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Antidiabetic and Hypolipidemic Effect of Ethanolic Seed Extract of *Prosopis juliflora* in Fructose Induced Hyperglycemia in Rats

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To evaluate the antidiabetic and hypolipidemic effect of ethanolic seed extract of Prosopis juliflora in fructose induced hyperglycemia in wistar albino rats in comparison with Metformin. 30 male wistar albino rats were divided equally into 5 groups. Group I and II were the normal and the disease control groups. While, groups III to V were the treatment groups. Animals in group I received regular drinking water; whereas, groups II to V received 20% fructose water for 8 weeks. After 8 weeks, animals in groups II to V had elevated fasting blood sugar, HOMA-IR, weight gain and dyslipidemia. From week 9 to 16 group I animals continued to receive regular drinking water, group II received 2ml of distilled water and groups III, IV and V received Metformin 200mg/kg, P.juliflora extract 400mg/kg and 600mg/kg respectively in addition to 20% fructose water. The animals were sacrificed at the end of 16 weeks and histopathological examination of pancreas was done. Biochemical and hematological assessments were done at baseline and at 16 weeks to assess safety of the interventions. When compared to the disease control group, animals in group III treated with metformin and groups IV and V treated with P.juliflora extract at doses of 400mg/kg and 600mg/kg showed a significant decrease in Fasting blood glucose, HOMA-IR and improvement in lipid profile. Even though both the doses of the extract showed significant pharmacological activity, 600mg/kg showed better activity equivalent to metformin. Histopathological examination of pancreas showed regenerative changes in the metformin and P.juliflora 600mg/kg treated groups. No significant abnormality was observed in the biochemical and haematological parameters at the end of the study. *P.juliflora* seed extract in the dose 400 mg/kg and 600mg/kg exhibited antidiabetic and hypolipidemic activity with no significant adverse events, in this study. Both the doses were having anti dyslipidemic effect similar to metformin whereas 600 mg/kg dose of P.juliflora was having better antidiabetic effect comparable to Metformin.

Keywords: Diabetes; Dyslipidemia; Fructose; Metformin, Metabolic syndrome; *P. juliflora*.

"Diabetes mellitus (DM) is a chronic heterogeneous metabolic disorder characterized by persistently elevated blood glucose levels (hyperglycemia) with disturbances in carbohydrate, protein and fat metabolism as a result of defect in insulin secretion, insulin action or both". The

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chronic hyperglycemia in diabetes can cause damage, dysfunction and failure of various organs particularly the kidneys, eyes, heart, nerves and blood vessels.[1]

Diabetes can be classified based on its etiology into type I and type II. Type I diabetes mellitus (T1DM) occurs as a result of absolute insulin deficiency due to autoimmune destruction of pancreatic beta cells. It is commonly seen among the children and adolescents.[2] Type II diabetes (T2DM) occurs as a result of impaired insulin secretion or increased insulin resistance due to beta cell dysfunction or insulin resistance due to reduced responsiveness of the target tissue like liver, skeletal muscle and adipose tissues towards insulin. It is commonly seen among the older individuals.[3] Insulin resistance in T2DM causes increased production as well as reduced clearance of triglyceride rich lipoproteins thereby causing altered lipid metabolism and diabetic dyslipidemia.[4]

Diabetes can be managed with both pharmacological agents and non-pharmacological approaches such as like lifestyle modifications. Proper glycemic control helps in preventing and delaying complications due to diabetes. T1DM can be managed with insulin replacement therapy, whereas T2DM can be managed with a combination of oral hypoglycaemic agents, dietary changes and exercise. In spite of the availability of several groups of drugs, most of the patients do not respond to mono drug therapy and might require a combination of drugs for optimal glycemic control.[5] Most of the conventional antidiabetic drugs are not devoid of adverse effects. Hence there is a constant search for newer agents including traditional medicines.

Currently many studies are being conducted on a large number of medicinal plants and some of these medicinal plants have proven their efficacy in treatment of DM. *Prosopis* species are commonly used in folk medicine for treatment of various diseases including DM.[6]

Prosopis juliflora (Sw) DC belongs to the family-fabacea and subfamily-mimosoideae. It is commonly called as mesquite.It is an aggressive invader and propagates through the seeds. Various parts of *P.juliflora* like the seeds, leaves and the fruits have been reported to have to have antibacterial[7-10], antifungal[11-14], antioxidant activity[15], anticancer[16-18] and antidiabetic properties.[19,20] Though the effect of *P.juliflora* seeds on blood sugar levels has already been reported by literature, there are no data available regarding the effect of *P.juliflora* seeds on insulin levels and lipid profile, which usually get altered in patients with diabetes. In this study we used fructose to induce diabetes and diabetic dyslipidemia in wistar rats and planned to evaluate the anti-diabetic and hypolipidemic effect of *P.juliflora* seeds. Fructose is a lipogenic sugar. Increased consumption of fructose causes hepatic lipogenesis, which raises plasma triglyceride levels, lowers VLDL clearance, activates genes involved in hepatic de novo lipogenesis, and promotes triglyceride accumulation in hepatocytes and skeletal muscle. Ectopic triglyceride accumulation in tissues eventually leads to dyslipidaemia and insulin resistance.[21]

In our study we used fructose solution to induce T2DM in wistar albino rats as this model will induce features of T2DM along with metabolic symptoms like obesity, dyslipidemia and hyperinsulinemia. Also, patients with T2DM often present with obesity, reduced insulin sensitivity and beta cell compensatory mechanisms like excess basal insulin secretion and hyperinsulinemia. Diabetes and metabolic syndrome are often interrelated and many times, metabolic disorders may coexist along with T2DM.

Also, this model overcomes the disadvantages of chemically induced diabetic models by streptozotocin and alloxan as they cause selective loss of pancreatic beta cells and hyperglycemia occurs predominantly due to the cytotoxic effects on beta cells.[22] Because of the spontaneous regeneration of beta cells, chemically induced diabetic models are often less stable and occasionally reversible. In addition to their cytotoxic effects on beta cells, these chemicals can also have toxic effects on other organs, especially the liver and the kidney. Despite being the most widely used model, chemical induction of T2DM has recently been criticized because it causes beta cell toxicity, which leads to rapid and accelerated destruction of beta cells causing insulin deficiency more than insulin resistance thus mimicking T1DM rather than T2DM.[23] Due to the limitations of chemically induced diabetic models, we attempted to induce insulin resistance along with dyslipidemia in wistar rats by substituting the regular drinking

water with 20% fructose solution and the model's stability was determined by monitoring the fasting glucose and insulin levels. We further evaluated the antihyperglycemic and hypolipidemic effect of *P.juliflora* seed extract in wistar rats with fructose induced hyperglycemia as this model induced diabetic dyslipidemia along with insulin resistance.

Objectives

i. To assess the antidiabetic effect of ethanolic seed extract of *Prosopis juliflora* with the following outcome measures:

• Fasting blood sugar (FBS), Homeostatis model assessment- estimated insulin resistance (HOMA-IR).

ii. To assess the hypolipidemic effect of ethanolic seed extract of *Prosopis juliflora* with the following outcome measures:

• Total cholesterol (TC), Triglycerides (TG), LDL cholesterol (low-density lipoprotein), VLDL cholesterol (very-low density lipoprotein) and HDL cholesterol (high-density lipoprotein).

MATERIALS AND METHODS

Seeds collection and extract preparation

P.juliflora pods were collected near Madipakkam, Chennai, Tamil Nadu, and the seeds were separated. The plant and the seeds were identified and certified by an authorized botanist before preparation of the extract. Seeds of *P.juliflora* (500g) were washed, shade dried, powdered and soaked in 1 liter of ethanol (99.9 % v/v) for 72 hours with periodic shaking. The supernatant was filtered using whatman filter paper no 1. The filtrate obtained was evaporated using water bath for 48 hours. Finally, a brown sticky extract was obtained. The percentage yield obtained was 3.9%. Extract is stored in airtight container in refrigerator at 2-8 °C for further use.

Phytochemical screening

Phytochemical analysis of the seed extract was performed to identify different phytochemicals such as saponins, alkaloids, glycosides, terpinoids, flavanoids and steroids using the methods described in literature. [24-28]

Animals

This study was carried out with Institutional Animal Ethics Committee (IAEC) approval (Ref no: IAEC 1/Proposal:58/A.Lr:41/ Dt:05.03.2021). 30 male Wistar albino rats weighing approximately 180-200g were housed in individual clean polypropylene cages. They were maintained at a temperature of 23-25 °C humidity 50-60% in alternate light and dark cycle with food and water.

Induction of hyperglycemia along with dyslipidemia

Hyperglycemia and dyslipidemia were induced by administering 20% fructose water daily for 16 weeks.[29-33] Each rat was housed in a separate cage. 20% fructose solution was prepared daily by dissolving 2g of fructose powder in 10 ml of drinking water. Fructose water was provided daily through animal feeding bottles which were fit to the cage and the amount of fructose water consumed by the animals was monitored daily. If the animals exhausted all the fructose water, then regular drinking water was provided for the rest of the day. Animals with FBS level > 150mg/dl were included for the experiment.

Dosage selection of interventions

The dose of Metformin was calculated based on the formula given in FDA draft guidance for calculating animal dose from the human equivalent dose. The maximum recommended dose of Metformin is 2000 mg/day in humans. For a 60 kg human, the dose will be 33.3 mg/kg/day. The animal equivalent dose is calculated by multiplying the factor 6.2 for rats (FDA).[34] The calculated animal dose of Metformin is 206.7 mg/kg/day. The value was rounded off to 200 mg/kg/day for Metformin in this study. The doses of the *P.juliflora* seed extract 400 mg/kg and 600 mg/kg was decided based on previous studies. [19,35] The interventions were be dissolved in 2 ml of distilled water and administered via orally using gavage.

Drugs and Reagents

D-fructose was purchased from Rankem laboratory India ; Metformin was obtained from Intas Pharmaceuticals Ltd, India; Enzyme Linked Immunosorbent Assay kit for estimating rat fasting insulin levels was purchased from Bioassay technology Pvt. Ltd; Ethanol (99.9%) was purchased from Changshu Hongsheng Fine chemicals Co.Ltd; FBS levels were measured using glucometer- Contour plus, Ascensia Diabetes Care India Pvt Ltd; Lipid profile parameters (TC, TG, LDL, VLDL and HDL) were assessed using

auto-analyser. Ketamine was purchased from Neo laboratories, India and Halothane was purchased from Raman & Weil Pvt Ltd, India.

Experimental design

30 male wistar albino rats divided into 5 groups and each group had 6 animals. The day of induction (starting fructose) was taken as day '0' (baseline).

Study assessments

All the parameters were assessed at baseline, 8 weeks and 16 weeks. For assessing all the biochemical parameters mentioned below except FBS, blood was collected via retro orbital route after administering ketamine anesthesia intra peritoneally (50mg/kg). For measuring FBS level blood samples were obtained from tail tip.

- FBS levels were measured after overnight fasting using glucometer.
- For the assessment of beta cell function and insulin resistance, serum insulin levels were measured after overnight fasting with rat insulin ELISA kit and HOMA-IR was calculated using the formula (fasting insulin (micro U/L) * fasting glucose (nmol/L)/22.5
- Lipid profile (TC, TG, LDL, VLDL and HDL) using auto-analyser.
- Body weight was measured using automatic weight scale.

All the rats were sacrificed using high dose halothane anesthesia at the end of 16 weeks and histopathological examination (HPE) of pancreas was done.

Statistical analysis

Graphpad instat version 3.0 was used for performing statistical analysis. All the continuous variables were summarized as mean + Standard deviation. For comparing the data within the groups and between the groups, inferential statistical tests such as paired t test& repeated measures ANOVA (for within group analysis) and one way ANOVA with Tukey's post hoc test (for between group analysis) were used. P value less than 0.05 was considered as statistically significant.

RESULTS

All the animals in the groups II to V developed features of T2DM and insulin resistance evidenced by increase in FBS levels and HOMA-IR. There was no mortality throughout the experimental duration.

Phytochemical screening

The phytochemical screening of the extract showed the presence of alkaloids, flavanoids and steroids.

Results of in vivo study

Body weight (BW)

All groups had similar BW at baseline. At 8 weeks all groups gained significant weight. At 16 weeks, the normal and disease control groups (I and II) gained significantly more weight compared to the baseline and 8 weeks, whereas the treatment groups (III to V) lost weight significantly when compared to 8 weeks (within group analysis,

Table 1. Details of the grouping and study interventions

Groups	No of rats	Treatment 0 to 8 weeks	Treatment 9 to 16 weeks
I (control)	6	Regular drinking water	Regular drinking water
II (Hyperglycemia control)	6	20% fructose solution in drinking water	20% fructose solution in drinking water + 2 ml distilled water
III	6	20% fructose solution in drinking water	20% fructose solution in drinking water + Metformin 200 mg/kg
IV	6	20% fructose solution in drinking water	20% fructose solution in drinking water + *P.juliflora* seed extract 400mg/kg
V	6	20% fructose solution in drinking water	20% fructose solution in drinking water + *P.juliflora* seed extract 600mg/kg

P<0.0001). Intergroup comparison of BW showed that, although all the groups gained weight after 8 weeks, animals in fructose supplemented groups (II to V) had significantly higher weight gain than the normal control group (I) (P<0.0001). At 16 weeks, Metformin and extract treated groups (400mg/kg and 600mg/kg) lost weight significantly when compared to 8 weeks. On the other hand, the normal and disease control groups (I and II) continued to gain weight. Nevertheless, group II gained significantly more weight than group I (P<0.001), and weight loss was similar and not statistically significant among groups III to V (P>0.05). Data on BW is showed in figure 1.

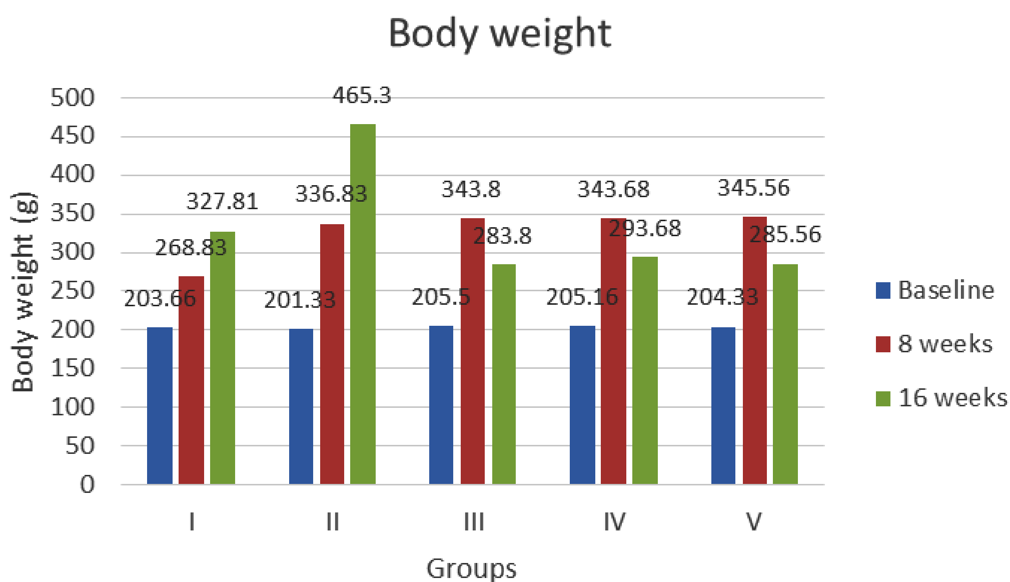
Fasting Blood sugar

All the groups (I-V) had similar FBS at baseline. In fructose fed groups (II to V) FBS increased at 8 weeks (all rats had FBS>150mg/

Table 2. Fasting blood sugar

Groups	Fasting blood Sugar (mg/dl)mean + SD			P value	
	Baseline	8 Weeks	16 Weeks	Within group comparison (Repeated measures ANOVA)	Inter group comparison (One way ANOVA)
I	8610.431	87.6610.78	87.59.50	0.27	< 0.0001 *
II	85.665.95	158.167.05	178.667.36	< 0.0001*	
III	875.17	159.666.31	127.165.52	< 0.0001*	
IV	84.835.45	163.335.46	150.56.38	< 0.0001*	
V	84.668.73	160.665.46	131.664.84	< 0.0001*	

Post hoc analysis:Group I (16weeks) Vs Groups III, IV and V (16 weeks)- P<0.001*, Group II (16 weeks) Vs groups III, IV and V (16 weeks)-P<0.001*, Group III (16weeks) Vs Group IV (16 weeks)-P<0.001*, Group III (16weeks) Vs Group V (16 weeks)- P>0.05, Group IV (16weeks) Vs Group V (16 weeks)-P<0.01*



Within group p-value (Repeated measures ANOVA): Groups I, II, III, IV and V- P<0.0001*; Inter-group comparison (one way ANOVA)- P<0.0001*

Post hoc analysis: Groups III, IV and V (8 weeks) Vs Groups III, IV and V (16 weeks)- P<0.001*, Group I (16weeks) Vs Group II (16 weeks)-P<0.001*, Groups III (16weeks) Vs Groups IV and V (16 weeks)- P>0.05, Group IV (16weeks) Vs Group V (16 weeks)-P>0.05

Fig. 1. Effect on body weight

dl). At 16 weeks, Metformin and extract treated groups (III to V) showed significantly reduced FBS compared to 8 weeks (within group analysis, P<0.0001) while the disease control group did not show any difference and the FBS remained elevated. When the reduction in FBS was compared among the groups, Metformin and extract (600mg/kg) showed similar reduction in FBS. Group IV (400mg/kg), though reduced the FBS levels before and after treatment, it was not comparable to Metformin and 600mg/kg extract. Although the FBS level decreased with treatment in the Metformin and extracts treated groups (III to V), their levels were still higher when compared to the normal control group (group I). The data of fasting blood sugar levels is shown in table 2.

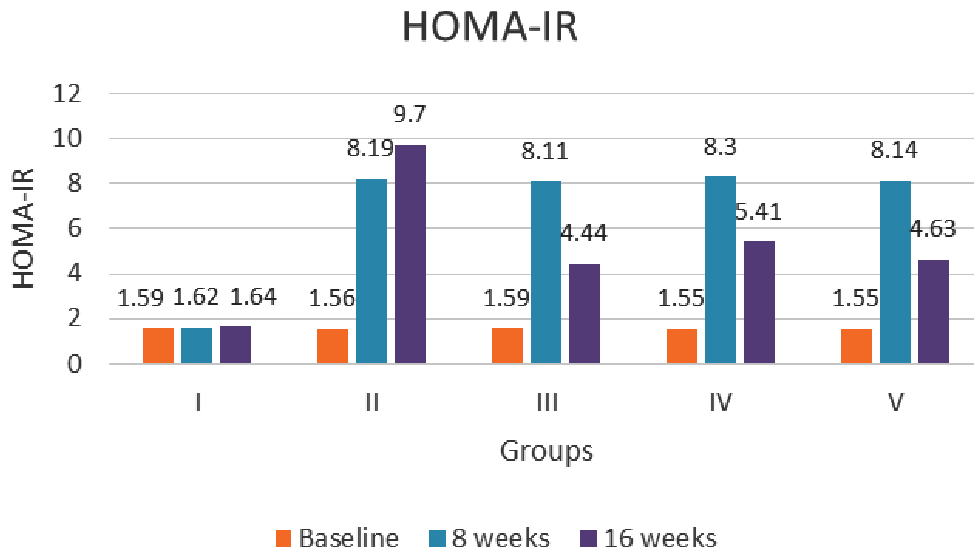
HOMA-IR

All the groups (I-V) had similar HOMA-IR levels at baseline. At 8 weeks, the HOMA-IR level increased significantly in the fructose

Table 3. Effect on HDL cholesterol

Groups	HDL cholesterol (mg/dl) mean± SD			P value	
	Baseline	8 Weeks	16 Weeks	Within group comparison (Repeated measures ANOVA)	Inter group comparison (One way ANOVA)
I	27.83±1.60	27.5±1.64	27.6±1.21	0.59	<0.0001*
II	27.5±1.87	22.5±1.37	19.5±1.37	<0.0001*	
III	27.66±1.50	20.5±1.87	23.5±1.37	<0.0001*	
IV	27.33±1.96	20.3±2.33	23±1.89	<0.0001*	
V	27.5±1.64	20.1±2.04	23.3±1.36	<0.0001*	

Post hoc analysis: Group I (16weeks) Vs Groups III, IV and V (16 weeks)- P<0.01*, Group II (16 weeks) Vs Groups III, IV and V (16 weeks)- P<0.01*, <0.05* and <0.05* respectively. Group III (16 weeks) Vs Group IV and V (16 weeks)- P>0.05, Group IV (16 weeks) Vs Group V (16 weeks)- P>0.05



Within group p-value (Repeated measures ANOVA): group I = 0.08 and groups II, III, IV and V <0.0001* Inter group p-value (one way ANOVA)- P<0.0001*

Post hoc analysis:Group I Vs III to V (16 weeks)- p<0.001*, Group II Vs III to V (16 weeks)-p<0.001*, Group III Vs IV (16 weeks)-P<0.001*, Group III Vs V (16 weeks)- p>0.05, Group IV Vs V (16 weeks)-P<0.05*

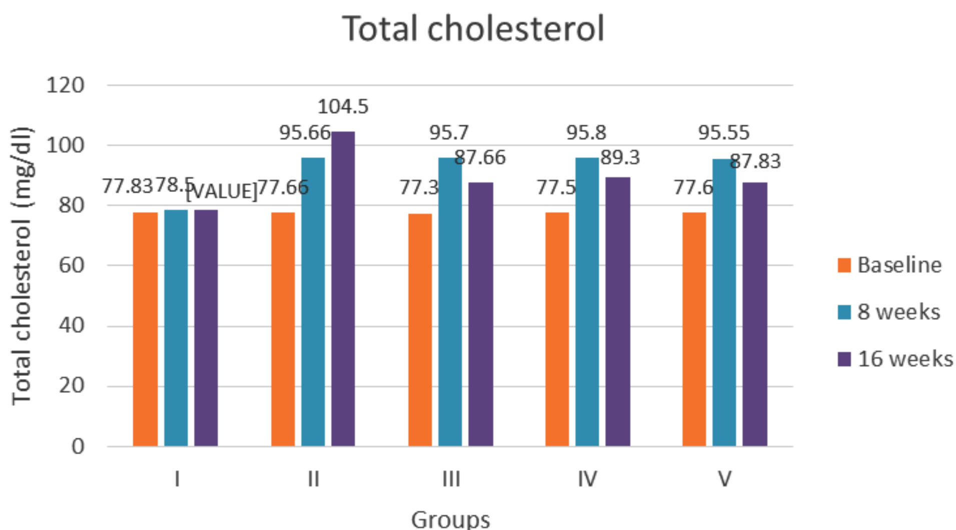
Fig. 2. Homeostasis model assessment- estimated insulin resistance

supplemented groups (II to V). At 16 weeks, Metformin and extract treated groups (III to V) showed significant reduction in the HOMA-IR levels compared to 8 weeks (within group analysis, P<0.0001). However, HOMA-IR remained elevated in disease control group (II) till the end of the study (within group analysis, P< 0.0001). Intergroup comparison of the reduction in HOMA-IR levels showed that, group III and V treated with Metformin and extract (600mg/kg) showed similar reduction in HOMA-IR level. Even though group IV (400mg/kg) reduced the HOMA-IR levels before and after treatment, it was not comparable to Metformin and 600mg/kg extract. Although HOMA-IR levels decreased with treatment in the Metformin and extracts treated groups (III to V), it still remained higher than the normal control group (group I). Data is shown in figure 2.

Table 4. Effect on Triglycerides

Groups	Triglycerides (mg/dl) mean± SD			P value	
	Baseline	8 Weeks	16 Weeks	Within group (Repeated measures ANOVA)	Inter group (One way ANOVA)
I	60.5±1.87	60.66±2.06	61.16±2.04	0.10	<0.0001*
II	60.16±3.06	73.5±3.45	78.83±2.92	<0.0001*	
III	60.66±1.86	73.66±1.50	70.16±1.47	<0.0001*	
IV	60.33±2.25	73.33±1.50	70±1.67	<0.0001*	
V	60.5±1.37	74.16±1.16	70.1±1.16	<0.0001*	

Post hoc analysis: Group I (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group II (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group III (16 weeks) Vs Group IV and V (16 weeks)- P>0.05 and Group IV (16 weeks) Vs Group V (16 weeks)-P>0.05



Within group P-value (Repeated measures ANOVA): Group I = 0.14 and Groups II, III, IV and V P<0.0001*, Inter group P-value (one way ANOVA)- P<0.0001*

Post hoc analysis: Group I (16weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group II (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group III (16weeks) Vs Group IV and V (16 weeks)- P>0.05, Group IV (16 weeks) Vs Group V (16 weeks)-P>0.05

Fig. 3. Effect on Total cholesterol

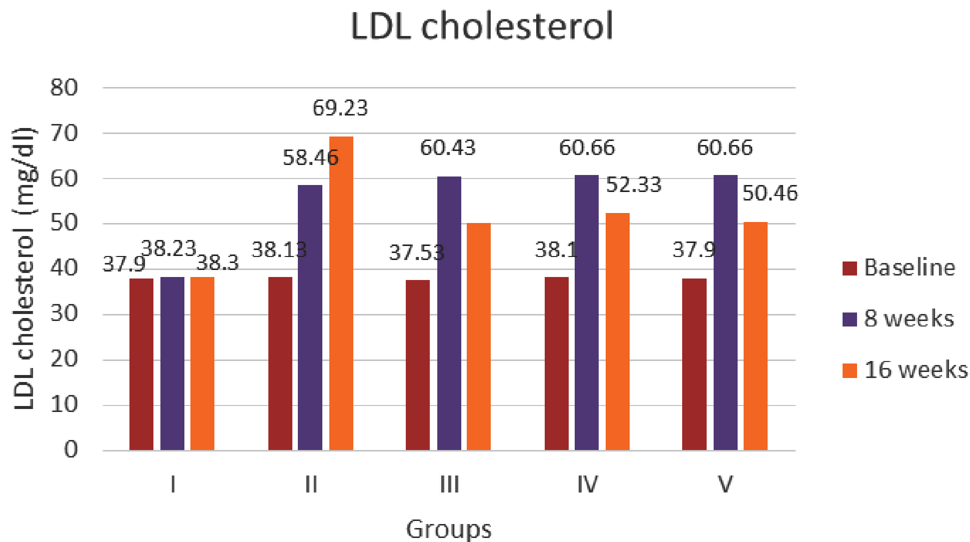
HDL cholesterol

HDL level in the normal control (group I) remained constant throughout the duration of the study. At 8 weeks, HDL levels decreased significantly in the fructose supplemented groups (II to V). At 16 weeks, HDL levels increased significantly in metformin and extract treated groups (III to V) when compared to 8 weeks due to the effect of the treatment, whereas it remained low in the disease control animals due to the progression of the disease (group II) (Within group analysis, P<0.0001). When the HDL levels were compared among the different groups, it was observed that the Metformin and extracts 400mg/kg and 600mg/kg treated groups (III to V) had significantly higher HDL levels at 16 weeks when compared to the disease control group (II). Also, the increase in the HDL levels in these groups were similar and comparable. However, when compared to the normal control group, HDL levels were lower

Table 5. Effect on VLDL cholesterol

Groups	VLDL (mg/dl) mean± SD			P value	
	Baseline	8 Weeks	16 Weeks	Within group (Repeated measures ANOVA)	Inter group (One way ANOVA)
I	12.1±0.37	12.13±0.41	12.23±0.40	0.10	<0.0001*
II	12.03±0.61	14.7±0.68	15.76±0.58	<0.0001*	
III	12.13±0.37	14.73±0.30	14.03±0.29	<0.0001*	
IV	12.06±0.45	14.66±0.30	14±0.33	<0.0001*	
V	12.1±0.27	14.83±0.23	14.03±0.23	<0.0001*	

Post hoc analysis: Group I (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group II (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group III (16 weeks) Vs Groups IV and V (16 weeks)- P>0.05 and Group IV (16 weeks) Vs Group V (16 weeks)-P>0.05



Within group p-value (Repeated measures ANOVA): Group I = 0.10 and Groups II, III, IV and V P<0.0001*, Inter group p-value (one way ANOVA)- P<0.0001*
Post hoc analysis: Group I (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group II (16 weeks) Vs Groups III, IV and V (16weeks)- P<0.001*, Group III (16 weeks) Vs Groups IV and V (16 weeks)- P>0.05 and Group IV (16 weeks) Vs Group V (16 weeks)-P>0.05

Fig. 4. Effect on LDL cholesterol

in these groups. Data of HDL levels are shown in table 3.

TC, TG, LDL and VLDL cholesterol

In the normal control animals (group I) TC, TG, LDL and VLDL levels remained constant throughout the experimental duration. These levels, on the other hand, were significantly elevated in the fructose fed groups (II to V) at the end of 8 weeks, and decreased after treatment in the metformin and extract treated groups (III to V) but remained elevated in the disease control animals (group II) (within group analysis, P<0.0001). Intergroups comparison showed that at the end of 16 weeks TC, TG, LDL and VLDL levels decreased significantly in the Metformin and extract treated (400mg/kg and 600mg/kg) groups when compared to the disease control animals (group II). But, when compared to the normal control animals (group I), the levels remained elevated. Furthermore, in the Metformin and extract treated (400mg/kg and 600mg/kg) groups the reduction in the levels of these parameters in these groups were almost similar and comparable. Data of TC and LDL cholesterol is shown in figure 3, 4 and data on TG and VLDL cholesterol is shown in table 4 and 5.

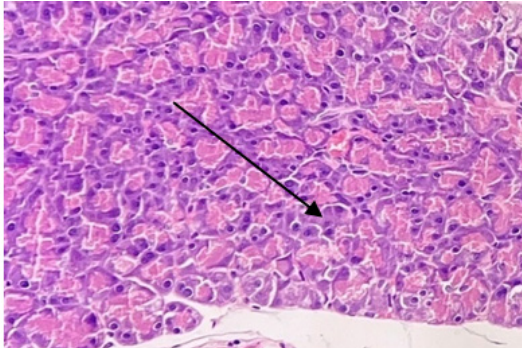


Fig. 5. Group I- Normal islet cells

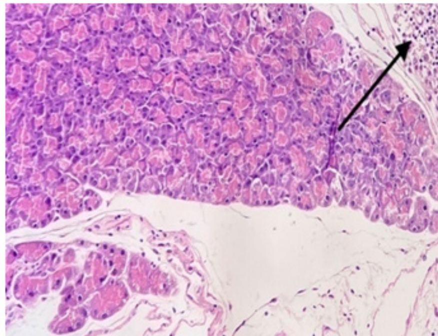


Fig. 6. Group II- Presence of inflammatory infiltrates

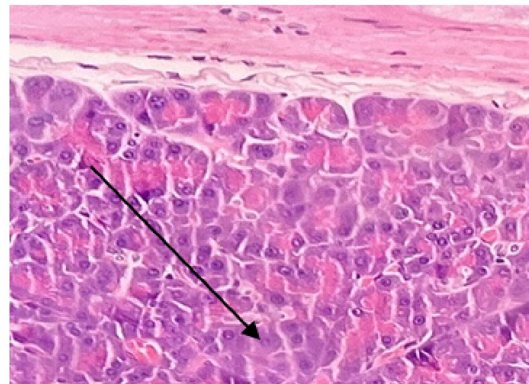


Fig. 7. Group III- Islet cells with pale large to ovoid beta cells with regenerative changes

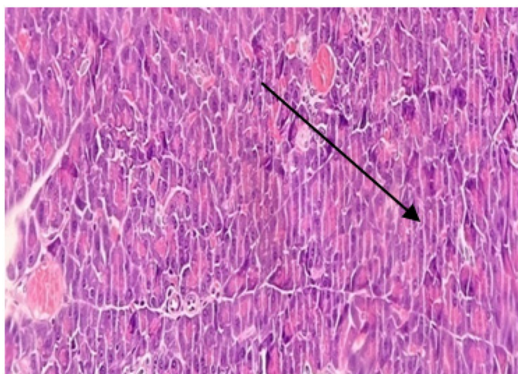


Fig. 8. Group IV- Islet cells with some degenerative changes

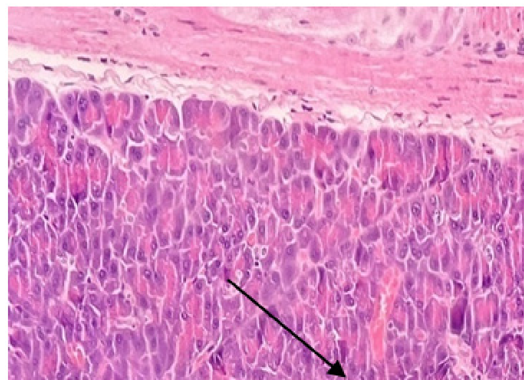


Fig. 9. Group V- Islet cells with pale and ovoid beta cells with regenerative changes

Histopathology

HPE of pancreas showed the following changes

In the normal control group (group I), HPE of pancreas did not show any significant changes (Figure 5). Whereas, in the disease control group (group II) islet cells were surrounded with inflammatory infiltrates such as lymphocytes, macrophages, and plasma cells (Figure 6). The metformin and *P.juliflora* extract 600mg/kg treated groups (group III and V) showed normal islets cells with large, pale and ovoid beta cells (Figure 7 and 9). While the *P.juliflora* extract 400mg/kg treated group showed some degenerative changes (Figure 8). These findings indicate that the islet cells may have recovered after treatment with *P.juliflora* extract 600mg/kg.

DISCUSSION

Lipid abnormalities such hypertrigly ceridemia and reduced HDL cholesterol levels as are commonly observed in patients with T2DM. Diabetic dyslipidemia can be treated with strict glycemic control and weight loss. Majority of the conventional anti-diabetic drugs are associated with unavoidable side effects.[36]An ideal anti-diabetic drug should be safe, effective in maintaining optimal blood glucose levels and also prevent long term complications. Alternative system of medicine is practiced by a large population for treatment of various diseases and ailments including DM.[37] Herbal medicine & plant components have relatively low toxicity and fewer side effects and therefore, they can be considered as a therapeutic option for DM.[38]

In this study, male wistar albino rats were fed with 20% fructose water daily for a period of 8 weeks presented with features of T2DM like fasting hyperglycemia, insulin resistance. In addition to T2DM, fructose supplementation also induced some of the features of metabolic syndrome like weight gain and altered lipid profile (dyslipidemia).

The anti-diabetic and hypolipidemic activity of the seed extract was compared with metformin. It was observed that both the doses of extract (400mg/kg and 600mg/kg) reduced the blood glucose levels. However, the dose 600mg/kg showed maximum reduction which was comparable to the effect produced by metformin. Therefore, it is clear that the ethanolic seed extract of *P.juliflora* significantly reduced the blood glucose levels in a dose-dependent manner. Similar findings were observed in another study where methanolic seed extract of *P.juliflora* in the dose 600mg/kg reduced the blood sugar levels in streptozotocin induced diabetic rat model.[19]

Phytochemical analysis showed the presence of alkaloids, flavonoids, terpenoids &steroids. Based on this, it is proved that *P.juliflora* seeds are rich in polyphenolic compounds like flavonoids. Flavonoids are biologically active secondary metabolites in the plants.[39] They have numerous beneficial effects on metabolic disorders such as cardiovascular disease, obesity, cancer and DM.[40]Chronic hyperglycemia in T2DM raises inflammatory cytokine levels which may cause endoplasmic reticulum stress, oxidative stress and lysosomal destabilization, which ultimately leads to beta cell death through apoptosis.[41] Flavonoids promote beta cell proliferation and decrease apoptosis, regulate glucose metabolism in the liver, reduce the hepatic glucose output, regulates the enzymes involved in the carbohydrate metabolism (inhibits α-glucosidase), exhances the expression of glucose transporters, promote insulin secretion and reduce insulin resistance.[42,43] According to many studies, flavonoids also act as insulin mimetics and insulin secretagogues.[44-46] Therefore, the hypoglycaemic effect of ethanolic seed extract of *P.juliflora* might be due to the presence of bioactive phytoconstituents like flavonoids that act separately or synergistically and either stimulates or mimics the action of insulin.

In our study, we discovered that animals in the fructose fed groups gained significantly more weight at the end of 8 weeks, when compared to the animals in the normal control group. Similar findings were observed in another study where wistar rats fed on 20% fructose water for 8 weeks had significantly higher body weight when compared to the control group.[47] Fructose promotes de novo lipogenesis in animals, resulting in increased body weight and adipocity. Obesity develops as a result of the dyslipidemia and increased body fat stores. Insulin resistance and obesity frequently coexist. This could explain the increased body weight and insulin resistance observed in our study[48-50]

We also observed that the levels of TG, LDL, VLDL and TC increased while HDL

cholesterol decreased after the induction of diabetes at 8 weeks. Similar findings were reported in some of the previous studies where wistar rats fed on 20% fructose water for 8 weeks and 21 weeks respectively, developed signs of dyslipidemia as well as insulin resistance.[29,30]

"Dyslipidemia is characterized by elevated levels of TC, LDL, VLDL and TG, as well as a decrease in HDL cholesterol"[51]. In our study, the lipid parameters remained unchanged in the disease control group at the end of treatment. In contrast, there was a significant increase in HDL levels and a decrease in the levels of TG, LDL, VLDL and TC in the metformin and extracts treatment groups (400mg/kg and 600mg/kg). Moreover, both the doses of extract were having anti dyslipidemic effect similar to metformin

Hyperglycemia is frequently associated with dyslipidemia in T2DM. Insulin activates the enzyme lipoprotein lipase, which causes triglyceride hydrolysis. Lipoprotein lipase is not activated in T2DM due to insulin resistance, and catabolism of triglyceride-rich lipoproteins is decreased, resulting in hyper-triglyceridaemia and other lipoprotein alterations.[52] Furthermore, the hypolipidemic effect of the *P.juliflora* seed extract can be attributed to the presence flavonoids and terpenoids which might cause inhibition of the enzyme lipoprotein lipase and thereby decreasing the levels of triglycerides, LDL, VLDL and total cholesterol.[53,54]

Biochemical and haematological assessments were done at baseline and at 16 weeks to assess safety of the interventions and no significant abnormality was observed.

HPE of pancreas showed regenerative changes in the metformin and extract 600mg/kg treated groups. These findings indicate that the damaged islet cells may have recovered after treatment with the plant extract.

One of the major goals in the management of diabetes is to maintain optimal blood glucose levels to prevent complications.[55] considering the current study's findings as well as the available evidence for anti-diabetic and hypolipidemic activity, *P.juliflora* seed extract can be used to treat hyperglycemia and dyslipidemia associated with T2DM. However, further studies are needed to determine the exact mechanism of such activity by isolating bioactive compounds and assessing their effect.

CONCLUSION

P.juliflora seed extract in the doses 400 mg/kg and 600mg/kg exhibited antidiabetic and hypolipidemic activity in terms of reduction in the fasting blood sugar, HOMA-IR and lipid levels. Both the doses, 400 and 600 mg/kg showed hypolipidemic activity similar to Metformin whereas the antidiabetic activity showed a dose dependent response i.e., 600 mg/ kg was having better antidiabetic effect compared to 400 mg/ kg and the reduction in blood sugar was similar to that of Metformin. Both the doses of the extract were safe as there were no significant abnormality observed in the hematological and biochemical parameters. Further, regenerative changes were noted in the pancreas of the animals treated with Metformin and 600 mg/kg of the extract. All these findings suggest that the seed extract of *P.juliflora* has a potential therapeutic benefit in diabetes and hyperlipidemia.

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Conflict of Interest

The author(s) declares no conflict of interest

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