Research Article

CHANGES IN SERUM BIOCHEMICAL PARAMETERS AND LIPID PROFILE IN NORMAL AND STZ INDUCED DIABETIC RATS WITH THE ADMINISTRATION OF ETHANOLIC EXTRACT OF POLYALTHIA CERASOIDES STEM BARK

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ABSTRACT

The present study was conducted to determine the changes in serum biochemical parameters and lipid profile by oral administration of ethanolic extract of Polyalthia cerasoides stem bark (PcEE 400 mg/kg b. wt) in streptozotocin (45 mg/kg b. wt) induced diabetic rats. Albino rats of weighing between 200-230 g were induced with single dose of streptozotocin (dissolved in 0.1 M ice cold citrate buffer (pH = 4.5) at a dose 45 mg/kg b. wt). Diabetes was confirmed after 48 h in streptozotocin induced rats showing fasting blood glucose levels ≥ 250 mg/dl. The rats were randomly divided into five groups (n = 6). Group I and II (normal and diabetic controls), Group III (normal rats fed with 400 mg of PcEE), Group IV (diabetic rats fed with 400 mg of PcEE), Group V (diabetic rats fed with 20 mg of Glibicinamide). After 21 days animals were sacrificed and blood samples were collected for biochemical analysis. The results showed that the extract significantly (p < 0.05) reduced serum ALT, AST and ALP levels when compared to the diabetic control. The urea and creatinine levels are also controlled significantly (p <0.05). Similarly TC, LDL, VLDL-cholesterol, TG and total lipids of serum were significantly (P <0.05) decreased in streptozotocin induced diabetic rats but HDL-cholesterol levels remained unchanged when compared to the diabetic control animals. The results clearly suggested that the extract has the ability to retain the altered biochemical parameters as normal in diabetic animals and it was effective as glibicinamide treated animals. Hence the study reveals the therapeutic use of PcEE on diabetes and its complications

Keywords: Diabetes mellitus, P. cerasoides, Streptozotocin, Glibicinamide, Serum lipid profiles.

INTRODUCTION

Diabetes mellitus is found in almost all nations of the world, so it is called as a global disease. Moreover the morbidity and mortality rate is increased continuously in worldwide, it has estimated 135 million people in the world with diabetes and it would rise to 380 million by the year 2025.¹ In particular hyperglycemia is the main characteristic feature of diabetes, due to decreased secretion or inefficient action of insulin secreted from β-cells of pancreas. It is thought to contribute various biochemical changes in cellular metabolisms, vascular complications, oxidative stress and alterations in circulating lipoproteins.² The altered metabolisms of carbohydrate, lipid and protein play vital role in diabetic complications like hypercholesterolemia and hypertriglyceridemia. Secondary complications are developed due to chronic hyperglycemia in diabetes which affecting eyes, kidney, artery and nerves³. STZ is widely used to induce experimental diabetes and it exhibit reduced response in hepatic and peripheral tissues. Hence it alters cellular metabolisms and some biochemical reactions in tissues.⁴ Commercially available oral hypoglycaemic drugs and insulin are recommended for treatment of diabetes mellitus. But there is a necessity to develop herbal medicine due to undesirable side effects, high cost and safety on long term use.⁵ There are several kinds of medicinal plants that exhibit antidiabetic and antioxidant activities, but there is lack of information regarding the biochemical, haematological and safety assessment of many plants in their applications and medicinal uses. Polyalthia cerasoides (Roxb) Bodd is a medicinal plant belongs to the family Annonaceae. Vernacular name is Guttidudduga and is distributed in India, China, Burma and Thailand. P. cerasoides roots are used as a tonic febrifuge⁶ stem bark is used as a pain relief and kidney disfunction⁷ P. cerasoides has potent biological activity as an inhibitor of the mammalian mitochondrial respiratory chain⁸ and it had significant reactive oxygen species (ROS) scavenging activity.⁹ The aim of this present study was to investigate the effect of P. cerasoides stem bark on serum lipoproteins and vital biochemical parameters in STZ induced diabetic rats. It has the potential antidiabetic activity.¹⁰

MATERIALS AND METHODS

Plant material

Stem bark of P. cerasoides used for the experiment were collected from herbal garden in Dravidian university and surrounding areas of Kuppam, A.P., India. The stem bark was dried in shade and pulverized in mechanical grinder. The powder (stem bark) was stored in airtight container and it is used for the extraction process.

Animal model

Male albino wister rats weighting 200-230 g were used for study. Rats were acclimatized to animal house in polypropylene cages and maintained under standard photoperiodic condition and temperature (26 ± 2°C) fed with standard pellet diet and provided water ad libitum. All the animal experiments were conducted according to the ethical norms approved the institutional ethical committee of Sri Padmavati Mahila Visva Vidyalayam, Tirupati, Andhra Pradesh, India (Ref.:1677/PO/a/12/CPCSEA).
Preparation of extract

The dried powder was extracted in a soxhlet extractor using ethanol at a temperature range of 55-60°C and the extract was dried in Rotary evaporator then the extract was used for the experimental study.

Induction of diabetes

The animals were fasted overnight and then induced diabetes by a single intra-peritoneal injection of streptozotocin (45 mg/kg b. wt). Streptozotocin was prepared just prior to injection in 0.1 M ice cold sodium citrate buffer pH = 4.5. After 48 h fasting blood glucose levels of rats were determined. The rats with fasting blood glucose levels (> 250 mg/dl) were selected for the drug treatment.

Experimental Design

In the present experimental study, the rats were divided into five groups for evaluation of serum biochemical changes and lipid profile with six animals in each group.

Group 1: Normal control rats (NC).
Group 2: Diabetic control rats (DC).
Group 3: Normal rats given PcEE (400 mg/kg b. wt).
Group 4: Diabetic rats given PcEE (400 mg/kg b. wt).
Group 5: Diabetic rats given Glb (20 mg/kg b. wt) for 21 days.

After 21 days of treatment, the rats were sacrificed by cervical dislocation; whole blood was collected into plain tubes via cardiac puncture using sterile syringes and needles, allowed to clot for about two hours. After the clotted blood was centrifuged at 3,500 rpm for 30 minutes; to recover serum from clotted blood. Serum was separated with sterile syringes and stored frozen for biochemical analysis.

Biochemical Assays

Protein determination

Total protein content was measured by using standard method.12

Alanine and aspartate aminotransferases determination

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method.13

Alkaline phosphatase determination

Alkaline phosphatase (ALP) activity was determined by using p-nitrophenylphosphate as substrate according to the method.14

Urea and creatinine determination

Urea and creatinine concentrations were determined by the standard methods.15

Estimation of serum lipid profile

Total cholesterol and triglycerides of serum were estimated by using standard methods.16,17 HDL cholesterol was determined by phosphotungstate/magnesium method.18 VLDL cholesterol was calculated as triglycerides/5 and LDL cholesterol was calculated by the equation:

LDL cholesterol = Total serum cholesterol – (HDL + VLDL)

Statistical Analysis

The data were expressed as mean ± SD and the difference between the groups and within the groups were tested by using one-way analysis of variance (ANOVA) with Duncan multiple range test (DMRT). Data was statistically handled by SPSS software version (16.0). P < 0.05 was considered as statistically significant.

RESULTS

The results of ALT, AST and ALP enzyme activities were summarized in Table 1. The ALT and AST levels in normal rats are 10.83 U/L and 33.33 U/L, whereas the diabetic control group increased the ALT and AST levels 34.33 U/L and 58.66 U/L respectively. The 400 mg/kg b. wt PcEE treated diabetic rats were decreased the ALT and AST (16.66 U/L and 37.33 U/L) enzyme concentration as greater than or equal to normal rats. Glb treated diabetic rats also decreased the ALT and AST levels (15.83 U/L and 34.83 U/L). ALP concentration was increased in diabetic rats (50.33 U/L); it was almost decreased in extract treated animals (38.50 U/L). The concentrations of ALT, AST and ALP were remained unchanged in normal extract treated rats. Table 2 showed total protein concentration, urea and creatinine levels in normal and diabetic treated rats. The total protein concentration was decreased in diabetic rats (64.01 g/L) when compared to the normal rats (84.15 g/L). These protein concentration was increased in PcEE treated (70.70 g/L) and Glb treated group (82.65 g/L). The extract treated diabetic animals were also decreased the urea and creatinine levels (35.50 and 0.71 mg/dL) when compared to the diabetic control animals (51.66 and 1.30 mg/dL). These urea and concentration were near to normal (23.16 and 0.58 mg/dL) in Glb treated diabetic rats. The lipid profile concentration was summarized in Table 3. In the entire study, increased concentrations of total cholesterol (TC 206.66 mg/dL), low density lipoproteins (LDL 142.28 mg/dL), very low-density lipoproteins (VLDL 29.83 mg/dL) and triglycerides (TG 149.83 mg/dL) were observed in diabetic untreated group of rats. They remained almost unchanged (no significance) in normal extract treated group. The significant (P < 0.05) decrease of the above parameters in extract treated diabetic group when compared with diabetic control group and was as effective as Glb treated diabetic group. High-density lipoproteins (HDL) were decreased in diabetic untreated group. Significant changes was not observed in normal extract treated group and significantly (P < 0.05) increased near to normal levels in diabetic extract treated group was as effective as Glb treated diabetic group (P < 0.05).

Table 1: Effect of PcEE on serum enzyme activities in normal and STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT  U/L</th>
<th>AST  U/L</th>
<th>ALP  U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10.83 ± 1.47</td>
<td>33.33 ± 3.98</td>
<td>25.50 ± 2.50</td>
</tr>
<tr>
<td>Normal rats treated with PcEE (400 mg/kg b. wt)</td>
<td>10.50 ± 1.04</td>
<td>31.16 ± 3.06</td>
<td>23.83 ± 1.60</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>34.33 ± 3.88</td>
<td>58.66 ± 4.32</td>
<td>50.33 ± 3.50</td>
</tr>
<tr>
<td>Diabetic rats treated with PcEE (400 mg/kg b. wt)</td>
<td>16.66 ± 2.65</td>
<td>37.33 ± 3.98</td>
<td>38.50 ± 3.72</td>
</tr>
<tr>
<td>Diabetic rats treated with Glb (20 mg/kg b. wt)</td>
<td>15.83 ± 2.13</td>
<td>34.83 ± 3.76</td>
<td>37.66 ± 1.50</td>
</tr>
</tbody>
</table>

Note: Values are expressed as Mean ± SD, n = 6, values with different superscript in same column are significantly different at (P < 0.05) when compared to the control groups.
Urea is a product of protein catabolism and is excreted through urine. The determination of the serum urea and creatinine showed any effect on enzyme activities.

Serum ALP decreased levels ALT and AST were observed in diabetic disease. Moreover AST and ALP were observed to increase the risk of coronary heart disease.

Table 2: Effect of PcEE on serum biochemical parameters in normal and STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea mg/dL</th>
<th>Creatinine mg/dL</th>
<th>Total Protein g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>23.16 ± 1.83a</td>
<td>0.58 ± 0.13c</td>
<td>84.15 ± 2.45a</td>
</tr>
<tr>
<td>Normal rats treated with PcEE (400 mg/kg b. wt)</td>
<td>24.66 ± 2.65a</td>
<td>0.55 ± 0.05a</td>
<td>85.95 ± 2.73a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>51.66 ± 4.92a</td>
<td>1.30 ± 0.24a</td>
<td>64.01 ± 0.99a</td>
</tr>
<tr>
<td>Diabetic rats treated with PcEE (400 mg/kg b. wt)</td>
<td>35.50 ± 3.01a</td>
<td>0.71 ± 0.04a</td>
<td>79.70 ± 1.27a</td>
</tr>
<tr>
<td>Diabetic rats treated with Glb (20 mg/kg b. wt)</td>
<td>36.16 ± 3.31a</td>
<td>0.70 ± 0.10a</td>
<td>82.65 ± 1.85a</td>
</tr>
</tbody>
</table>

Note: Given values are represent as mean ± SD (n = 6 rats per group). The values with different letter of superscript were statistically P < 0.05 deviated when compared to the control groups.

Table 3: Effect of PcEE on serum lipid profile in normal and STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol mg/dL</th>
<th>HDL-C mg/dL</th>
<th>VLDL-C mg/dL</th>
<th>LDL-C mg/dL</th>
<th>Triglycerides mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>106.66 ± 4.22a</td>
<td>61.33 ± 7.18a</td>
<td>17.65 ± 1.08a</td>
<td>28.66 ± 2.92a</td>
<td>86.16 ± 5.42a</td>
</tr>
<tr>
<td>Normal rats treated with PcEE (400 mg/kg b. wt)</td>
<td>105.83 ± 6.74a</td>
<td>60.16 ± 5.69a</td>
<td>17.86 ± 1.49a</td>
<td>28.50 ± 4.34a</td>
<td>87.83 ± 3.18a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>206.66 ± 3.90f</td>
<td>35.33 ± 5.49f</td>
<td>29.83 ± 1.30a</td>
<td>142.28 ± 4.20a</td>
<td>149.83 ± 5.36f</td>
</tr>
<tr>
<td>Diabetic rats treated with PcEE (400 mg/kg b. wt)</td>
<td>109.90 ± 4.72a</td>
<td>56.33 ± 4.98f</td>
<td>18.98 ± 1.91f</td>
<td>35.78 ± 4.85f</td>
<td>93.83 ± 5.75f</td>
</tr>
<tr>
<td>Diabetic rats treated with Glb (20 mg/kg b. wt)</td>
<td>106.70 ± 4.94a</td>
<td>57.83 ± 4.29a</td>
<td>19.95 ± 2.50a</td>
<td>30.61 ± 3.91f</td>
<td>95.33 ± 6.42a</td>
</tr>
</tbody>
</table>

Note: Given values are represent as mean ± SD (n = 6 rats per group). The values with different letter of superscript were statistically P < 0.05 deviated when compared to the control groups.

DISCUSSION

Diabetes is a chronic disorder affecting the carbohydrate, protein and lipid metabolisms. Diabetes exhibited much higher glucose levels or hyperglycemia it is the main characteristic symptom of diabetes. In addition to hyperglycemia systemic biological elevations may contribute to molecular metabolisms and vascular wall function in diabetic patients. Various herbal remedies are known in folk medicine and used for treatment and management of diabetes due fewer side effects. But there is no scientific evidence to exert biological action against diabetic complications. Our study evaluated the effect of PcEE on serum biochemical changes and lipid profile in STZ induced diabetes in albino rats. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are excellent makers for diagnostic purpose, which play a main role in the conversion of amino acid to ketoacid. AST was found in many tissues like liver, kidney, heart, brain and skeletal muscle it is not specific liver enzyme; but ALT is more specific liver enzyme found in large amount in liver when compared to other tissues. Serum ALP is a sensitive detector for intra-hepatic and extra-hepatic bile obstruction; the presence of bile obstruction and all bone diseases. Moreover ALT, AST and ALP are marker enzymes for liver function and integrity, which are liberated into the serum whenever liver cells are damaged and the serum enzyme activity is increased. The increased level of ALT, AST and ALP were observed in the diabetic control. Hence it indicates that diabetes may induce hepatic dysfunction in rats. These elevations are also associated with cell necrosis of many tissues. Increase in the activities of AST, ALT in serum may be due to the leakage of these enzymes from liver cytosol to blood stream which gives an indication on the hepatotoxic effect of STZ. The serum levels of ALT and AST were reduced significantly (P < 0.05) in 400 mg/kg b. wt of PcEE treated group when compared to the diabetic control group. Similarly, the decreased levels ALT and AST were observed in Glb treated diabetic rats. It was also observed the significant reduction of the ALP level in PcEE treated and Glb treated diabetic rats. The obtained results are similar with the plant P. amarus lower level of serum transaminases. The normal rats treated with PcEE do not showed any effect on enzyme activities when compared to normal control. The effect of PcEE on the kidney function was assessed by the determination of the serum urea and creatinine. Urea is the end product of protein catabolism and is excreted through urine. Creatinine is an end product of creatine metabolism. Creatine is synthesized in the liver and passes into the circulation. Urea and creatinine are regarded as reliable markers of renal function. The concentrations of urea and creatinine were significantly (P < 0.05) higher in diabetic rats when compared to the normal rats. Hence it was the indication that the STZ diabetes may lead to renal dysfunction. The treatment of STZ induced diabetic rats with 400 mg/kg b. wt of PcEE significantly (P < 0.05) reduced the urea level in serum when compared to the diabetic group. Similarly the elevation of creatinine level in serum was caused by diabetes and it was reduced after administration of 400 mg PcEE (P < 0.05) compared to the diabetic control. The urea and creatinine levels were significantly (P < 0.05) increased in Glb treated diabetic rats. Total protein concentration was also decreased in the serum of STZ induced diabetic rats. The reduction of total protein is due to an increased conversion glycogenic amino acid to CO₂ and H₂O. Diabetic rats treated with PcEE attained the protein level near to normal. Similarly it was also observed in the Glb treated rats. Alterations serum lipid profiles are well known in diabetes, which lead to increase the risk of coronary heart disease CAD. Diabetes characterizes the hyperlipidemia due to uninhibited actions of lipolytic enzymes and absence of insulin. Results of the present study showed an increased level of total cholesterol in STZ induced diabetic rats. LDL levels were also increased on the diabetic control group. Low-density lipoprotein LDL is an important event in the development of vascular disease. The administration of 400 mg PcEE in STZ induced diabetic rats were significantly (P < 0.05) reduced the serum TC and LDL levels. This effect may be due to the gut inula-lumen interactive effect of saponins. Saponins are known anti nutritional factors which reduce the uptake of certain nutrients including glucose and lipid especially cholesterol. Hence saponins have hypcholesteremic effect similarly the same results were observed in Glb treated diabetic rats. The PeEe was does not altered the normal TC and LDL levels in normal rats. The high level of high-density lipoprotein HDL protect against cardiovascular disease. HDL removes cholesterol from antheroma within arteries and transports it back to liver for excretion or re-utilization. Lower cholesterol may have contributed the high serum HDL in the animals. Total 30 % of blood cholesterol was carried in the form of HDL. The present study showed that HDL concentration was decreased in diabetic control rats due to the increased total cholesterol. The PeEe extract treated group showed the significant (P < 0.05) increase in HDL levels. Which are near to similar the Glb treated group. The normal HDL levels remained unchanged. In the same way the triglycerides TG and very low-density lipoproteins VLDL were increased in diabetic rats. These levels were...
significantly (P<0.05) lowered in PcEE extract treated group. The same results were observed in Glib treated rats and normal levels were remained unchanged in PcEE treated normal rats.

CONCLUSION

The present study clearly demonstrated the treatment of PcEE extract showed the hypolipidemic effect in STZ induced diabetic rats. Thus it can be concluded from our findings that the levels of total serum cholesterol, triglycerides, VLDL and LDL-cholesterol which are raised in STZ induced diabetes, can be lowered with the treatment of PcEE extract. In addition the extract is capable of protecting the liver and kidney functions in STZ induced diabetic rats as shown the activities of serum enzymes (ALT, AST and ALP) and other biochemical parameters (urea and creatinine) examined. Further research is required on identification and isolation of components which are responsible for the suppression of serum parameters and enzyme activities.

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