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Original article

Modulation of antioxidant defense system by polyherbal extract mixture which ameliorated the pathophysiological alterations in streptozotocin induced diabetic rats

Shaul Ahmed R., Urvesh D. Patel, Dixita H. Bhadarka, Harshad B. Patel and Chirag M. Modi
Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh-362 001, Gujarat, India

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Abstract

Diabetes mellitus is described as a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism, resulting from defects in insulin secretion, insulin action, or both. Looking into the pharmacological properties of different plant varieties like *Allium cepa*, *Trigonella foenum-graecum*, *Tinospora cordifolia*, *Gymnema sylvestre*, *Syzygium cumini* and *Momordica charantia*, the study was carried out to evaluate the effect of polyherbal extract mixture (PHEM) on blood glucose level, lipid profile and other pathophysiological alterations in streptozotocin induced diabetic rats. Thirty Sprague-Dawley rats were randomly divided based on body weight in five groups (C1, C2, C3, T1 and T2). Rats of four groups (C2, C3, T1 and T2) were injected with streptozotocin to induce diabetes. Rats of group C1, C2, and C3 were kept as normal, diabetic and standard control, respectively. Rats of group C3 were administrated with glibenclamide (5 mg/kg, PO for 28 days). Rats of group T1 and T2 were treated with PHEM at 100 and 200 mg/kg, respectively orally for 28 days. Administration of PHEM significantly reduced the blood glucose level in T1 and T2 experimental groups after 28 days of treatment. In lipid profile, mean values of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in rats treated with PHEM were found comparable to those of control rats. Altered biochemical enzymes like AST, ALT, ALP and bilirubin were normalized by administration of PHEM in diabetic rats. Antioxidant enzymes which got altered in diabetic rats were also normalized in rats under the treatment of PHEM. In conclusion, administration of polyherbal extract mixture at the dose rate of 200 mg/kg, PO for 28 days have shown ameliorating effect against STZ induced alterations in glucose level, lipid profile, oxidative status and other pathological changes in rats.

Key words: Glucose level, blood parameters, histopathological evaluation, polyherbal extract mixture, diabetic rat model

1. Introduction

Diabetes mellitus is a chronic heterogeneous metabolic disorder characterized by loss of glucose homeostasis which results from defect in insulin secretion or an insulin action or sometimes both (Jha *et al*., 2018). It is a major endocrine disorder, fast gaining the status of a potential epidemic in India (Kumar *et al*., 2006). The metabolic abnormalities that characterize diabetes, such as hyperglycemia, increased free fatty acids and an insulin resistance can provoke molecular mechanisms that contribute to vascular dysfunction (Creager and Lusher, 2003). Diabetes is a major healthcare problem, since it increases the risk of heart disease, stroke and microvascular complications such as blindness, renal failure and peripheral neuropathy (Frances and Patrik, 2012). The phenomenon of oxidative stress resulting from the imbalance of a critical balance of pro-oxidants and antioxidants in the organism has scientifically been established as a vital player in the pathology of chronic ailments (Gul *et al.*, 2016; Rajeshwari *et al*., 2013, Tayyab and Lal, 2016). Production of reactive oxygen species (ROS) in oxidative stress is proposed as the important cause of progression of β-cell dysfunction, insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus (Wright *et al.*, 2006).

In addition to insulin, oral hypoglycemic agents like sulphonylureas, thiozolidinediones, biguanides, D-phenylalanine derivatives, meglitinides and α-glucosidase inhibitors are the conventional drugs used for the treatment of diabetes. However, use of these allopathic drugs for prolong period may cause side effects like hypoglycemia, nausea, vomition, hyponatremia, flatulence, gastrointestinal disturbance, headache, weight gain, lactic acidosis, pernicious anemia, dyspepsia, dizziness and joint pain. Hence, instead of allopathic drugs, herbal drugs are good option for management of diabetes which has no or lesser side effects (Kokar and Mantham, 1998).

It is estimated that around 30000 plants species are known to have importance and of which 15000 plants are known to have worldwide use as drugs (Manoharachary and Nagaraju, 2016). Many herbal

Author for correspondence: Dr. Urvesh D. Patel
Assosiate Professor, Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh-362 001, Gujarat, India
E-mail: urvesh1981@yahoo.com
Tel.: + 91-9725003818

plants have been evaluated so far for having antidiabetic effects and also used by people for prevention and treatment of diabetes in India and all over the world. Different formulations of herbal origin are also available in the market for the treatment of diabetes. Despite the availability of many marketed herbal antidiabetic products, the formulation having antidiabetic effect along with protective role against diabetic complication is needed. *Gymnema sylvestre* (Retz.) Schult., *Allium cepa* L., *Momordica charantia* L., *Trigonella foenum-graecum* L. (Ghorbani, 2013) *Tinospora cordifolia* (Willd.) Miers (Shaul *et al.,* 2017) and *Syzygium cumini* L. Skeels (Nayak and Subrata De, 2013) have been individually reported to have role in diabetes. We hypothesized that the mixture of extracts of all these plants might produce good results in diabetes as well as such study with mixture containing all these adaptogens have not been evaluated for evaluation of antioxidant mediated protective role in diabetes. Thus, above all plants have been selected to prepare polyherbal extract mixture (PHEM) and used to evaluate its effect on blood glucose level, lipid profile with other blood parameters and histopathological changes in STZ-induced diabetic rodent model.

2. Materials and Methods

2.1 Experimental animals

The study was carried out on 30 male Sprague-Dawley rats of 6-8 weeks of age. Rats were procured from registered breeder. All experimental animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Institute (Protocol No. JAU/JVC/IAEC/SA/16/2017).

2.2 Animal husbandry

The polypropylene cages with stainless steel top grill was used to house rats. The cages were changed at least thrice in a week. During entire study period, the animals were housed in the cool environmental temperature (23°C to 26°C) with relative humidity ranged between 40 to 55%. Twelve hour light-dark cycle was maintained in laboratory animal room. Rat pelleted feed (VRK biological system, India) containing 18% protein was provided *ad libitum* to animals throughout the study period.

2.3 Plant materials and chemicals

Plant materials (leaves of *Gymnema sylvestre* (Retz.) Schult.,peels of *Allium cepa* L., fruit of *Momordica charantia* L., stem of *Tinospora cordifolia* (Willd.) Miers and dried seeds of *Trigonella foenum-graecum* L. and *Syzygium cumini* (L.) Skeels were collected from nearby area of Junagadh and authenticated by Mr. Punit Bhatt, Pharmacognosist, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, India. The specimens of above plants were submitted to the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, Gujarat (Specimen No. JVC/VPT/SP/15/2017 to JVC/VPT/SP/20/2017). Each plant material was subjected to shade dry at room temperature and was finely powdered using electric grinder and stored in air tight glass containers for further use. Streptozotocin (STZ) and glibenclamide (GLB) with lot No. CMS1758 and BCBN1690V, respectively were procured from Sigma Aldrich. Standard kits (Diatek Health Care Pvt. Ltd.) were used for analysis of serum biochemical parameters. Hydro-alcoholic (50:50) extract of each powder was prepared and mixed in equal proportion to prepare polyherbal extract mixture (PHEM).

2.4 Induction of diabetes

Citrate buffer previously adjusted to pH 4.5 with 0.1 M citric acid was used to dissolve streptozotocin (STZ). This solution was administered by single intra-peritoneal injection at a dose rate of 50 mg/kg body weight to all animals except animals of group C1 (Normal control). Diabetes was confirmed after 48 h by measuring the level of blood glucose using glucometer (Alere GI, India). Rats with blood glucose level above >150 mg/dl were considered as diabetic and were used in the experiment (Kotadiya *et al.,* 2017ab; Kotadiya *et al.,*2018). All rats were randomly divided into different experimental groups based on their blood glucose level and body weight.

2.5 Experimental design

Completely Randomized Design (CRD) was employed with five different treatment groups having six rats in each treatment group. Rats of group C1, C2, and C3 were kept as normal, diabetic and standard control, respectively. Rats of group C3 were administrated with glibenclamide (5 mg/kg, PO for 28 days). Rats of group T1 and T2 were treated with PHEM at 100 and 200 mg/kg, respectively orally for 28 days.

2.6 Physical examinations, body weight and feed consumption

All rats during the entire study period were examined daily for any abnormal physical and behavioral changes. The body weight of individual rat was recorded daily. Feed offered to each group was accurately recorded daily in the morning and the residual feed given day before was also accounted. Based on these data, amount of feed consumed by rats of each group was calculated on daily basis.

2.7 Blood glucose levels and oral glucose tolerance test (OGTT)

Blood glucose level was estimated on day 0, 15, 22 and 29 of experiment, using glucometer (Alere GI, India). Oral glucose tolerance test (OGTT) was also carried out on day 15 of experimental period to check the status of diabetes. Glucose (2 g/kg) was administered to overnight fasted animals of all groups. Control animals (Group 1) were administered with equal volume of water only. Blood samples were withdrawn from retro-orbital plexus of each animal after glucose administration at 0, 30, 60, 90 and 120 min to know blood glucose level (Hepcy *et al*., 2012).

2.8 Hematological evaluation

Hematological parameters like hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count, (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were estimated by using automated hematology analyzer (Abacus Junior Vet 5, Diatron, Hungary) at Department of Veterinary Pathology, Junagadh Agricultural University, Junagadh, India.

2.9 Biochemical parameters

Biochemical parameters like blood glucose, total cholesterol (TC), HDL cholesterol, LDL cholesterol, triglyceride (TG), alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline

phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRT), total protein (TP), albumin (ALB), globulin (GLB) and total bilirubin (TB) were estimated by using standard kits with fully automatic biochemistry analyzer (Diatek Health Care Pvt. Ltd.).

2.10 Oxidative stress markers

2.10.1 Collection and preparation of samples

All rats were humanely sacrificed on 29th day of experiment and tissues of pancreas, liver and kidney were collected in phosphate buffer for evaluation of parameters of oxidative stress. Blood sample (50 µl) was mixed with 450 µl of RBC lysis buffer (Sigma Aldrich, Lot no. RNBG 0536) and kept for 5 min for efficient erythrocyte lysis. The resultant blood lysate was used for evaluation of catalase and glutathione (GSH) antioxidant enzymes, whereas the direct serum sample was used for analysis of superoxide dismutase (SOD). Pancreas, liver and kidney samples (100 mg) were collected from all rats and immediately stored in ice cold 0.1 M (1 ml) phosphate buffer (PB, pH:7.4) for evaluation of catalase and GSH, whereas pancreas, liver and kidney samples (0.5 g) were separately collected in Tris-EDTA buffer (pH:8.2) for analysis of SOD. Protein estimation in pancreas, liver and kidney was carried out using the standard method (Bradford, 1976). These data were used to calculate catalase activity in liver and kidney tissues.

2.10.2 SOD activity in serum sample

Serum (Cu-Zn) SOD activity was determined by a simple and rapid method based on the ability of the enzyme to inhibit the autoxidation of pyrogallol (Marklund and Marklund, 1974). The autoxidation of pyrogallol was investigated in the presence of Tris-EDTA at pH range 7.9-10.6. The rate of autoxidation increases with increasing pH. The autoxidation of pyrogallol in the presence of Tris-EDTA buffer at pH range 8.2-8.5 is 50%. For control reading: To 2.9 ml of Tris-EDTA buffer, 0.1 ml (20 mM) of pyrogallol solution was added, mixed and reading was taken at 420 nm, exactly after 1 min 30 sec and 3 min 30 sec. The absorbance (A) per two minutes difference was recorded, which shows rate of autoxidation of pyrogallol. For sample reading: To 2.8 ml of Tris-EDTA buffer, 0.1 ml of serum sample was added, mixed and started the reaction by adding 0.1 ml of pyrogallol solution (as per control). It was read at 420 nm exactly after 1 min 30 sec and 3 min 30 sec and absorbance (B) per 2 min difference was recorded. Units of SOD/3 ml of assay mixture was calculated from $(A - B) \times \frac{100}{A \times 50}$.

Unit × 10 = Units /ml of sample solution. One unit of superoxide dismutase is described as the amount of enzyme required to cause 50 % inhibition of pyrogallol auto oxidation per 3 ml assay mixture.

2.10.3 SOD activity in liver and kidney tissues

Superoxide dismutase (SOD) activity in tissues was determined according to the method described previously (Marklund and Marklund, 1974). All tissue homogenates were prepared in Tris-EDTA buffer centrifuged for 40 min at 10000 rpm at 4°C; the supernatant was used for the enzyme assay. Tris-EDTA (2900 µl) and 100 µl pyrogallol (2 mM) were taken in the cuvette and scanned for 3 min at 420 nm wavelength. Tris-EDTA buffer (2890 µl, pH 8.2), 100 µl pyrogallol and 10 µl of tissue homogenate were taken and scanned for 3 min at the same wavelength. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50% and was expressed as units/mg protein/min. The enzyme unit can be calculated by using the following equations:

$$\% \text{ of inhibition} = \frac{(A - B) \times 100}{B} .$$

$$\text{Enzyme unit (U)} = \frac{(\% \text{ of inhibition}) \times \text{common dilution factor (100)}}{50\ \% \text{ inhibition is similar to 1 U.}}$$

50% inhibition is similar to 1 U.

2.10.4 Catalase activity in blood

Twenty µl of blood lysate was mixed with 1980 µl PB (0.1 M PB, pH 7.5) in a test tube. Then 1 ml of 30 mM H_2O_2 was added to it and absorbance of reaction was taken at 240 nm in a spectrophotometer for 1 min, against blank having mixture of PB and blood lysate only without H_2O_2. Unit activity of catalase was expressed in molar/min (Aebi *et al.*, 1974).

2.10.5 Catalase activity from tissue

Tissue samples (100 mg) of pancreas, liver and kidney were homogenized using 1 ml PB (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 5 min. Then 20 µl of supernatant was taken out and mixed with 1980 µl PB (0.1 M, pH 7.4). One ml of H_2O_2 (30 mM) was added to it and absorbance of test sample was taken at 240 nm against blank having mixture of PB and tissue homogenate only. Activity of catalase was calculated using the molar extinction coefficient of 43.6 cm^{-1}(Aebi *et al.*, 1974). mmoles of H_2O_2 decomposed/min/mg protein was calculated with formula as (ΔA/min × 1000 × 3)/(43.6 × mg protein in sample). ΔA/min is mean absorbance change per minute.

2.10.6 GSH level in blood

Blood lysate (10 µl) was mixed with 2970 µl of PB (0.1 M PB, pH 7.5) in a test tube and dTNB (20 µl, 30 mM) was added in to it. The mixture was allowed for reaction up to 45 min and absorbance was taken at 412 nm against blank having mixture of PB and blood lysate only without dTNB using spectrophotometer. Concentration of GSH was expressed in molar (Ellman, 1959).

2.10.7 GSH levels in pancreas, liver and kidney tissues

Tissue samples (0.5 g) of liver and kidney were homogenized using 1 ml PB (0.1 M, pH 7.4). Tissue homogenate (0.5 ml) was added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA for precipitation of the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 10,000 rpm. The supernatant (200 µl) then transferred to a new set of test tubes and added with 1.8 ml of the Ellman's reagent (5, 50-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.1 M phosphate buffer with 1% of sodium citrate solution). All test tubes were made up to the volume of 2 ml. After completion of the total reaction, absorbance was measured at 412 nm against blank having mixture of PB and supernatant. Absorbance values were compared with a standard curve generated from known concentration of GSH (Ellman, 1959).

2.11 Pathological examination

All rats were humanely sacrificed using CO_2 and dressed on 29th day of the experiment in a confined disinfected laboratory and were subjected to gross pathological examination by systemic approach. Major organs like, pancreas, liver, spleen, kidney, heart and lungs were collected in 10% formalin for histopathological analysis. The formalin fixed tissues were subjected to paraffin wax embedding for tissue sectioning. Sections of each tissue collected were cut at 6-8 microns thickness with automatic section cutting machine, semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with hematoxylin and eosin (H and E) stain (Luna, 1968). The H and E stained slides were observed under microscope and microscopic pathological lesions were recorded.

2.12 Statistical analysis

All numerical data were presented as Means ± standard error (SE) and have been subjected to statistical analysis. Data were analyzed statistically by ANOVA, followed by Duncan's multiple range test (DMRT) to observe difference among the means of each parameter in different groups (Snedecor and Cochran, 1982).

3. Results and Discussion

3.1 Symptoms, body weight and feed consumption

After induction of diabetes with STZ, symptoms like dullness, sluggish movement, weight loss, polyuria, polydipsia and polyphagia were observed in rats of diabetic control group. Four animals of diabetic control group showed signs of polyuria and progressive reduction in body weight. These symptoms were mild to moderate in all other treatment groups except normal control group. Reduction of body weight in diabetes might be due to breakdown of tissue proteins in diabetic rats (Andulla and Varadacharyulu, 2003).

In our study, feed consumption was progressively increased in diabetic control rats (Figure 1). The feed consumption in diabetic rats treated with glibenclamide was significantly lower during 3rd and 4th week of experiment as compared to those observed in diabetic control group. The increased feed consumption in diabetic rats was not reduced with treatment of PHEM during 1st, 2nd and 3rd week of experiment. However, the mean value of feed consumption in diabetic rats treated with PHEM was reduced significantly (p<0.05) during 4th week of experimental period. This finding is supported by previous reports by Deepak *et al*. (2006) and Chaturvedi (2005) that extract of *Gymnema sylvestre* (Retz.) Schult.,and *Momordica charantia*L. alter the feed intake. Higher body weight was observed at the end of study period in C1, C2 and C3 experimental animal groups (Figure 2). Body weight was significantly (p<0.05) lower in rats treated with high dose of PHEM at the end of experimental period and the results obtained are in agreement with findings of Sujin *et al*. (2008) and Abdullah *et al*. (2007) that the administration of *Gymnema sylvestre* (Retz.) Schult.,and *Tinospora cordifolia* (Willd.) Miers herbal extracts reduces the body weight in diabetic rats, respectively. Kumar *et al*. (2008) reported that water soluble fraction of *Syzygium cumini* (L.) Skeels extract (120 mg/kg, p.o. for 21 days) in streptozotocin induced diabetic rats reduced the body weight.

3.2 Glucose levels

Streptozotocin (STZ) enters the pancreatic cell *via* a glucose transporter-GLUT2 (Glucose transporter 2) and causes alkylation of DNA. Further, it has been reported that STZ induces activation of poly adenosine diphoshate ribosylation and nitric oxide release, as a result of STZ action; pancreatic cells are destroyed by necrosis and finally induce insulin dependent diabetes (Patel *et al*., 2006). After diabetes induction with STZ, the blood glucose levels in rats of different groups were progressively increased up to 4 weeks during the experiment but treatment with glibenclamide and PHEM for a period of 28 days after induction of diabetes significantly (p<0.05) reduced blood glucose level especially at 4th week (Table 1). xBlood glucose levels were reduced up to 41.97 and 35.48% at day 29 compared to those values on day 22 in diabetic rats treated with PHEM at 100 and 200 mg/kg for 28 days, respectively. However, the blood glucose levels in diabetic rats treated with glibenclamide were lower at day 15, 22 and 29 compared to those values of rats treated with PHEM at both doses which indicates satisfactory antidiabetic effect of glibenclamide. Surprisingly, dose of STZ used in the study produced high level of blood glucose in the present study but the mixture prepared from individual plant extract and administered in diabetic rats showed remarkable result related to reduction on blood glucose level on day 29 of experiment which demonstrated its efficacy in diabetes. Eyo *et al*. (2011), Chetan *et al*. (2012), Khedekar *et al*. (2015), Rajesh *et al*. (2015), Kumar *et al*. (2008) and Perumal *et al*. (2015) also reported hypoglycemic action of *A. cepa, T. foenum-graecum, T. cordifolia,, G. sylvestre, S. cumini* and *M. charantia* plant extracts, respectively but those were observed at high dose. Presence of S-methylcysteine, kaempferol-3-O-β-D 6{P-coumaroyl} glucopyranoside and flavonoids in *A. cepa* (Ikechukwu and Ifeanyi, 2016); trigonelline, trigocoumarin, and trimecoumarine alkaloids in *T. foenum-graecum* (Al-Habori and Raman, 1998); tannins, saponins, and steroids in *T. cordifolia* (Singh *et al*., 2003); gurmarin, gymnemanol and gymnemic acids in *G. sylvestre* (Rao and Sinsheimer, 1971); mycaminose, jamboline and gallic acid in *S. cumini* (Sharma *et al*., 2008); charantin, insulin-like peptides and alkaloids in *M. charantia* (Raman and Lau, 1996), have been reported to be responsible for such property.

Table 1: Blood glucose (mg/dl) levels at different time interval in different treatment groups

Group	Day 0 (Mean ± SE)	Day 15 (Mean ± SE)	Day 22 (Mean ± SE)	Day 29 (Mean ± SE)
C1	93.17 ± 5.08^{a}	111.33 ± 5.33^{a}	106.67 ± 8.2^{a}	90.83 ± 3.50^{a}
C2	289.33 ± 62.28^{b}	368.00 ± 51.28^{b}	442.50 ± 45.4^{b}	419.00 ± 44.69^{c}
C3	290.33 ± 43.84^{b}	226.17 ± 41.25^{ab}	190.50 ± 26.7^{a}	167.50 ± 28.79^{ab}
T1	282.33 ± 34.07^{b}	404.50 ± 53.60^{b}	518.67 ± 81.6^{b}	301.00 ± 37.99^{bc}
T2	288.67 ± 27.84^{b}	410.50 ± 59.42^{b}	450.00 ± 71.7^{b}	290.33 ± 42.76^{bc}

Values with different superscript in a column were significantly different (p<0.05).

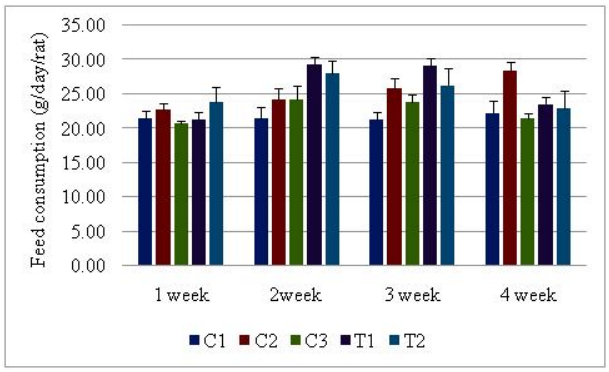

Figure 1: The average feed consumption (g/day/rat) of experimental animals in different groups.

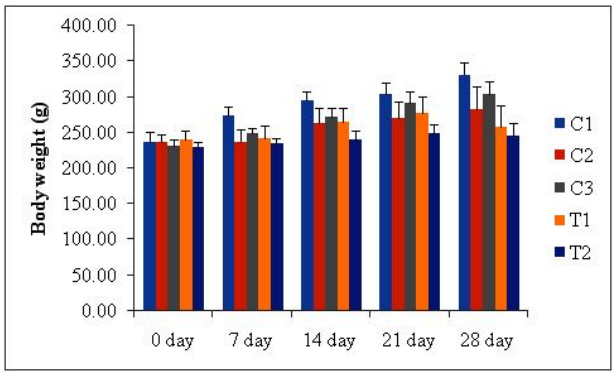

Figure 2: Body weight (g) of experimental animals of different groups.

On day 15, oral glucose tolerance test (OGTT) was carried out to evaluate the status of diabetic condition and it was observed that the blood glucose levels in rats of different treatment groups were significantly (p<0.05) higher at 30 min after oral glucose administration at dose of 2 g/kg as compared to normal rats (Figure 3). The values of glucose levels were significantly (p<0.05) reduced at 90 min and found significantly (p<0.05) lower at 120 min after glucose load which were comparable to the values at pre-glucose administration in C1 and C3 groups. The slight higher blood glucose levels in group C2, T1 and T2 indicates inability of PHEM to reduce the increased blood glucose level at day 15. This result was supported by no effect of PHEM treatment on blood glucose level up to 3rd week of the experiment.

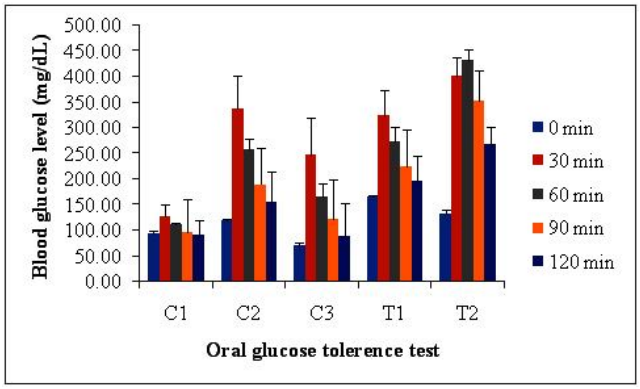

Figure 3: Glucose levels (mg/dl) in rats under oral glucose tolerance test (OGTT) at day 15 of experiment.

3.3 Haematological and biochemical parameters

Haematological parameters like Hb, PCV, TEC, TLC, MCV, MCHC, MCH and DLC were not significantly altered in diabetic rats (Table 2). The mean values of Hb, PCV and TEC were significantly higher in PHEM treated rats as compared to normal and diabetic control rats which showed little positive effect of PHEM in diabetes. Punithavathi *et al.* (2008) reported increased level of glycated hemoglobin in diabetic rats with subsequent decrease in total hemoglobin. In diabetes mellitus, hyperglycemia is accompanied with dyslipidemia that is characterized by increase in TC, LDL cholesterol and decrease in HDL cholesterol. The abnormally high concentration of serum lipids (TG) in diabetics is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots (Bopama *et al.*, 1997; Gupta *et al.*, 2009). In the present study, mean levels of TC and TG were non-significantly (p>0.05) increased and level of HDL-cholesterol was slightly decreased with significant (p<0.05) increase in level of LDL-cholesterol in diabetic control group as compared to other treatment groups (Table 3). Mean values of TC and TG were lower in both glibenclamide and PHEM treated groups. Also, mean values of HDL-cholesterol were significantly (p<0.05) increased, while mean values of LDL-cholesterol were significantly (p<0.05) decreased in both glibenclamide as well as in PHEM treated groups, showed their potential to have hypolipidemic action. Ozougwu (2011) also reported a significant dose dependent lowering of serum total lipid levels in streptozotocin induced diabetic rats when treated with *A. cepa*. Hypolipidaemic effect of *A. cepa* may be related to its active ingredient, allyl propyl disulphide (Kumari and Augusti, 2002). Presence of trigonelline in fenugreek seeds might be responsible for hypolipidaemic action which mediates its action by increased excretion of cholesterol and total bile acids into the feces (Muraki *et al.*, 2011). Also presence of gymnemic acids in *G. sylvestre* (Grijesh *et al.*, 2009); mycaminose in *S. cumini* (Kumar *et al.*, 2008) and charantin in *M, charantia* (Chaturvedi, 2005) might be responsible for hypolipidaemic effect of PHEM in the present experiment at lower dose in combination.

In biochemical parameters, non-significant increase in the values of ALT and AST has been observed in diabetic control rats (Table 3), which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes. However, PHEM treatment significantly reduced the elevated levels of AST in diabetic rats. Supporting to our findings, Arkkila *et al.* (2001) reported elevated activities of serum aminotransferases (ALT, AST, ALP) in liver and cardiovascular diseases are observed more frequently among people with diabetes. Increased levels of ALT and AST in diabetic rats indicates that diabetes may induce hepatic dysfunction. Supporting to our findings, it has been reported by Ohaeri (2001) that structure of liver was altered in diabetic rats. Therefore, the increase in the activities of ALT, AST and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Concepcion *et al.*, 1993). ALT and AST levels in diabetic rats treated with PHEM (T_1 and T_2) were lower as compared to those in diabetic rats which indicate partial corrective effect by herbal treatment in diabetic rats in the present study. Results obtained from the present study are clearly in agreement with previous reports by Renuka *et al.* (2009), Stanely *et al.* (2000) and Aziza *et al.* (2013) related to hepatoprotective activity of *T. foenum-graecum*, *T. cordifolia*, *G. sylvestre* herbal extracts, respectively in restoring back the elevated levels of ALT, AST and ALP in diabetes.

However, level of ALP in diabetic rats treated with PHEM was not significantly reversed which might be due to differences of doses or amount uses in the study. The mean BUN value (mg/dl) was increased in diabetic rats which was significantly lower in glibenclamide treated rats. However, the mean BUN (mg/dl) level of PHEM treated animals at day 29 of experiment were not significantly corrected which indicate partial effect of PHEM on kidney damage caused by STZ in rats. The mean value of creatinine (mg/dl) in diabetic control group was non-significantly (p>0.05) increased as compared to control group. No significant alteration (p>0.05) was observed in creatinine levels of rats of all other treatment groups as compared to diabetic control group. The mean value of bilirubin (mg/dl) in diabetic control group was non-significantly (p>0.05) increased as compared to control group. Whereas, the mean values of bilirubin (mg/dl) were non-significantly (p>0.05) lower in glibenclamide as well as in PHEM treated groups.

Table 2: Hematological parameters in rats of different treatment groups

Parameters	Treatment groups				
	C1	C2	C3	T1	T2
HB (g/dl)	17.10 ± 0.21^{a}	18.51 ± 0.43^{abc}	18.06 ± 0.45^{ab}	19.98 ± 0.61^{bc}	20.13 ± 0.48^{c}
PCV(%)	46.71 ± 0.42^{a}	50.88 ± 1.04^{ab}	49.33 ± 1.14^{ab}	51.33 ± 1.53^{b}	52.52 ± 1.57^{b}
TEC (10^{6}/µl)	9.28 ± 0.18^{a}	10.12 ± 0.26^{ab}	9.81 ± 0.23^{ab}	10.11 ± 037^{ab}	10.70 ± 0.31^{b}
WBC (10^{3}/cmm)	9.94 ± 1.20^{bc}	8.60 ± 0.28^{abc}	11.97 ± 1.62^{c}	7.37 ± 1.16^{ab}	5.22 ± 1.00^{a}
MCV (fl)	50.39 ± 0.63^{a}	50.16 ± 0.60^{a}	50.50 ± 0.76^{a}	50.66 ± 0.42^{a}	49.00 ± 0.52^{a}
MCHC (%)	36.65 ± 0.43^{a}	36.80 ± 0.28^{a}	36.45 ± 0.20^{a}	38.98 ± 1.04^{b}	38.41 ± 0.33^{ab}
MCH (pg)	18.46 ± 0.31^{a}	18.48 ± 0.15^{a}	18.40 ± 0.33^{a}	19.80 ± 0.59^{b}	18.85 ± 0.28^{ab}
Lymphocyte (%)	84.30 ± 2.11^{a}	83.48 ± 1.78^{a}	73.00 ± 6.64^{a}	77.41 ± 2.73^{a}	72.25 ± 8.56^{a}
Neutrophils (%)	12.85 ± 1.67^{a}	13.13 ± 1.16^{a}	23.33 ± 5.75^{a}	17.95 ± 1.14^{a}	24.65 ± 6.73^{a}
Eosinophil (%)	0.33 ± 0.21^{a}	0.33 ± 0.21^{a}	0.17 ± 0.17^{a}	0.33 ± 0.21^{a}	0.17 ± 0.17^{a}
Monocytes (%)	2.85 ± 1.10^{a}	3.03 ± 2.07^{a}	3.63 ± 1.61^{a}	4.65 ± 2.56^{a}	3.10 ± 1.99^{a}

Values with different superscript in a row were significantly different (p > 0.05).

Table 3: Biochemical parameters in rats of different treatment groups

Parameters	Treatment groups				
	C1	C2	C3	T1	T2
Total cholesterol (mg/dl)	129.67 ± 11.51^{a}	151.00 ± 18.50^{a}	134.67 ± 12.93^{a}	140.17 ± 13.16^{a}	142.83 ± 7.88^{a}
Triglycerides (mg/dl)	116.50 ± 9.67^{a}	123.33 ± 16.45^{a}	114.17 ± 6.94^{a}	116.00 ± 11.69^{a}	108.50 ± 14.66^{a}
HDL–cholesterol (mg/dl)	45.76 ± 3.79^{a}	42.36 ± 3.98^{a}	48.68 ± 9.01^{a}	71.27 ± 7.16^{b}	63.10 ± 4.48^{ab}
LDL–cholesterol (mg/dl)	17.27 ± 2.45^{ab}	21.03 ± 1.18^{b}	18.81 ± 3.86^{b}	15.52 ± 2.98^{ab}	9.45 ± 1.59^{a}
AST (IU/l)	136.33 ± 7.28^{ab}	148.24 ± 14.39^{b}	127.22 ± 15.75^{ab}	119.87 ± 8.19^{ab}	112.59 ± 8.96^{a}
ALT (IU/l)	41.13 ± 2.64^{a}	60.39 ± 7.32^{a}	55.12 ± 12.68^{a}	52.65 ± 9.10^{a}	45.79 ± 4.90^{a}
ALP (IU/l)	169.33 ± 7.60^{a}	349.85 ± 70.12^{ab}	299.02 ± 93.02^{ab}	476.75 ± 80.88^{b}	403.58 ± 92.33^{ab}
BUN (mg/dl)	25.00 ± 1.05^{ab}	40.70 ± 5.98^{b}	22.48 ± 1.87^{a}	42.92 ± 8.48^{b}	41.42 ± 3.85^{b}
Creatinine (mg/dl)	0.43 ± 0.05^{a}	0.49 ± 0.08^{a}	0.40 ± 0.09^{a}	0.60 ± 0.10^{a}	0.46 ± 0.11^{a}
Total protein (g/dl)	6.51 ± 0.10^{a}	6.17 ± 0.34^{a}	6.37 ± 0.13^{a}	6.34 ± 0.09^{a}	6.35 ± 0.21^{a}
Albumin (g/dl)	3.48 ± 0.03^{b}	3.08 ± 0.16^{ab}	3.06 ± 0.12^{a}	3.25 ± 0.05^{ab}	3.01 ± 0.17^{a}
Globulin (g/dl)	3.03 ± 0.09^{a}	3.09 ± 0.17^{a}	3.31 ± 0.15^{a}	3.09 ± 0.07^{a}	3.34 ± 0.23^{a}
Total bilirubin (mg/dl)	0.29 ± 0.01^{a}	0.60 ± 0.14^{a}	0.44 ± 0.14^{a}	0.46 ± 0.08^{a}	0.41 ± 0.17^{a}

Values with different superscript in a row were significantly different (p<0.05).

Table 4: Mean values of organ weight (pancreas, liver, kidneys, heart and spleen) in ratsunder different treatment groups

Organ	Organ weight (g)				
	C1	C2	C3	T1	T2
Pancreas	1.75 ± 0.14[a]	1.31 ± 0.27[a]	1.41 ± 0.16[a]	1.31 ± 0.15[a]	1.38 ± 0.13[a]
Liver	12.30 ± 1.26[a]	10.82 ± 1.35[a]	12.27 ± 0.53[a]	13.10 ± 1.28[a]	10.78 ± 1.28[a]
Kidneys	2.25 ± 0.13[a]	2.50 ± 0.13[a]	2.51 ± 0.13[a]	2.73 ± 0.22[a]	2.56 ± 0.25[a]
Heart	1.10 ± 0.06[a]	1.05 ± 0.13[a]	1.08 ± 0.05[a]	1.04 ± 0.11[a]	0.89 ± 0.07[a]
Spleen	0.61 ± 0.03[a]	0.51 ± 0.07[a]	0.67 ± 0.10[a]	0.47 ± 0.08[a]	0.44 ± 0.08[a]

Values with different superscript in a row were significantly different (p<0.05).

Table 5: SOD activity in different samples collected fromrats under different treatments

Sample	SOD (U/ml)				
	C1	C2	C3	T1	T2
Serum	11.05 ± 0.55[a]	8.67 ± 0.79[b]	9.52 ± 1.06[ab]	11.62 ± 0.64[a]	10.10 ± 1.01[a]
Liver	55.10 ± 9.11[a]	43.54 ± 4.46[a]	53.84 ± 4.96[a]	48.57 ± 4.98[a]	61.12 ± 4.94[a]
Kidney	42.52 ± 2.55[ab]	41.50 ± 1.36[a]	51.02 ± 3.16[c]	52.04 ± 1.56[c]	49.66 ± 1.80[bc]
Pancreas	65.99 ± 4.55[a]	59.76 ± 6.02[a]	67.59 ± 3.48[a]	65.44 ± 2.79[a]	69.15 ± 5.69[a]

Values with different superscript in a row were significantly different (p<0.05).

Table 6: Catalase activity in different samples collected from rats under different treatments

Sample	Catalase				
	C1	C2	C3	T1	T2
Blood (molar/min)	2.007 ± 0.151[a]	1.990 ± 0.144[a]	2.134 ± 0.173[a]	2.070 ± 0.110[a]	2.271 ± 0.139[a]
Liver (U/mg protein)	0.057 ± 0.006[ab]	0.043 ± 0.008[a]	0.057 ± 0.006[ab]	0.077 ± 0.008[b]	0.075 ± 0.007[b]
Kidney (U/mg protein)	0.181 ± 0.015[a]	0.157 ± 0.004[a]	0.192 ± 0.021[a]	0.191 ± 0.017[a]	0.188 ± 0.013[a]
Pancreas (U/mg protein)	0.465 ± 0.098[a]	0.329 ± 0.024[a]	0.361 ± 0.038[a]	0.331 ± 0.026[a]	0.389 ± 0.051[a]

Values with different superscript in a row were significantly different (p < 0.05).

Table 7: GSH levels in different samples collected from rats under different treatments

Sample	GSH				
	C1	C2	C3	T1	T2
Blood (molar)	9.42 ± 0.30[ab]	8.52 ± 0.26[a]	9.55 ± 0.67[ab]	10.17 ± 0.82[ab]	10.67 ± 0.49[b]
Liver (μg/mg of tissue)	0.18 ± 0.01[a]	0.14 ± 0.01[a]	0.22 ± 0.02[a]	0.15 ± 0.02[a]	0.17 ± 0.03[a]
Kidney (μg/mg of tissue)	0.28 ± 0.01[a]	0.25 ± 0.02[a]	0.28 ± 0.01[a]	0.25 ± 0.05[a]	0.28 ± 0.02[a]
Pancreas (μg/mg of tissue)	0.19 ± 0.01[ab]	0.16 ± 0.02[a]	0.24 ± 0.01[c]	0.21 ± 0.01[bc]	0.22 ± 0.01[bc]

Values with different superscript in a row were significantly different (p<0.05).

3.4 Weight and gross observations of organs

Effect of daily oral administration of PHEM (100 and 200 mg/kg, P.O) on organ weight in rats of different experimental groups are presented in Table 4. No significant (p>0.05) alterations in organ weight in any treatment group have been observed as compared to those of control rats. Upon gross examination of pancreas, no appreciable gross lesions in all treatment groups have been observed. Macroscopic examination of liver and kidneys of experimental rats of diabetic control group shown congestion, paleness and slight enlargement. No appreciable macroscopic lesions have been observed in the spleen, heart and lung of rats of different treatment groups.

3.5 Antioxidant defense system

The activity of SOD was significantly decreased in serum and non-significantly decreased in liver and pancreas of animals, treated with STZ, compared to those observed in control rats. These alterations were not observed in diabetic rats treated with glibenclamide and PHEM at both doses (Table 5). The catalase activity in liver, kidney and pancreas were found non-significantly lower in diabetic control rats compared to those in normal control rats. Compared to diabetic control rats, higher values of catalase activity have been observed in liver (significant) and serum, kidney and pancreas (non-significantly) (Table 6). The levels of GSH in blood, liver, kidney and pancreas were non-significantly lower in diabetic rats compared to those estimated in normal control rats. The values of GSH level in blood, liver, kidney and pancreas were improved in diabetic rats treated with glibenclamide as well as PHEM at both doses (Table 7).

Superoxide dismutase (SOD) belongs to a family of antioxidant enzymes that catalyze the dismutation of superoxide to yield hydrogen peroxide and oxygen. More production of superoxide ion leads to elevation in SOD activity to overcome the auto oxidation and oxidative stress. Catalase is a ubiquitous enzyme which is present in the peroxisomes that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic reactive oxygen species (ROS) production (Kohen and Nyska, 2002). In toxicity condition, the blood catalase utilized more due to production of more ROS. Catalase is inducible enzyme in tissues like liver, kidney and pancreas. During the exposure to xenobiotics, there is more accumulation of chemical in these organs leads to more metabolite production which increase more ROS and oxidative stress. GSH is an intra-cellular reluctant and plays a major role in catalysis, metabolism, and transport. It protects cell against free radicals, peroxides, and other toxic compounds (Aydemir *et al.*, 2000). Several studies have demonstrated that hyperglycemia in STZ induced diabetes has been associated with increased formation of reactive oxygen species (ROS) and oxidative damage to tissue components (Alireza *et al.*, 2009). Also oxidative stress in STZ induced diabetic animals is due to glucose auto-oxidation, protein glycation, formation of advanced glycation products and the polyol pathway that generates free radicals (Atalay and Laaksonen, 2002). Results related to improved oxidative stress markers in diabetic animals treated with PHEM in the present study are in agreement with previous findings reported by Coskun *et al.* (2005), Xue *et al.* (2011), Gupta and Sharma. (2011) and Aziza *et al.* (2013) related to antioxidant effects of *A. cepa*, *T. faenum-graecum*, *T. cordifolia* and *Gymnema sylvestre*, respectively.

3.6 Histopathological evaluation

Histopathological findings in pancreas and kidney of rats of all five groups are shown in Figures 4a to 4e and Figures 5a to 5e, respectively. The histopathological changes of pancreas of rats of diabetic control group (C2) revealed degenerative changes with loss of normal architecture of parenchyma. Islets of langerhans were shrunken with loss of its normal cell cord arrangement and degeneration in many of serous acini and islet of langerhans. While histopathological changes in pancreas collected from rats treated with PHEM (higher dose) shown an apparent increase in the size of an islet of langerhans with few cords of normal endocrine cells compared to diabetic control group. Histopathological changes of kidney of diabetic control rats showed increase bowman's capsular space, vacuolar degeneration and necrosis of tubular epithelium. While kidneys of rats treated with PHEM were with less degenerated epithelium in glomeruli as well as tubules compared to diabetic control group. No appreciable histopathological lesions have been observed in the liver, spleen, heart and lung of rats in all treatment groups.

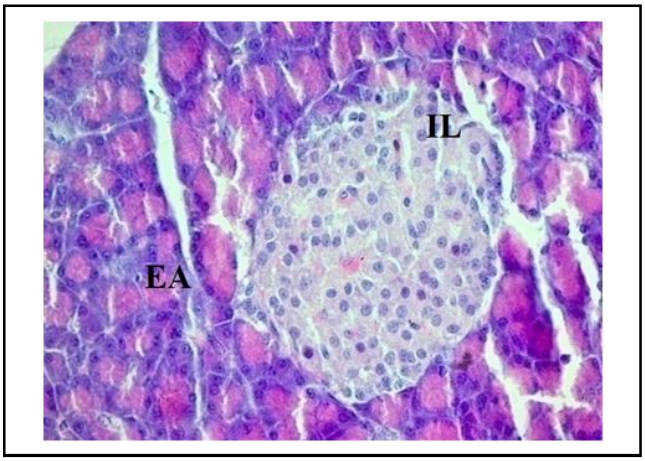

Figure 4(a): Microscopic view of pancreas in control group (C1) showing well-defined islet of langerhans (Il) surrounded by exocrine acini (EA) (H and E stain, 400X).

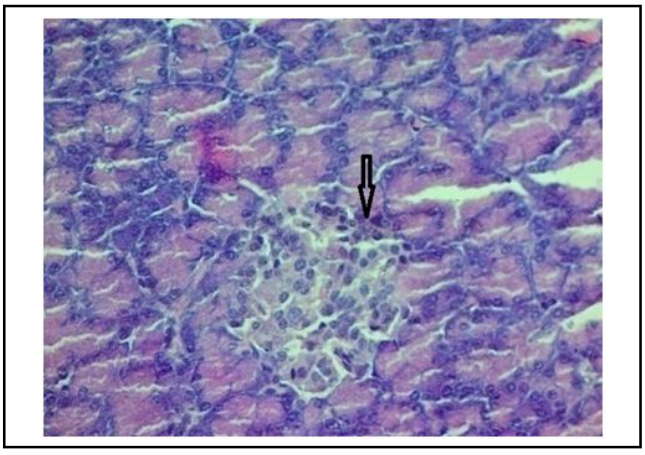

Figure 4(b): Microscopic view of pancreas in diabetic control (C2) group showing shrunken islets of langerhans with loss of its normal cell cord arrangement compare to control group (Arrow) (H and E stain, 400X).

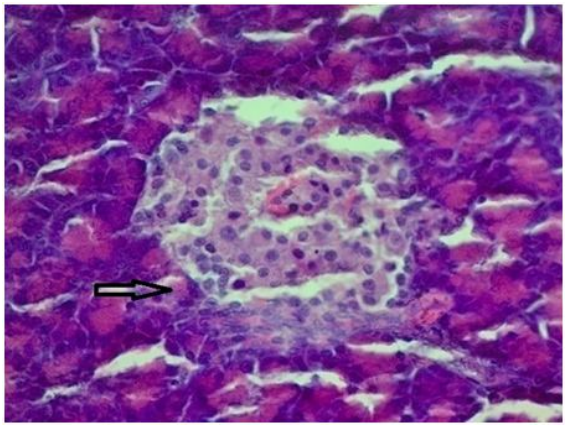

Figure 4(c): Microscopic view of pancreas in glibenclamide treated group (C3) showing an apparent increase in the size of an islet of langerhans (Arrow) (H and E stain, 400X).

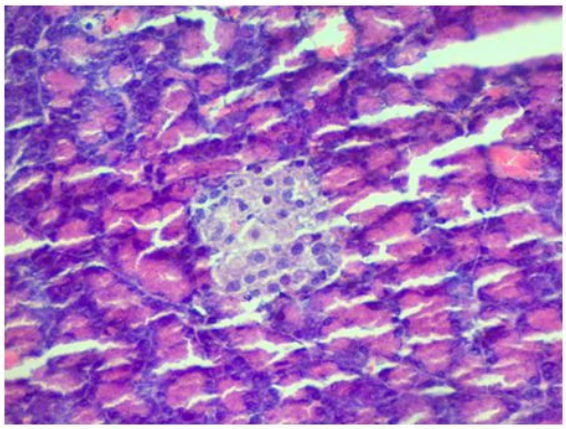

Figure 4(d): Microscopic view of pancreas in PHE treated group (T1) showing moderate cellularity (Arrow) (H and E stain, 400X).

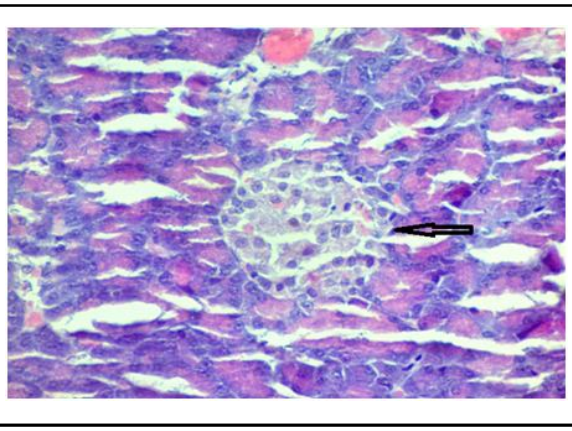

Figure 4(e): Microscopic view of pancreas in PHE treated group (T2) showing an apparent increase in the size of an islet of langerhans (Arrow) (H and E stain, 400X).

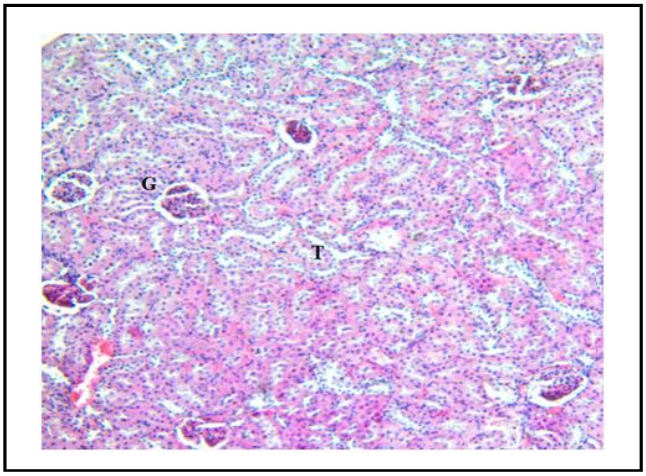

Figure 5(a): Microscopic view of kidney in control group (C1) showing normal architecture of glomeruli (G) as well as tubular (T) epithelium (H and E stain, 100X).

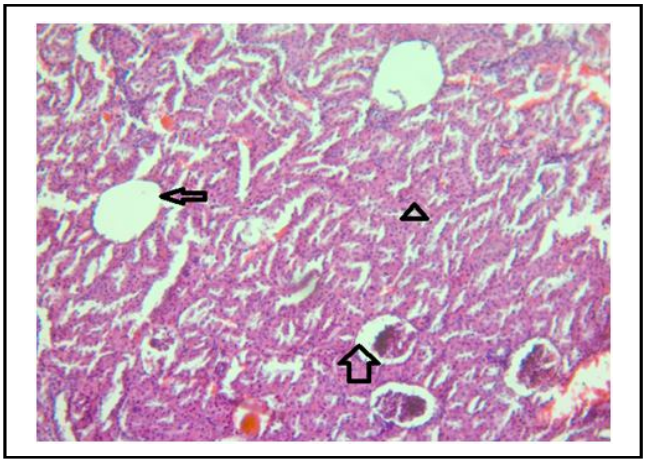

Figure 5(b): Microscopic view of kidney in diabetic group (C2) showing loss of glomerular structure (Thin arrow), increased bowman's capsular space (Thick arrow), vacuolar degeneration and necrosis of tubular epithelium (Arrow head) (H and E stain, 100X).

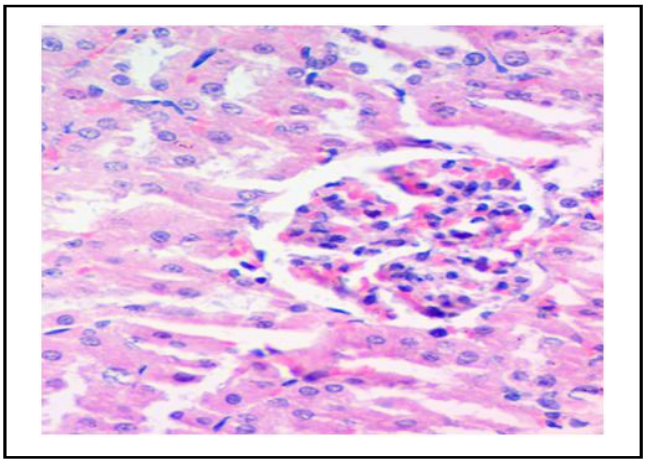

Figure 5(c): Microscopic view of kidney in group C3 showing normal architecture of glomeruli as well as tubular epithelium (H and E stain, 400X).

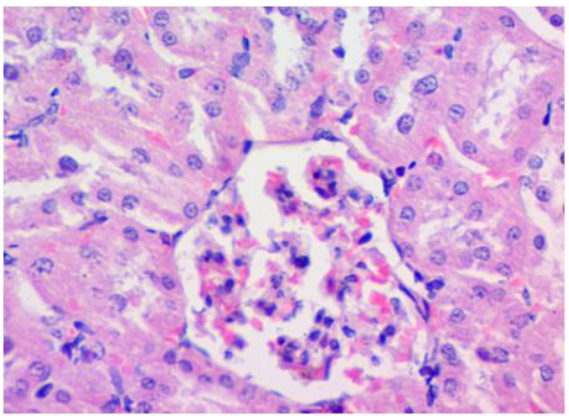

Figure 5(d): Microscopic view of kidney in group T1 showing normal architecture of glomeruli as well as tubular epithelium (H and E stain, 400X).

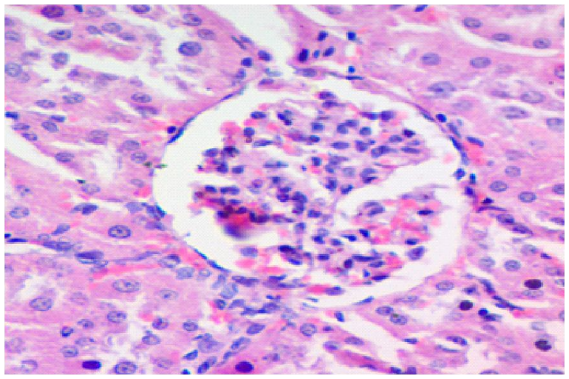

Figure 5(e): Microscopic view of kidney in group T2 showing normal architecture of glomeruli as well as tubular epithelium (H and E stain, 400X).

4. Conclusion

Polyherbal extract mixture with equal part of hydro-alcoholic extracts of leaves of *G. sylvestre*,peels of *A. cepa*, fruits of *M. charantia*, stem of *T. cordifolia* and dried seeds of *T. foenum-graecum*. and *S. cumini* at 200 mg/kg showed ameliorating effect against streptozotocin induced alterations in diabetic rats due having active principles with antioxidant potential. Further, efficacy of such extract mixture needs to be evaluated in other models of diabetes along with safety studies.

Conflict of interest

We declare that we have no conflict of interest.

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