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Studies on the effect of moringa leaf extract on blood glucose and insulin levels in streptozotocin induced diabetic rats

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Abstract
The present study was undertaken to evaluate the effect of Moringa leaf extract on blood glucose, body weights and insulin levels in streptozotocin (STZ) induced diabetic rats for a period of 21 days. The efficacy of the extract was compared with that of glibenclamide, a standard hypoglycaemic drug.

A total of 30 male Wistar albino rats were divided into 5 groups with 6 animals in each group. *Viz*: Normal control (NC) (Group-I), diabetic control (DC) (Group-II), diabetic rats treated with glibenclamide@0.6mg/kg bw (Group-III), diabetic rats treated with *Moringa oleifera* (MO) leaf extract @300 mg/kg bw (Group-IV) and MO leaf extract @400 mg/kg bw (Group-V). All group of rats were subjected to evaluation of body weight, blood glucose and serum insulin levels on day 0, 7, 14 and 21 of the experiment. There was significant (P<0.05) decrease in body weight and serum insulin and significant (P<0.05) increase in blood glucose level in DC rats compared to NC rats. In the Present study, daily oral administration of MO leaf extract at dose rate of 300 and 400 mg/kg bw and glibenclamide at 0.6mg/kg bw in diabetic rats for 21 days showed a progressive improvement in body weight, blood glucose and serum insulin concentration.

Histopathological alterations in pancreas and kidney were studied in control and experimental group rats which were sacrificed on 21st day of the experiment. Microscopic examination of pancreas from DC rat's revealed destruction, decreased number, altered distribution of α and β cells and damaged acinar cells. While, treated groups revealed a moderate restoration in damaged histoarchitecture. Similarly, sections of kidney from DC rats showed a minimal degenerative changes in both pancreas and kidney, suggestive of regenerative changes in treated group.

The overall inference of the study is that, the Moringa leaf extract at dose rate of either 300 or 400 mg/kg bw used showed protective effect against the damage caused by streptozotocin induced diabetes in decreasing blood glucose and improving body weight and insulin hormone levels, thus suggestive of its therapeutic value in Type 2 Diabetes mellitus.

Keywords: DM, STZ, moringa leaf extract, glibenclamide, blood glucose, insulin

Introduction
Diabetes mellitus (DM) is one of the most prevalent metabolic disorder affecting both human and animals, characterized by relative or absolute defects in both insulin secretion and insulin action and consequent disturbances in carbohydrate, fat and protein metabolism [1]. Besides humans, DM is also a common disease in dogs and cats [2]. This disease has been virtually reported from all species of lab animals including non-human primates [3].

There are two types of DM, type I and type II. Type I is caused due to insulin insufficiency, because of lack of functional beta cells. Patients suffering from type I DM are therefore totally dependent on exogenous source of insulin, while type II DM which is the most common type, is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion.

The therapeutic management of DM with minimal side effects remains a clinical challenge. There is growing interest in the potential use of medicinal plant products as an alternative treatment for DM as these are commonly cheaper, less toxic and with fewer side effects [4].

Moringa oleifera (MO), commonly called 'drumstick' belongs to the family Moringaceae, a multipurpose tree found almost all over Asian and African countries. Most of the parts of the plant possess antimicrobial activity [5]. They are also known for their pharmacological actions and are recommended for varied conditions such as hepatotoxicity, rheumatism, venomous bites and also for Type II DM [6, 7].

Hence the present study was aimed with the following objectives.

1. To induce DM in rats using Streptozotocin.
2. To assess the efficacy of *Moringa oleifera* leaf extract in two different doses in mitigating the changes due to DM induction in lowering blood glucose level and Increase in insulin levels.
3. To compare the hypoglycaemic effect of *Moringa oleifera* leaf extract with that of standard drug Glibenclamide used in the treatment of type 2 DM.

Materials and Methods

In the present study, a total of 30 male albino *Wistar* rats weighing 200-250 g were procured from M/S Jeeva life sciences Pvt. Ltd, Hyderabad. All rats were housed in polypropylene cages at the Laboratory Animal Facility in an environmentally controlled room with 22± 3º C temperature and 30-70% relative humidity and Light/dark cycles of 12/12 hours were provided throughout the study period. Sterilized rice husk was used as bedding material. All the rats were provided with standard pellet diet procured from National institute of Nutrition (NIN), Hyderabad and deionized water at *ad libitum* throughout the experimental period. All protocols, as per CPCSEA guidelines on the care and use of laboratory animals, were followed, with prior approval of the Institutional Animal Ethics Committee vide ref No. (III /2019-07/ IAEC/ C. V. Sc., Hyd, Dated 17/04 /2019).

Drugs and chemicals

Streptozotocin (STZ) was purchased from M/S SRL Pvt. Ltd., Mumbai. Glibenclamide was purchased from M/S Qualigens Pvt. Ltd., Mumbai. Rat INS (Insulin) ELISA kit was purchased from M/S Nexgen Scientifics, Rangareddy, Telangana. Moringa leaf extract capsules were procured from M/s Inlife Healthcare, Inlife-Pharma Pvt. Ltd., Hyderabad, Telangana. Whereas Stains and chemicals for histopathology of pancreas and kidney were obtained from M/S Qualigens Pvt. Ltd., Mumbai.

Streptozotocin, Glibenclamide and Moringa leaf extract formulation

STZ of required quantity was dissolved in ice cold 0.1 M citrate buffer (pH 4.5) to give a final concentration of 30 mg/Kg body weight. This preparation was readily injected intraperitoneally to rats in order to avoid degradation.

Glibenclamide (Daonil®, 5 mg) was dissolved in distilled water (82.33 ml) to give a concentration of 60 µg/ml. This was used as a stock solution and administered orally at a dose of 600 µg/Kg body weight [8].

M. oleifera leaf extract capsules were dissolved in distilled water and administered at dose of 300mg/kg bw and 400mg/kg bw respectively. Solutions were administered per oral to rats using an oral gavage needle attached to a 2 ml syringe.

Experimental Design

All the groups were maintained as per the following schedule for 21 days.

Group I: Normal control

Group II: Diabetic control: Streptozotocin i.p.30mg/kg BW

Group III: Standard: Streptozotocin i.p. + 0.6mg/kg BW Glibenclamide (oral)

Group IV: Streptozotocin i.p.+300mg/kg BW *Moringa oleifera* (oral).

Group V: Streptozotocin i.p.+400mg/kg BW *Moringa oleifera* (oral).

Experimental induction and conformation of diabetes

The animals were fasted overnight and diabetes was induced in group II to V by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (30 mg/kg b w) in 0.1 M cold citrate buffer of p^H 4.5 [8]. Control (Group I) animals received citrate buffer alone. The blood glucose levels were estimated 72 hours post STZ injection using Accu-Check Glucometer in order to confirm the diabetic state in animals. The animals with blood glucose levels above 200 mg/dL were considered as diabetic. After confirmation of diabetic state, all the groups received their respective treatments daily for 21 days.

Collection of blood and Tissue samples

During the experimental period, blood samples were collected on day 0, 7, 14, and 21 from all the animals by puncturing the retro-orbital plexus under light ether anesthesia with the help of capillary tube [9]. Blood samples (1 ml) collected in centrifuge tubes without anticoagulant were allowed to clot at room temperature (26 ± 2 ℃). Serum was harvested by centrifugation at 1500× g for 10 minutes at 15 ºC and stored at -80 ºC for biochemical analysis. After collection of blood samples on day 21, all the rats were sacrificed by cervical dislocation. Tissue samples *viz.*, kidneys and pancreas were collected and preserved in 10% formalin solution for histopathological examination.

Recording of Body weights

Body weights of all the animals were taken using electronic weighing balance, initially on the day of initiation of treatment (Day 0) and subsequently on day 7, 14 and 21 of experiment.

Determination of Blood Glucose and Insulin Levels

Blood samples were collected from the orbital sinus of the rats. Determination of the blood glucose levels was done by the glucose-oxidase principle [10]. Accu-Check (Roche Diagnostics, Manheim, Germany) was used for the determination of the blood glucose levels of the animals and results expressed as mg/dl [11]. Fasting insulin was determined using Elisa method (Insulin ELISA test kits, Wuhan Fine Biotech Co., Ltd. China).

Statistical analysis

Data obtained (body weights, blood glucose and insulin hormone) was subjected to statistical analysis by applying Two-way ANOVA and using statistical package for social sciences (SPSS) version 25.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P<0.05$.

Results

Body weight

The mean values of body weights (g) in different groups (I, II, III, IV and V) were ranged from 239.5±3.69 to 240.5±6.85 on 0^{th} day, 234.3±3.90 to 249.1±5.84 on 7^{th} day, 224.8±6.05 to 260±7.64 on 14^{th} day and 222.8±6.43 to 275.5±8.89 on 21^{st} day of experiment. Significantly ($P<0.05$) lower mean values of body weights were observed in group II rats when compared to groups (I, III,IV and V) on 14^{th} and 21^{st} day of experiment (Table 1).

Blood glucose

The mean blood glucose levels (mg/dL) in different groups (I, II, III,IV and V) were ranged from 98.3±1.11 to 340.5±5.52 on 0th day, 99.5±2.17 to 346±5.10 on 7th day, 98±1.09 to 351±3.86 on 14th day and 98.8±1.30 to 359.8±2.56 on 21st day of experiment. Significantly (P<0.05) higher mean values of blood glucose levels were observed in group II rats when compared to groups (I, III,IV and V) on7th, 14th and 21st day of experiment (Table 2).

Insulin hormone

The mean serum insulin levels (µIU/mL) in different groups (I, II, III,IV and V) were ranged from 8.13±0.14 to 18.27±1.19 on 0th day, 7.81±0.16 to 18.66±1.15 on 7th day, 6.975±0.21 to 17.82±1.09 on 14th day and 6.14±0.06 to 18.73±1.06 on 21st day of experiment. Significantly (P<0.05) lower mean values of serum insulin levels were observed in group II rats when compared to groups (I, III,IV and V) on7th, 14th and 21st day of experiment (Table 3).

Histopathological Study

Pancreas

The pancreas from control group rat showed Normal architecture of islet of Langerhans and pancreatic acini. The section of pancreas from DC rats showed shrunken ILH, loss of demarcation between α and β cells and degeneration of exocrine acinar epithelial cells. Similarly, pancreas from Group III (DM+G) showed reconstructive appearance of ILH and exocrine acinar cells. Group IV (DM+MO 300) rats showed rejuvenation of β cells of ILH with normal appearance of acinar cells. Group V (DM+MO 400) rats showed regenerative appearance of islets of Langerhans and pancreatic acini.

Kidney

The section of kidney of normal control rat showed a normal architecture and uniform size of glomerulus and renal tubules. Kidney of DC rats showed an increase in bowman's space, shrunken and atrophied glomerulus and cystic dilatation with distorted glomerulus. There were degenerative necrotic changes in these epithelium. Group III (DM+G) rats showed a reconstructive appearance of glomerular tufts and renal tubules with mild tubular epithelial degeneration. Group IV (DM+MO 300) rats showed mild variation in size and shape of glomerulus and renal tubules and Group V (DM+MO 400) rats showed rejuvenation of glomerular tufts, mild degenerative changes in tubular epithelial cells and few areas with cystic inter tubular dilatations with hyper cellularity and restored architectural details of tubules.

Table 1: Body weights (g) in different experimental groups of rats

Groups		Days post-treatment		
	0	7	14	21
Group-I (NC), Normal Control	240 ± 6.80^{a,q}	249.1 ± 5.84^{a,q}	260 ± 7.64^{a,pq}	275.5 ± 8.89^{a,p}
Group-II (DC), Diabetic Control (DC)	240 ± 4.32^{a,p}	234.3 ± 3.90^{a,pq}	224.8 ± 6.05^{b,pq}	222.8± 6.43^{c,q}
Group-III (DM+G), DM+ Glibenclamide @0.6mg/kg	239.5 ± 3.69^{a,q}	240.1 ± 2.85^{a,pq}	246.3 ± 3.92^{a,pq}	251.6 ± 4.57^{b,p}
Group-IV (DM+MO 300), DM+Moringa oleifera@ 300mg/kg	240.5 ± 6.85^{a,p}	243.2 ± 7.11^{a,p}	245 ± 6.74^{a,p}	250.5 ± 6.94^{b,p}
Group-V (DM+MO 400), DM+Moringa oleifera @400mg/kg	239.8 ± 1.64^{a,q}	241.6 ± 2.23^{a,q}	243.8 ± 2.21^{a,pq}	248.1 ± 2.24^{b,p}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)

^{a,b,c} Means sharing different superscripts in a column differ significantly ($P \leq 0.05$)

^{p,q} Means sharing different superscripts in a row differ significantly ($P \leq 0.05$)

Table 2: Blood glucose concentration (mg/dL) in different experimental groups of rats

Groups		Days post-treatment		
	0	7	14	21
Group-I (NC), Normal Control	98.3 ± 1.11^{b,p}	99.5 ± 2.17^{c,p}	98 ± 1.09^{c,p}	98.8 ± 1.30^{d,p}
Group-II (DC), Diabetic Control	336.3 ± 7.16^{a,q}	346.0 ± 5.10^{a,pq}	351 ± 3.86^{a,pq}	359.8 ± 2.56^{a,p}
Group-III (DM+G), DM+ Glibenclamide @0.6mg/kg	340.5 ± 5.52^{a,p}	322.5 ± 4.44^{b,q}	301.3 ± 3.57^{b,r}	281.50 ± 3.28^{c,s}
Group-IV (DM+MO300), DM+Moringa oleifera @ 300mg/kg	337.6 ± 6.61^{a,p}	329.6 ± 5.33^{b,pq}	312.8 ± 7.71^{b,qr}	296.17 ± 7.03^{b,r}
Group-V (DM+MO 400), DM+Moringa oleifera @400 mg/kg	338.8± 5.61^{a,p}	326.6 ± 5.15^{b,p}	310.1 ± 5.70^{b,q}	292.1 ± 3.74^{bc,r}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)

^{a,b,c,d} Means sharing different superscripts in a column differ significantly ($P \leq 0.05$)

^{p,q,r} Means sharing different superscripts in a row differ significantly ($P \leq 0.05$)

Table 3: Insulin concentration (µIU/mL) in different groups of rats

Groups		Days post-treatment		
	0	7	14	21
Group-I (NC), Normal Control	18.27 ± 1.19^{a,p}	18.66 ± 1.15^{a,p}	17.82. ± 1.09^{a,p}	18.73 ± 1.06^{a,p}
Group-II (DC), Diabetic Control	8.13 ± 0.14^{b,p}	7.81 ± 0.16^{c,p}	6.975 ± 0.21^{d,q}	6.14 ± 0.06^{d,r}
Group-III (DM+G), DM+ Glibenclamide @0.6mg/kg	8.41 ± 0.11^{b,s}	9.89 ± 0.14^{b,r}	11.86 ± 0.14^{b,q}	13.35 ± 0.16^{b,p}
Group-IV (DM+MO300), DM+Moringa oleifera @300mg/kg	8.16 ± 0.10^{b,s}	8.86 ± 0.13^{bc,r}	10.22 ± 0.10^{c,q}	11.45 ± 0.09^{c,p}
Group-V (DM+MO 400), DM+Moringa oleifera @400 mg/kg	8.26 ± 0.12^{b,s}	9.25 ± 0.14^{bc,r}	11.09 ± 0.78^{bc,q}	12.88 ± 0.36^{bc,p}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)

^{a,b,c,d} Means sharing different superscripts in a column differ significantly ($P \leq 0.05$)

^{p,q,r,s} Means sharing different superscripts in a row differ significantly ($P \leq 0.05$)

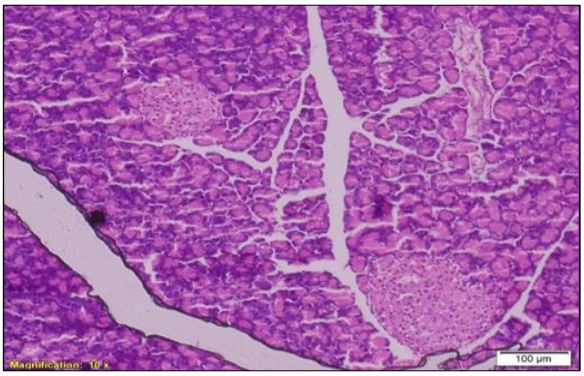

Fig 1: Photomicrograph of pancreas showing normal architecture of islets of langerhans and pancreatic acini (Group I). H&E X 100 µm

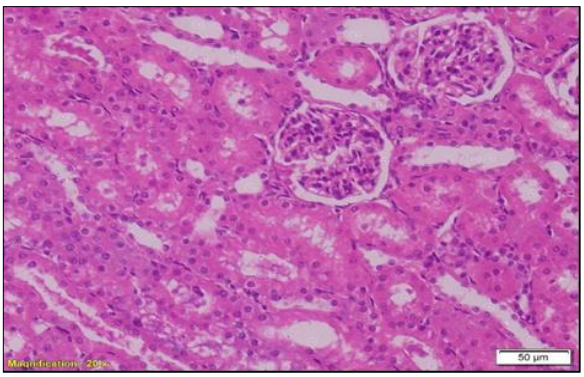

Fig 2: Photomicrograph of kidney showing uniform size glomerulus and normal renal tubules (Group I). H&E X 50 µm

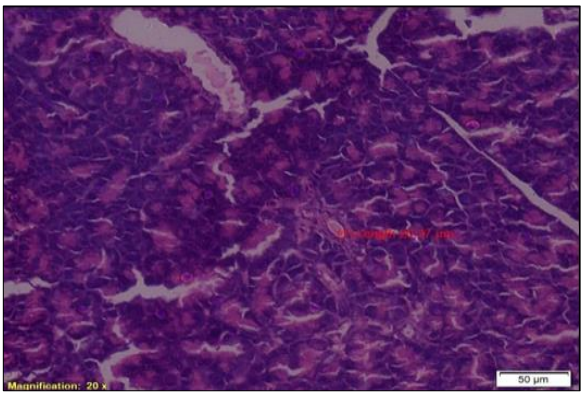

Fig 3: Photomicrograph of pancreas showing shrunken ILH, loss of demarcation between α and β cells and degeneration of exocrine acinar epithelial cells. (Group II). H&E X 50 µm

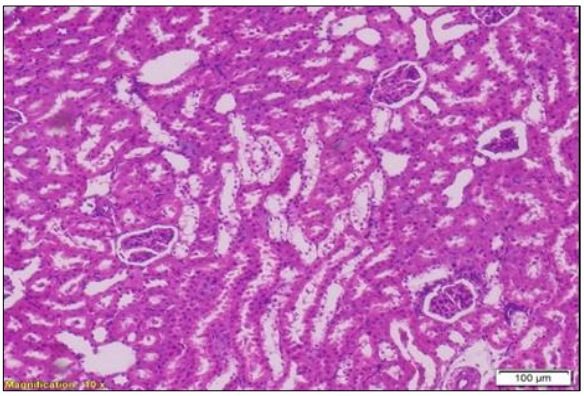

Fig 4: Photomicrograph of Kidney showing shrunken and distorted glomerulus with increased bowman's space, cystic dilatation, cast in the tubular lumen and degenerative necrosis of tubular epithelium (Group II). H&E X 100 µm

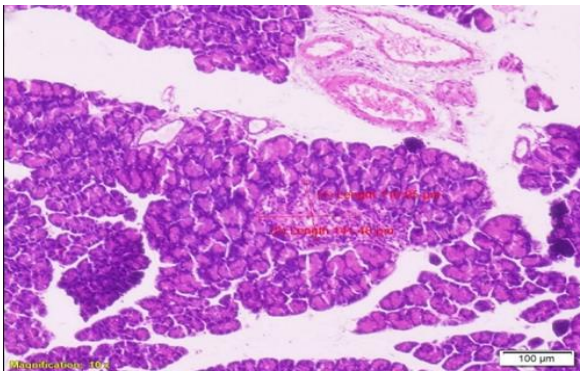

Fig 5: Photomicrograph of pancreas showing reconstructive appearance of ILH and exocrine acinar cells (Group III). H&E X 100 µm

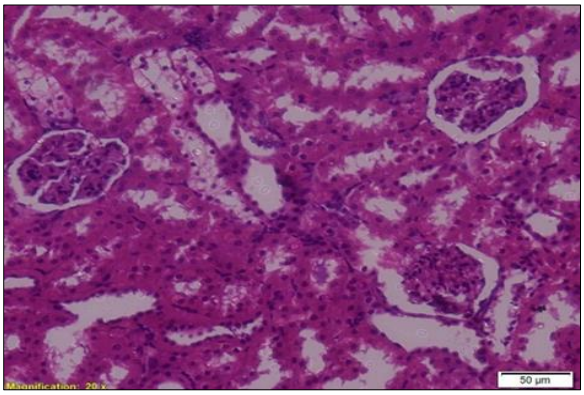

Fig 6: Photomicrograph of kidney showing swollen and shrunken glomerulus and mild tubular epithelial degeneration (Group III). H&E X 50 µm

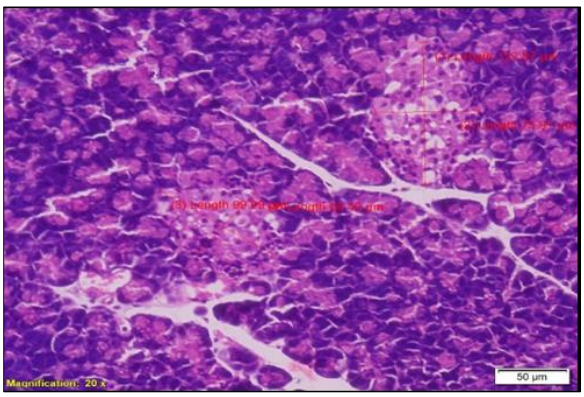

Fig 7: Photomicrograph of pancreas showing increase no of β-cells and normal histo-architecture of islets of langerhans and acinar cells (Group IV). H&E X 50 µm

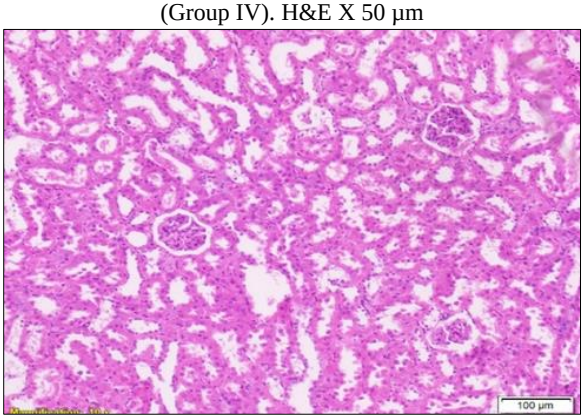

Fig 8: Photomicrograph of kidney showing variation in size and shape of glomerulus (Group IV). H&E X 100 µm

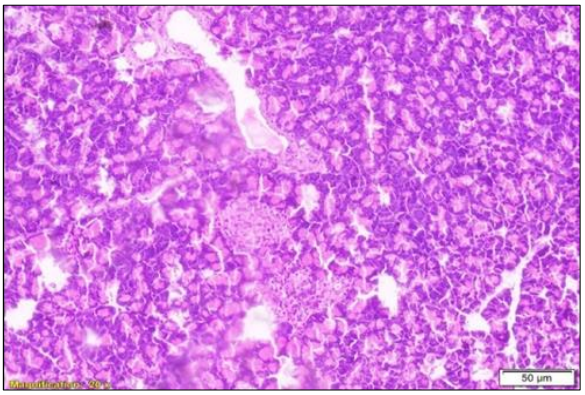

Fig 9: Photomicrograph of pancreas showing regenerative appearance of ILH and pancreatic acini (Group V). H&E X 50 µm

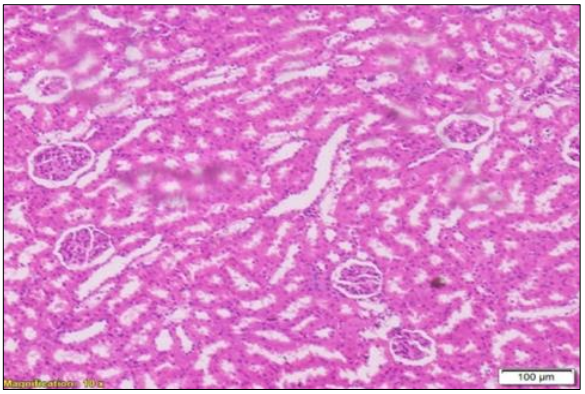

Fig 10: Photomicrograph of kidney showing rejuvenation of glomerular tufts, mild degenerative changes in tubular epithelial cells and few areas with cystic inter tubular dilatation (Group V).H&E X 100 µm.

Discussion

In our study, significantly (P<0.05) lower mean values of body weights were observed in group II rats when compared to groups (I, III,IV and V) on 14th and 21st day of experiment (Table 1). Since insulin is an anabolic hormone, its deficiency in DM group may have resulted in catabolism of carbohydrates (CHO), proteins and fats leading to loss of body weight. Further the reduced body weight may also be due to decreased protein synthesis in the absence of insulin and dehydration due to glycosuric polyuria [12, 13].

There was significant (P<0.05) increase in blood glucose was observed in STZ induced diabetic rats and when diabetic rats administered orally with moringa leaf extract (300mg/kg & 400 mg/kg) and standard drug glibenclamide (0.6 mg/kg), a reduction in blood glucose levels were observed. The glibenclamide administration appears to be more effective in regulating blood glucose when compared to MO groups as also reported by [14, 15]. Further, moringa at higher concentration appears to have no significant advantage over lower dose.

There was significant (P<0.05) decrease in serum insulin levels in STZ induced diabetic rats and when diabetic rats administered orally with moringa leaf extract (300mg/kg & 400 mg/kg) and standard drug glibenclamide (0.6 mg/kg), a progressive increase in serum insulin levels were observed. MO may have potentiated glucose induced insulin secretion from existing β cells or preventing damage of these cells due to the presence of phytochemical constituents like quercetin and kaempferol [16], flavonoids and phenol, terpenoids, glycoside and alkaloids [17].

The section of pancreas from DC rats showed a destruction of number of β cells while effecting both exocrine and endocrine components of the gland. The progressive decrease in the size of pancreas may be attributable to the cytotoxic effect of streptozotocin on β-cells of islets as well as damage to exocrine portion [18, 19]. Similarly, section from kidney of DC rats showed an increase in Bowman's space, shrunken and atrophied glomerulus and cystic dilatation with degenerative changes in these epithelium. Similar findings were reported [18, 19] in STZ induced DM. The changes in the kidney may be attributed to the damaging effect of STZ on kidney cells since these cells also express GLUT2 transporter [20].

The histopathological studies with MO supplemented Group (IV & V) and glibenclamide (0.6 mg/kg) were suggestive of marked rejuvenation of β cells of islets of Langerhans with normal appearance of acinar cells of pancreas. Further, sections of kidney showed reconstructive appearance of glomerular tufts and renal tubules with mild tubular epithelial degeneration. Reports of other workers [18, 19] also indicated similar observations.

The present study was conducted for a period of 21days and this may be the reason for the glucose and insulin levels in experimental groups have not returned to near normal values. Therefore, it is necessary to conduct the work over an extended period to ascertain conclusive benefits of MO in treatment of Type II DM.

Conclusion

The hypoglycemic activity of MO leaf extract in the present study is well compared with standard drug glibenclamide and further suggested that there is only a marginal or no benefit by increased in moringa supplementation from 300mg/kg bw to 400mg/kg bw. In conclusion, the moringa leaf extract may be considered as an alternative remedy in regulating Type II DM.

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