation and urine) profiles. The animals were watched for 14 days after a 24 h period to see if they died (5).

In vivo antidiabetic activity in rats with Streptozotocin induced diabetes

In the current study total 30 Wistar albino male rats (180-220 g) were used for evaluation of in-vivo antidiabetic activity. The animals were adapted for 7 days and kept till end of experimentation

The animals were divided into 5 groups of 6 animals each:

Group I: Normal control
Group II: Diabetic control
Group III: Rats with diabetes given PHE (250 mg/kg)
Group IV: Rats with diabetes given PHE (500 mg/kg)
Group V: Rats with diabetes given Glibenclamide (0.25 mg/kg)

One intra-peritoneal (*i.p.*) shot of afresh synthesized Streptozotocin (STZ) 60 mg/kg b.w. in 0.1 M citrate buffer (pH 4.5, 0.5 mL/kg b.w) was injected for induction of diabetes in overnight starved rats. STZ-induced rats' fasting blood sugars were evaluated 48 h post onset to validate diabetes. To prevent hypoglycaemia death, the rats received a 5 % w/v glucose solution (2 mL/kg b.w.) after a 24 h STZ dosage. Diabetic animals exhibiting fasting blood glucose more than 200 mg/dL were classified into four groups at random. The standard (glibenclamide) and polyherbal preparations were administered once per day administered orally in 1% w/v carboxy-methyl cellulose (CMC) for 21 days. On the first, seventh, fourteenth, and twenty-first days of drug administration, blood was collected by pricking into the tail veins of rats and used immediately away to measure blood glucose. The change in body weight was recorded on weekly basis. Blood samples were drawn across all the rats via retro-orbital plexus and stored in ethylene diamine tetra acetic acid tubes in order to perform the biochemical studies. Following anaesthesia with diethyl ether, the rats were slaughtered, and tissues from the liver, pancreas, and kidneys were removed and biochemically and pathologically evaluated. A part of the tissue sample was retained frozen for biochemical analysis, while the rest was preserved in a 10% formalin solution for histopathologic evaluation (23).

Biochemical analysis

The collected blood was centrifuged at 2000×g (20 min), separated serum was analysed for different haematological parameters; Glucose level, Hb, HbA1c, Creatinine, Liver glycogen, Urea, Protein, Serum triglyceride, High Density Lipoprotein cholesterol, Total serum cholesterol, Serum glutamate Oxaloacetate Transaminase and Serum Glutamate Pyruvate Transaminase (Based on kit method). The bio analyser from Micro lab 300-Merck, was used for above mentioned investigations.

Meanwhile, estimation of insulin, was done using standard radioimmunoassay technique with the help of standard kit. The experiment was performed in RIA (Radio Immune Assay) laboratory.

Histopathological analysis

A liver, kidney and pancreatic section was well-preserved in formalin (10%) for two days. Alcohol was used to dehydrate the liver, kidneys, and pancreas for 12 h each (70, 80, 90%, and absolute alcohol). Before being infiltrated with paraffin in an automated tissue processing machine, the tissues were rinsed again in xylene for at least 15-20 min. The prepared tissue slabs were cut with the help of a microtome so as to generate 5µm thick slices. The slices were cut on a microscope slide that had been coated with egg albumin (a sticky substance) and dried. At last, the staining of slide was done with haematoxylin and eosin (a basic and acidic stain respectively). Afterwards, the tissues were washed using xylene for another 15-20 min using a microtome, the tissue slabs were produced and sliced into 5m thick slices. On a glass slide that had been coated with egg albumin (a sticky material) and dried, the slices were

cut. Finally, eosin and haematoxylin were used to stain the slides (an acidic and basic stain respectively) (24).

Statistical analysis

The mean value and standard error in mean were used to write all of the data (SEM). To evaluate statistical significance seen between groups, a one-way ANOVA was used, accompanied by a Dunnett's test post-hoc test.

Results and Discussions

Organoleptic and microscopic evaluation

All the four drugs were analysed organopleptically and microscopically and the parameters obtained from the study are mentioned in Table 1.

Phytochemical analysis

The phytochemical analysis marked the occurrence of various phytoconstituents like alkaloids, glycosides flavonoids, tannins, sterols, in all four herbal drugs. The detailed description is given in the Table 2.

HPLC analysis

The phytochemical investigation report of individual ethanolic extracts of as well as polyherbal extract for phenolic as well beta carotene has been shown in Table 3 and Figure 1, Figure 2, Figure 3, Figure 4, Figure 5. Out of total fifteen standard mentioned there in procedure seven are found there in *Azadirachta indica* leaves extract. They are present as follow in decreasing order of their concentration:

Beta carotene>ferulic acid>Chlorogenic acid> Quercetin> Rutoside>Benzoic acid> Caffeic acid.

In *Pterocarpus marsupium* there are 13 compounds in order as

Beta carotene>hesperidin> Catechin > tannic acid> chlorogenic acid>caffeic acid> rutin> Gallic acid> Ferulic acid>Ellagic acid> Quercetin

In *Picrorhiza kurroa* there are 12 compounds in order as

Beta carotene> Rutoside> Hesperidin> Ellagic acid> tannic acid> ferulic acid> Chlorogenic acid> Benzoic acid>caffeic acid> Quercetin

In *Withania coagulans* there are 12 compounds in order as

Beta carotene> benzoic acid> tannic acid> Gallic acid> Chlorogenic acid> Rutin> Caffeic acid> ferulic acid> quercetin > Ellagic acid

In Polyherbal extract there are 13 compounds in order as per data cited from (Kaur & Sharma, 2022).

Beta carotene> Rutoside> Hesperidin > tannic acid> Ellagic acid> caffeic acid> chlorogenic acid> gallic acid> ferulic acid> benzoic acid > quercetin

From above results it has been observed that beta carotene is present in maximum amount in all samples and quercetin follows the routine of minimum. The beta carotene content was found maximum in polyherbal formulation, although the *Pterocarpus marsupium* won the race in context of hesperidin, catechin and tannic acid.

It has been observed that all the compounds which are found in maximum content show very good antioxidant activities. Therefore, they may play a major role in treatment of diabetes due to their antioxidant effects.

Physical evaluation of raw material

Foreign matter indicates either the matter other than the specified parts of the same drug, some other organisms or traces of it or the material adhering to the main drug which include sand, soil and dust.

Microbial load analysis determined the limits of pathogens that can cause the risk to human life or health. In general, a certain level of microbial load is permitted for herbal drugs but when it rises above the limit the sample is discarded. The four antidiabetic drugs showed the foreign matter, pH value, microbial analysis, ash value and extractive values within range of standard quality the result are shown in Table 4.

Pharmacological studies

Acute oral toxicity

Acute toxicity studies did not indicate any death up to 2000 mg/kg dose, administered as a solo oral dose. As a result, the study was done at doses of 250 and 500 mg/kg.

In vivo antidiabetic activity

Biochemical studies

The diabetic rats displayed substantial changes during the research. The body weight in experimental animals was found lower than that of the control animals. The polyherbal extract and glibenclamide, on the other hand, inhibited the weight loss caused by diabetes. When compared it is here to notice that the polyherbal extract worked in dose dependent manner, the higher dose lead to lower body weight loss. Whereas in case of glibenclamide the loss in weight was least which can be easily seen in Table 5. When compared the diabetic control animals had substantial hyperglycaemia in comparison to regular animals. The average amount of blood glucose in diabetic animals on day 0 was 265±3.47 mg/dL, and on day 21, 348±3.06 mg/dL glucose levels were recorded.

A considerable impact of polyherbal extract on blood sugar levels was discovered. It was found to be dose dependent as the levels reduced to normal but reduction was up to128±2.21 mg/dL in low dose of 250 mg/kg and 115±2.44 mg/dL in higher dose of 500 mg/kg. The effectiveness of higher dose was just equal to the standard drug glibenclamide on 21st day as it was 115±1.74 mg/dL.

The diabetic animals had significantly lower plasma insulin, HbA1c and haemoglobin levels in comparison to control. In diabetic rats, the polyherbal extract and glibenclamide reversed insulin depletion and restored normal haemoglobin and HbA1c values Table 6.

In diabetic animals it was found that the liver glycogen as well as total protein levels have markedly gone down, but the polyherbal extract and glibenclamide-treated groups had restored the glycogen levels of liver and total protein to normal. The constituents like phenols, tannins and flavonoids present in polyherbal extract, well known for their antioxidant capacity may be one of the reasons for the reversal of any kind of distortion in biochemical parameters in the diabetic animals. Table 7 shows the effects of polyherbal extract and glibenclamide on diabetic rats' liver and kidney indicators.

Serum lipid profiles in diabetic rats increased significantly (P 0.001) but therapy group restored it back to normal ranges by the end in Table 8.

The standard drug glibenclamide and polyherbal extract have succeeded in bringing all the disturbed parameters like creatinine, SGOT, SGPT, protein back to their normal levels in Table 9.

Histopathological study of rats

Pancreas: The Langerhans islets were found to be big and regular, and their boundaries were clearly defined. Langerhans islet cells were found to have a normal histologic appearance. In rats with STZ induced diabetes, histopathologic examination of pancreas has shown significant congestion, fibrosis, inflammatory cell infiltrations and a large reduction in the number of islets of Langerhans The islets had significant cell loss, the cellular order had been disturbed, the islets had atrophied, and the structure had degraded. The cytoplasm of degenerative cells showed hydropic degeneration and degranulation. Only a few cells in the Glibenclamide therapy group had degenerative and necrotic alterations and leads to moderate congestion and improvement in the amount of β cells in islets of Langerhans and mild lymphocytic infiltration. In the PHE 250 and 500 mg/kg treatment group, there was a substantial growth in the islet of Langerhans Figure 6.

Liver: In the liver sections of DM group rats, extensive hydropic degeneration in hepatocytes and necrosis were detected. In the cytoplasm of degenerated hepatocytes, large to small irregularly-edged, partly spherical vacuoles have been detected. In portal regions bile duct growth, mild fibrosis and inflammatory cell infiltration were seen. There was a significant fatty alteration, as well as mild portal inflammation, sinusoidal dilation, congestion, fibrosis, severe feathery bowel syndrome necrosis and degeneration. These results were shown to be considerably reduced in the livers of rats treated with Glibenclamide, as well as localized degeneration and necrosis in hepatocytes. Furthermore, abnormalities were shown to be considerably decreased in rats given PHE 250 and 500 mg/kg Figure 7.

Kidney: The control group showed a normal histological picture of the kidney. Hydropic degeneration and necrosis were detected in tubulus epithelial cells in the kidneys of DM rats. In the intertubular regions, there were a few foci of inflammatory cells. Diabetic rats' kidneys revealed thickening of the Bowman's capsule basement membrane, oedema and hyper-cellularity of the proximal tubules, necrosis, and hyaline deposits. When the polyherbal extract was given to the experimental animals, these characteristics were shown to be reversed. The polyherbal extract protected the diabetic kidney from oxidative damage by acting as an antioxidant. In diabetic nephropathy, polyherbal extract is used to reduce oxidative damage. In the kidneys of Glibenclamide-treated rats, mild hydropic degeneration and necrosis were seen in certain tubular epithelial cells Figure 8.

The polyherbal mixture was created by combining all the four ethanolic extracts in a 1:1:1:1 ratio.

Oxidative stress, an imbalance of production as well as destruction of very reactive species, may cause impairment of lipid and protein oxidations, insulin sensitivity, impaired glucose tolerance, β-cells' dysfunction leading to NIDDM. All those conditions can be counter acted by use of antioxidants (26).

While doing HPLC analysis of ethanolic extracts of various drugs, it has been found that all the four drugs as well as the polyherbal extract contain much amount of beta carotene followed by hesperidin, catechin and tannic acid, ferulic acid, caffeic acid, chlorogenic acid *etc*. As it is found in literature that all the above mentioned phytoconstituents are very good antioxidants. Therefore, the antioxidant activity may be found responsible for the antidiabetic effect of all the above-mentioned samples and polyherbal formulations. Hesperidin help by defending against hepatotoxicity caused by STZ induced diabetes.

β-carotene significantly chelate the reactive singlet oxygen and free radicals the conjugation is responsible for its activity, more the conjugation in structure more is the ability of a carotene to capture ROS. It may eliminate up to 1000 ROS. Hyperglycaemia leads to production of ROS, where β-carotene control the redox balance, inhibiting the production of new ROS as well as lipid peroxidation (27).

It has been seen in literature that Ferulic acid treatment leads to drop in plasma sugar levels, increase in insulin. It helped in increasing glucokinase activity and decreasing glucose-6-phosphatase and phosphor-enol-pyruvate carboxykinase activities in liver.

Caffeic acid has been found to show hypoglycaemic activity, improvement in insulin level and glucose tolerance in diabetic animals. Chlorogenic acid protect against retinopathy via inhibiting retinal neo-angiogenesis. It drops the fasting plasma glucose as well as levels of HbA1c. Tannic acid stimulates insulin-like glucose transport in adipocytes (28).

There may be other factors too which may contribute to the antidiabetic effect of various drugs and consequently to the antidiabetic effect of polyherbal extract.

The antidiabetic effect of neem is found to be due to the improvement in the expression of the insulin signaling molecule and GLUT4 proteins. The antidiabetic activity of the neem leaf may be attributed to synergistic effect of terpenoids, glycosides and

flavonoids present in it (29), (30), (31). The antidiabetic effect of *Withania coagulans* may be due presence of alkaloids, steroids and withanolide glycoside in it along with some trace minerals like Mg and Ca which helps in lowering serum glucose levels and other complications related to it (32), (33), (34).

The antidiabetic activity of *Picrorhiza kurroa* is due to the glycosides present in it and which helps in beta cell redevelopment and insulin production enhancement leading to the antihyperglycaemic effect (35). *Pterocarpus marsupium* is the tree of which heart is used and have bioactive principle pterostilbene, pterosupin, (-) epicatechin, marsupin, tannins leading to its anti-diabetic activity (36). Individual plant phyto-constituents are inadequate to generate the therapeutic effects necessary. By concurrently acting on numerous targets, the combination of precise ratio of different herbs will provide a better efficacy and lower toxic effect, resulting in improved patient compliance and therapeutic success. All of these benefits contributed to PHF's commercial success as compared to single herbal products. They have a high level of effectiveness, a wide therapeutic window, cost-effectiveness, easy to approach and have few adverse effects. These benefits have prompted many to turn to polyherbal for treatment and prevention, of illness claiming that they have synergistic, potentiated, and agonistic/antagonistic effects (5), (6). Up to 2000 mg/kg, the polyherbal extract showed no mortality or side events. As a result, the trial was conducted at doses of 250 mg/kg and 500 mg/kg. A poisonous glycoside STZ was derived from the gram-positive bacteria *Streptomyces achromogenes*. It gathers in cells of pancreas and suppresses their expression through the glucose transporter 2 (GLUT2). The alkylating capabilities of the STZ cause insulin-dependent diabetes by modifying biological macromolecules, fragmenting DNA, and destroying cells (23). Severe body weight loss was seen in the diabetes control group, which might be credited to increase in muscle and tissue protein harm. The polyherbal extract and glibenclamide treated group in this study gained a significant amount of weight, showing that they reduce muscle loss due to hyperglycaemia.

The drop-in levels of sugar could be owing to an upsurge in plasma insulin levels and blood glucose conveyance in peripheral tissue. The polyherbal extract elevates insulin blood levels and has potential anti-diabetic capabilities, according to our data. HbA1c rates were increased in diabetic mice owing to enhanced glucose synthesis in the blood, which interacts with blood haemoglobin to form HbA1c.

Hyperglycaemia is caused by elevated lipid peroxides and reactive oxygen species in rats with Streptozotocin induced diabetes. The complications caused by STZ on liver, pancreas and kidney got reversed in rats treated with a polyherbal extract. This might be attributable to the distinct herbs in the polyherbal extract. The free radical scavenging characteristics in polyherbal extract, reduced the diabetes caused by STZ.

Histopathological study of the pancreas in diabetic rats exhibited a substantial drop in the number of β-cells and islets of Langerhans and fibrosis, as reported before. The livers of STZ diabetic rats had significant fatty changes, sinusoidal dilatation, congestion, severe feathery degeneration, fibrosis, moderate portal inflammation and necrosis. Hepatocyte hypertrophy caused by an enhancement in intra-cytoplasmic eosinophilic granules might be the cause of the hepatic abnormalities (23). The effects mentioned above were found to be significantly reduced by glibenclamide and our polyherbal extract in dose dependent manner.

The results of our research show that the polyherbal extract has significant anti-diabetic impact. At doses of 250 and 500 mg/kg, the PHE was tested. The polyherbal extract's anti-diabetic potential is equivalent to glibenclamide, as demonstrated it has also expressively decreased the blood glucose levels, total cholesterol, HbA1c, triglyceride levels as well as urea, creatinine, SGOT, and density lipoprotein (LDL) cholesterol and SGPT levels. The extract also contributed in increasing the plasma insulin, HDL cholesterol, and liver enzymes along with amounts of glycogen and total proteins. The histo-pathological study also showed the improvement at cellular levels in the liver, pancreas and kidney of the rats. Further research can be done here to check the dosage form for the particular polyherbal extract combination so that its pharmacokinetic benefits can also be encashed.

Conclusion

A polyherbal ethanolic extract of *Azadirachta indica* leaves, *Pterocarpus marsupium* heartwood, *Picrorhiza kurroa* rhizomes, and *Withania coagulans* berries and fruit coat in a 1:1:1:1 ratio was prepared and tested for its *in vivo* antidiabetic activity. The extract showed significant results as antidiabetic. The antidiabetic activity may be due to the polyphenolic, flavonoid and beta carotene contents found in HPLC study. Further research can be done in order to prepare a dosage form which can provide good pharmacokinetic and pharmacodynamics profile to the drug.

Conflict of interest

None

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Table 1. Organoleptic and microscopic evaluation of various herbal drugs

Parameters	*Azadirachta indica*	*Pterocarpus marsupium*	*Picrorhiza kurroa*	*Withania coagulans*
Plant part	Leaves	Heart wood	Rhizome	Berries with fruit coat
Colour	Green	Yellowish brown	Deep greyish brown	Yellowish to dark brown
Odour	Indistinct	Odorless	Unpleasant	Characteristic
Taste	Bitter	Bitter	Bitter	Bitter
Extra features	Leaves are alternate, pinnate and petiolate Leaflets are 6-9 cm long and 1-2 cm broad, sickled in shape and have denticulate margin	Rough, fissured and scaly	3-6 cm in length, 0.5-1 cm in diameter it has wrinkles and annulation on surface, fracture is tough	Diameter 5-30mm Globose shape and surface is glaborous
Powder microscopy	lignified fibers, vascular bundles, covering trichomes, epidermal cells	Lignified cells, fibers	Lignified tissues, fibers, epidermal layer,	Lignified fibers, epidermal layer, lignified cellular bundles

Table 2. Phytochemical analysis of herbal drugs

Chemical Test	*Azadirachta indica*	*Withania coagulans*	*Picrorhiza kurroa*	*Pterocarpus marsupium*
Alkaloids	+	-	+	+
Glycosides	++	-	+	+
Anthraquinones	-	+	+	+
Flavonoids	+	++	++	++
Saponins	++	-	+	-
Sterols and terpenoids	+	++	+	++
Tannins and Phenols	++	+	+	++
+: present; -: absent				

Table 3. HPLC analysis of ethanolic extracts of various drugs

Standards	*Azadirachta indica*		*Picrorhiza kurroa*		*Pterocarpus marsupium*		*Withania coagulans*		Polyherbal(25)	
	RT	Conc. ug/g of DPE	RT	Conc.ug/g of DPE	RT	Conc. ug/g of DPE	RT	Conc. ug/g of DPE	RT	Conc. ug/g of DPE
Gallic acid	-----	----	----	---	2.2	9.51	2.18	4.23	2.2	7.14
Chlorogenic acid	14.5	1.04	14.6	5.99	14.52	17.91	14.51	2.49	14.52	7.58
Caffeic acid	18.03	0.20	17.81	4.79	17.87	16.58	17.99	1.42	17.91	7.77
Catechin	----	----	----	----	20.5	42.59	----	----	-----	----
Ellagic acid	----	----	26.87	27.99	26.68	4.85	27.16	0.001	26.87	12.73
Rutoside	27.88	0.38	28.83	39.24	----	----	----	----	28.82	17.20
Ferulic acid	29.88	1.20	29.91	7.56	29.37	9.11	29.41	1.01	29.95	5.81
Rutin	----	----	-----	----	30.03	14.10	30.07	1.80	----	----
Benzoic acid	32.5	0.21	31.4	5.76	----	----	32.45	5.23	32.06	2.18
Tannic acid	----	----	35.99	13.99	35.19	32.20	35.22	4.56	35.22	14.69
Hesperidin	----	----	34.09	37.11	35.19	55.70	----	----	34.11	16.83
Quercetin	44.39	0.43	42.99	0.29	44.29	0.09	44.7	0.11	44.43	0.35
β-carotene	50.45	243.2	50.49	215.62	50.46	245.94	50.49	224.56	50.46	251.50

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RT: Retention time

Table 4. Physical evaluation of herbal antidiabetic drugs

Drugs	FM	pH 1% sol	M L	%age TA	%age WSA	%age AIA	WSEV	ASEV
Azadirachta indica	1.24%	6.48	Ab	9	0.94	0.86	16.71	13.56
Withania coagulans	1.17	4.37	Ab	6.2	1.56	1.13	21.35	11.85
Picrorhiza kurroa	1.157	4.18	Ab	5	1.23	0.63	42.68	22.84
Pterocarpus marsupium	0.64	4.21	Ab	2.5	1.46	0.37	15.74	14.23

FM: Foreign Matter; ML: Microbial Load; TA: Total ash; WSA: Water soluble ash; AIA: Acid insoluble ash; WSEV: Water soluble extractive value; ASEV: Alcohol soluble extractive value

Table 5. The effect of polyherbal extract on body weight in STZ treated rats

Treatment	0th day	7th	14th day	21st day
NC	205.8±6.337	207.5±6.270	220.5±6.350	227.8±4.881
DC	204.8±5.023	203.3±5.463	196.8±4.847*	189.5±4.985***
PHE 250mg/kg	206.2±5.173	204.8±5.250	211±5.859	219.3±5.852***
PHE500mg/kg	200.7±5.383	206.0±4.940	217.2±4.520*	229.5±4.372***
Glibenclamide	202.3±4.731	207.5±4.372	217.5±4.566*	230.2±3.483***

NC: Normal control, DC: Diabetic control, PHE: Polyherbal extract. The values are expressed as mean±SEM (n=6) *P<0.05, ***P<0.001 when compared to diabetic control (one way ANOVA followed by Dunnet's test) was applied.

Table 6. The effect of polyherbal extract on blood glucose levels in STZ treated rats

Treatment	0th day	7th day	14th day	21st day
NC	90.3±0.882	95.2±.946	95.7±1.26	94.2±.946
DC	265±3.47	288±2.23	322±2.36	348±3.06
PHE 250mg/kg	263±3.76	221±2.40***	165±3.04***	128±2.21***
PHE500mg/kg	263±3.56	192±2.50***	151±2.70***	115±2.44***
Glibenclamide	264±2.73	180±3.17***	135±2.09***	115±1.74***

NC: Normal control, DC: Diabetic control, PHE: Polyherbal extract. The values are expressed as mean±SEM (n=6) ***P<0.001 when compared to diabetic control (one way ANOVA followed by Dunnet's test) was applied.

Table 7. The effect of polyherbal extract on insulin levels in STZ treated rats

Treatment	Insulin levels (µIU/mL)				Hb (mg/dL)	Hb1Ac (mg/g of Hb %)
	0th day	7th day	14th day	21st day	21st day	
NC	21.5±1.2	20.7±0.42	21.0±0.57	21.8±0.60	13.8±0.30	0.328±0.01
DC	6.83±0.6	6.00±0.51***	5.33±0.42***	4.50±0.22***	5.67±0.33***	2.08±0.47***
PHE 250 mg/kg	8.17±0.4	11.0±0.36***	14.2±0.47***	17.5±0.47***	8.83±0.47***	1.22±0.08***
PHE500 mg/kg	8.67±0.3	14.5±0.42***	18.3±0.33***	19.7±0.33***	13.3±0.21***	0.45±0.01***
GBD	9.0±0.25	16.0±0.36***	19.5±0.42***	20.0±0.36***	13.7±0.33***	0.32±0.01***

NC: Normal control, DC: Diabetic control, PHE: Polyherbal Extract. GBD: Glibenclamide, Hb: Hemoglobin, Hb1AC: Glycosylated hemoglobin. The values are expressed as mean±SEM (n=6) ***P<0.001 when compared to diabetic control (one way ANOVA followed by Dunnet's test) was applied.

Table 8. The effect of polyherbal extract on serum lipids levels in STZ treated rats

Treatment	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	LDL (*mg/dL*)	HDL (*mg/dL*)	VLDL (*mg/dL*)
NC	69.3±1.54	12.7±1.31	36.0±1.91	36.0±1.93	82.2±2.12
DC	164±2.29***	41.0±1.34***	87.0±2.57***	16.2±1.35***	154±3.34***
PHE 250mg/kg	63.8±2.50***	20.2±1.30***	40.7±1.48*	28.5±1.77***	86.5±1.95***
PHE500mg/kg	62.0±2.07***	17.7±1.67***	35.2±0.17***	31.7±1.82***	77.2±2.04***
Glibenclamide	59.7±1.43***	14.2±0.83***	33.0±1.91***	33.3±1.94***	77.2±2.20***

NC: Normal control, DC: Diabetic control, PHE: Polyherbal extract, HDL: High density lipoproteins, LDL: Low density lipoproteins, VLDL: Very low density lipoproteins. The values are expressed as mean±SEM (n=6) *P<0.05, ***P<0.001 when compared to diabetic control (one way ANOVA followed by Dunnet's test) was applied.

Table 9. The effect of polyherbal extract on liver glycogens, total protein, urea, creatinine, SGOT and SGPT levels in STZ treated rats

Treatment	Liver glycogen g/100mg wet tissue	Total protein (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	SGOT (IU/l)	SGPT (IU/l)
NC	5.50±0.42	8.67±0.422	22.8±0.87	0.468±0.01	102±1.62	81.2±0.94
DC	2.67±0.33***	4.50±0.42***	51.5±1.18***	1.31±0.16***	159±1.65***	158±2.74***
PHE 250 mg/kg	3.50±0.22	6.83±0.30***	38.3±0.88***	1.06±0.04***	128±2.86***	128±2.73***
PHE 500 mg/kg	5.17±0.30***	8.33±0.33***	21.3±0.66***	0.435±0.01***	109±1.15***	83.8±2.41***
GBD	5.83±0.30***	8.83±0.30***	23±0.57***	0.363±0.01***	102±1.14***	80±1.37***

NC: Normal control, DC: Diabetic control, PHE: Polyherbal extract, GBD: Glibenclamide; SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase. The values are expressed as mean± SEM (n=6) ***P<0.001 when compared to diabetic control (one way ANOVA followed by Dunnet's test) was applied.

Figure 1. HPLC analysis of ethanolic extract of *Azadirachta indica* leaves

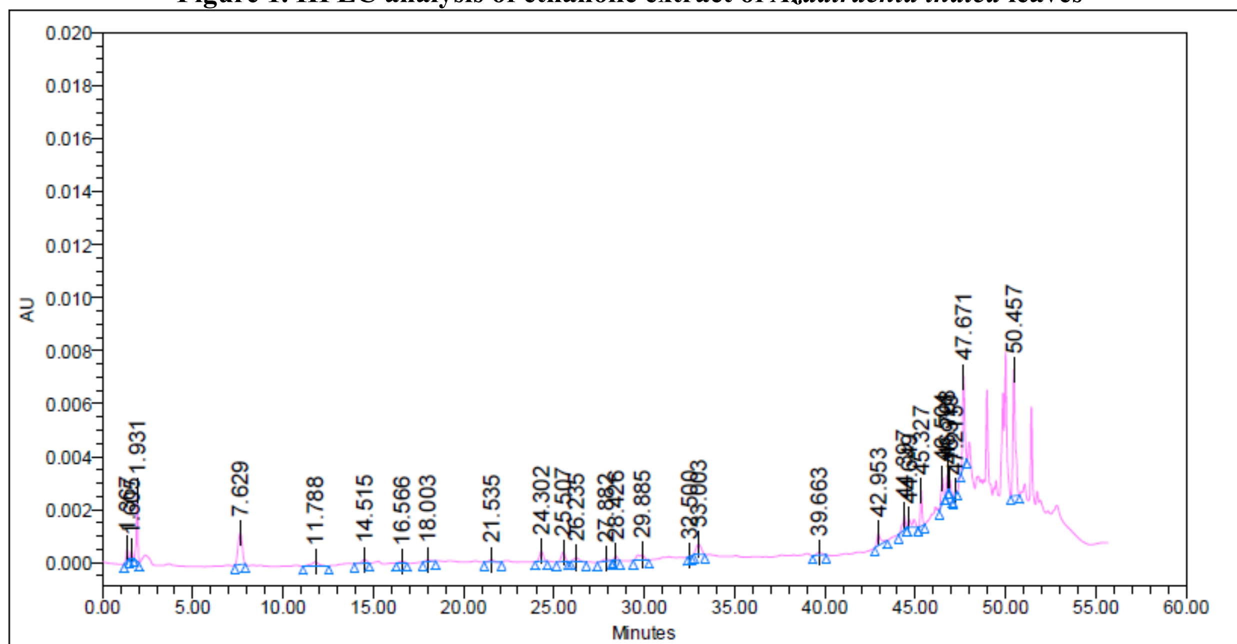


Figure 2. HPLC analysis of ethanolic extract of *Picrorhiza kurroa* rhizomes and roots

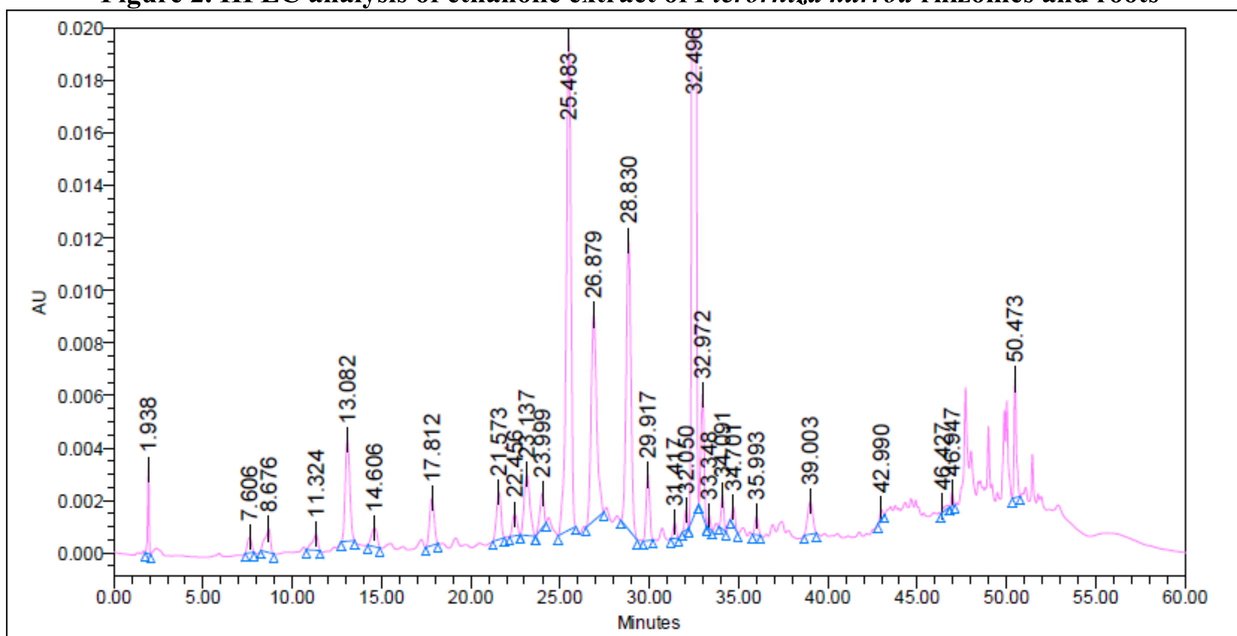


Figure 3. HPLC analysis of ethanolic extract of *Pterocarpus marsupium* wood

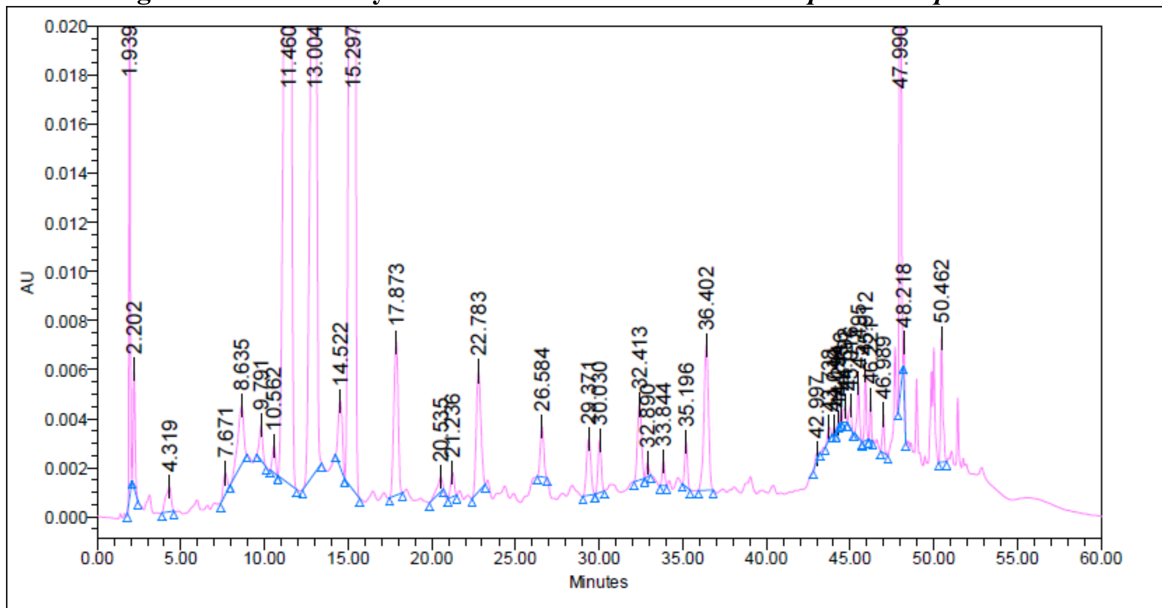


Figure 4. HPLC analysis of ethanolic extract of *Withania coagulans* fruit

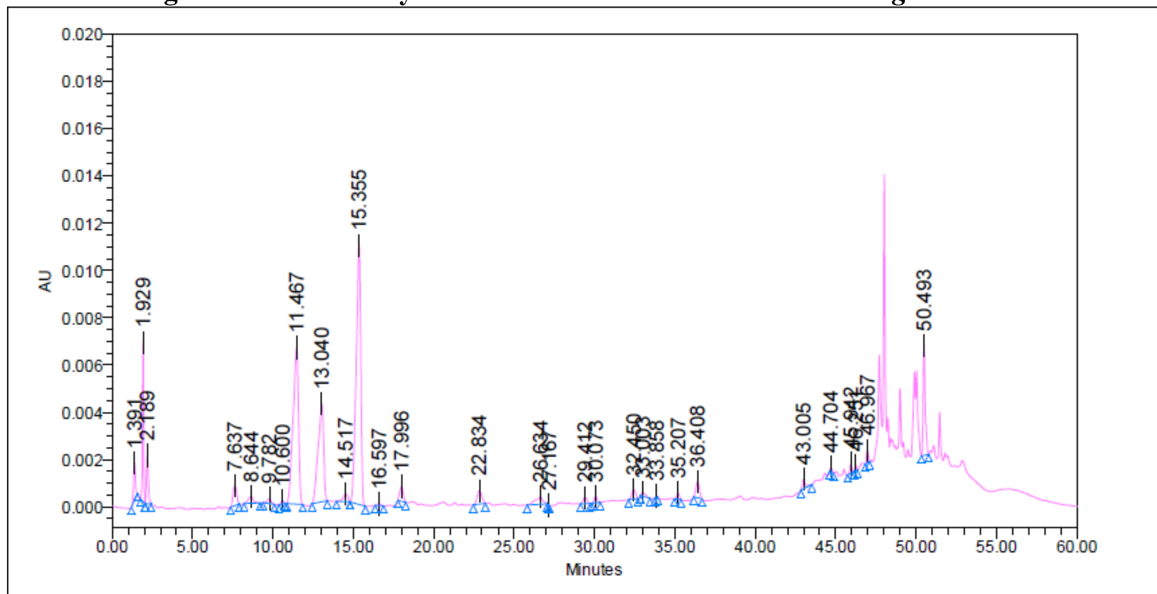


Figure 5. HPLC analysis of ethanolic polyherbal extract (25)

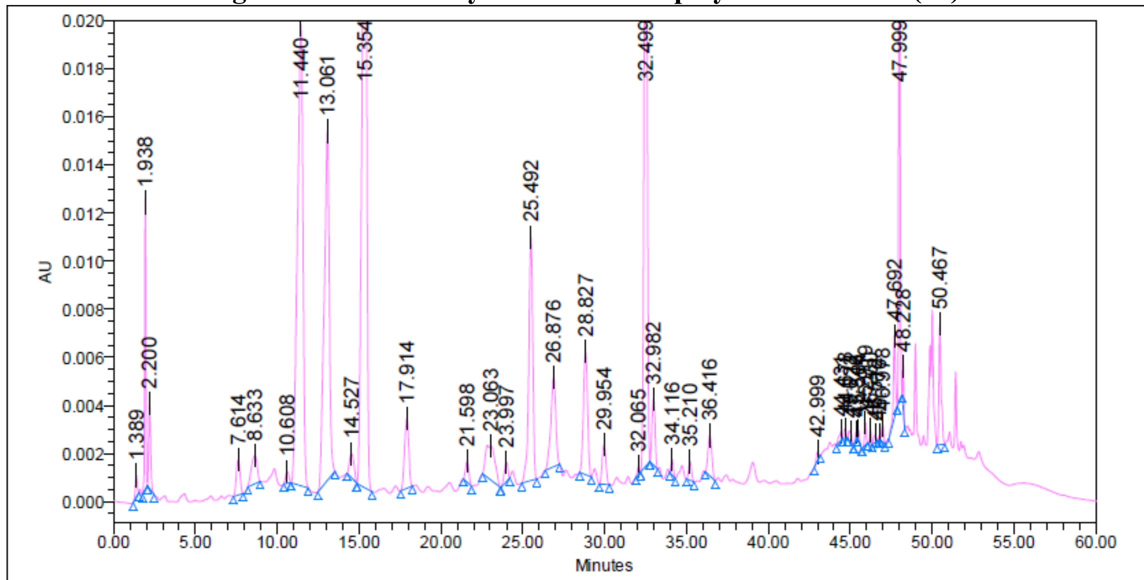


Figure 6. Histopathology of Pancreas of rats, section stained with Eosin and Hematoxylin (x400)
PHE: Polyherbal extract

Normal control	Diabetic control	Glibenclamide	PHE 250 mg/kg	PHE 500 mg/kg

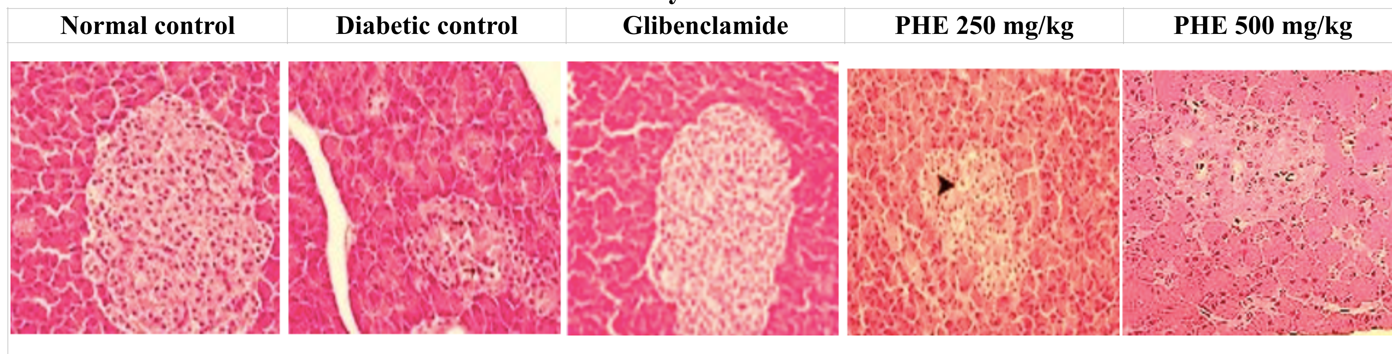


Figure 7. Histopathology of liver of rats, section stained with Eosin and Hematoxylin (x400)
PHE: Polyherbal extract

Normal control	Diabetic control	Glibenclamide	PHE 250 mg/kg	PHE 500 mg/kg

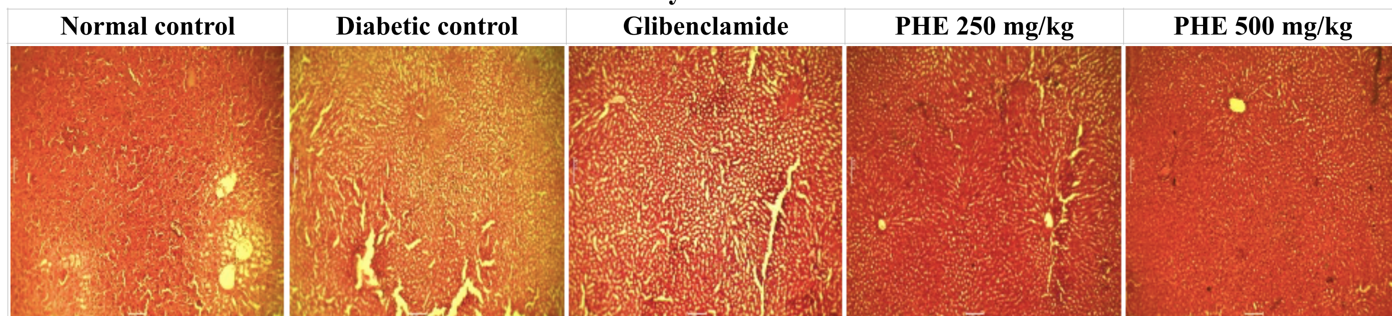
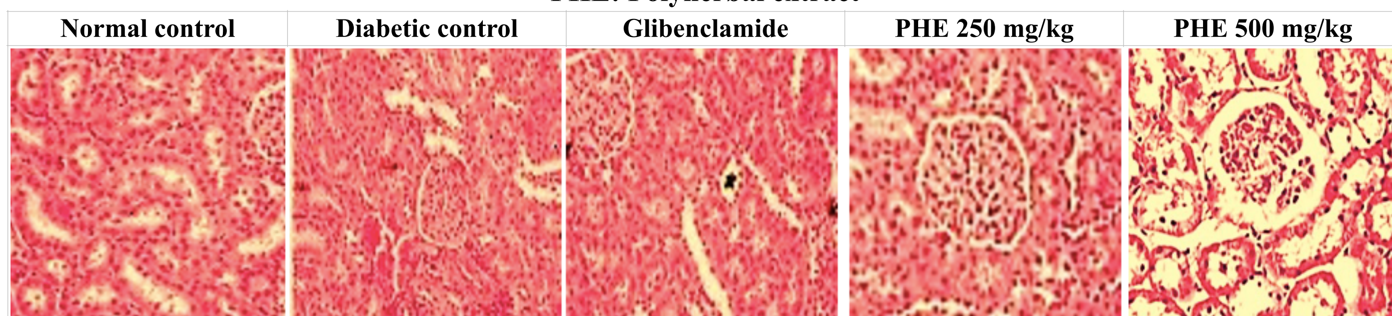


Figure 8. Histopathology of kidney of rats, section stained with Eosin and Hematoxylin (x400)
PHE: Polyherbal extract

Normal control	Diabetic control	Glibenclamide	PHE 250 mg/kg	PHE 500 mg/kg



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