PROTECTIVE EFFECT OF ETHANOLIC ROOT EXTRACT OF COMMIPHORA CAUDATA AGAINST DIABETIC-INDUCED RATS IN HIGH-FAT DIET-STREPTOZOTOCIN MODEL

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ABSTRACT

Objective: The present study was to explore the phytochemical analysis and antidiabetic potential of the root of Commiphora caudata in high-fat diet (HFD) streptozotocin-induced diabetic rats.

Methods: The ethanolic root extract of C. caudata at a dose of 400 mg/kg and 200 mg/kg was administered to diabetic rats. Glibenclamide (5 mg/kg) was used as standard drug.

Results: The data were statistically assessed using one-way ANOVA followed by Dunnett’s multiple comparison tests. To unfold the mechanism, we studied all the biochemical parameters glucose, total cholesterol, triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very LDL (VLDL) and histopathological examination of the pancreatic tissue section. The ethanolic extracts of root of C. caudata showed significant reduce of the level of cholesterol, TG, LDL, VLDL, and significant increase in the serum level of HDL at 400 mg/kg rather than 200 mg/kg.

Conclusion: Further studies should look into the characterization and isolation of the constituents to know the exact mechanism of hypoglycemic activity. Statistical analyses of this screening method confirm that the proposed method is appropriate and it can be expected to improve basic idea to the researcher who is working in area-like antidiabetic activity.

Keywords: Commiphora caudata, Glibenclamide, High-fat diet streptozotocin, Cholesterol, Triglycerides, Low-density lipoproteins, and Very low-density lipoproteins.

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic syndrome characterized by either deficiency of insulin production or showing resistance toward insulin. Type 2 DM is a condition in which cells fail to respond to insulin property [1]. As the disease progresses a lack of insulin, formerly called as non-insulin-dependent DM or adult-onset diabetes.

Commiphora caudata is a deciduous tree growing from 12 to 20 m tall. The bole can be 15–25 cm in diameter. The tree is sometimes harvested from the wild for local medicinal use. It is occasionally used as an avenue tree and is often planted as an ornamental. The endosperm obtained from four or five fresh or dried seeds is taken two times a day for 2–3 days to relieve stomach ache [2]. The heartwood is gray with darker streaks; the sapwood is white. In addition, these major compounds extracted from seeds of C. caudata have important pharmacological effects, thereby aiding in the understanding of the physiology of organisms and in the treatment of various pathologies [3,4]. It is claimed to possess astringent, sweet, cooling, aphrodisiac, diuretic, and antidiabetic activities. It is used for fever, strangury, the leaves are useful in rheumatagia [5,6]. By considering all the facts, the purpose of the present study was to unlock the antidiabetic activity of the C. caudata in the high-fat diet (HFD) streptozotocin (STZ)-induced rats.

METHODS

Plant material and authentication

Roots of C. caudata was collected in November from Tirumala hills, Chittoor district of Andhra Pradesh and authenticated by Professor N. Yacodamma, Department of Botany, Sri Venkatkeswara University, Tirupati, India and compared to that of the standard Herbarium SVUTY, Department of botany with specimen voucher.

Chemicals and reagents

STZ, glibenclamide (GLB), and the biochemical estimation kits were procured from Aldrich Sigma Ltd., Bangalore.

Extraction

Collected roots were shade dried; powdered and crude substance 500 g was taken and subjected to run in Soxhlet apparatus for 72 h with ethanol as a solvent, respectively. The extract obtained was concentrated in Rotary flask evaporator under reduced pressure at 65°C; yield was found to be 4.89% and stored in the desiccators for the further use.

Preliminary phytochemical screening

The ethanolic root extract of Commiphora caudate (EECC) was subjected to preliminary phytochemical analysis for the detection of various phytoconstituents such as alkaloids, glycosides, saponins, flavonoids, tannins, phenolic compounds, triterpenoids, carbohydrates, and proteins using standard procedures as per mentioned [7,8].

Experimental animals

The male albino Wistar rats weighing between 180 and 200 g were taken for the study which were procured and maintained in a well-ventilated room with 12:12 h light/dark cycle in polypropylene cages. Standard pellet feed (hindustan lever ltd., bangalore) and drinking water was provided ad libitum throughout the experimentation period. Rats were acclimatized to laboratory conditions 1 week before the initiation of experiments. Ethical Committee clearance was obtained from the Institutional Animal Ethical Committee (IAEC) of Committee for Control and Supervision of Experiments on Animals and bearing SWCP/IAEC/2018.
Acute toxicity studies
As per OECD 423 guidelines, the EECC was administered to albino Wistar rats by oral route starting from 5 mg/kg to 2000 mg/kg. The animals were monitored for any changes in fur balking, eyes, sign of tremors, lethargy, motor activity, and itching, behavioral parameters continuously, observed for lethality and desired dose of 400 mg/kg and 200 mg/kg was selected [9].

Experimental design
The experimental design comprised five groups each consisting of five rats fed by normal standard pellet for acclimatization, then 2 weeks of dietary manipulation, the groups of rats were fed by HFD and the administration was continued for 21 days and on the 21st day STZ (45 g/kg) was administered and the fasting blood glucose was measured before and after 3 days after the vehicle or STZ injection. 20% glucose was administered after 4 h of STZ injection and ethanolic extract of C. caudata low dose and high dose was continued for 21 days after STZ injection [10]. The rats with the fasting blood glucose ≥200 mg/dl were considered diabetic and selected for further pharmacological studies. Group I: Normal control (NC) rats were administered 0.1% CMC in saline daily (NC). Group II: Diabetic rats were administered HFD + STZ (45 mg/kg). Group III: Diabetic control rats were administered HFD + STZ (45 mg/kg) and GLB (5 mg/kg). Group IV: Diabetic control rats were administered HFD + STZ (45 mg/kg) and EECC (200 mg/kg per body weight [BW]). Group V: Diabetic control rats were administered HFD + STZ (45 mg/kg) and EECC (400 mg/kg per BW). BW was measured weekly. At the end of the experimental period, the diets were removed from the cages 12 h before the animals were euthanized [11]. Blood samples were collected by the retroorbital method and centrifuged to obtain serum after the collection of blood, the rats were euthanized, and liver, pancreas, and kidney were excised immediately, rinsed with phosphate buffer saline, and weighed [12]. The samples were undergone for biochemical estimations and pancreas was stored in 10% formalin solution for histopathological studies.

Measurement of body weight of the rats
Initial and final BW of the rats was measured using weighing machine and the data obtained was represented.

Measurement of biochemical parameters
Determination of the serum biochemical parameters such as the total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very LDL (VLDL) were measured using the commercially available standard kits [13] according to the instructions and the results were calculated as per the formula.

Histopathological studies
After inducing diabetic condition, rats from each group were anesthetized and pancreas was removed from the abdominal region of each rat and was excised quickly and fixed in 10% buffered-formaldehyde at room temperature [14]. After dehydration using graded ethanol, pieces of tissues were embedded in paraffin; 5-µm thick sections were cut by a rotator microtome and mounted on glass slides. Sections were then deparaffinized with xylene, counterstained with hematoxylin and eosin [15]. After the tissue sections were stained with hematoxylin and eosin and then viewed were examined using Olympus BX51 microscope model U-LH100HG and the results were shown. The normal architecture and the damaged architecture were studied of liver, pancreas, and kidney was assessed and analyzed in all the histopathological sections.

Statistical analysis
The statistical analysis was carried out using the latest Graph pad prism software. All values were expressed as mean ± standard error of the mean. Data analysis was performed by one-way ANOVA followed by Dunnett’s multiple comparison tests. Difference level at p<0.05 was considered a statistically significant condition.

RESULTS AND DISCUSSIONS

Preliminary phytochemical analysis
The phytochemical screening of EECC was revealed the presence of flavanoids, terpenoids, saponins, proteins, alkaloids, and these chemical constituents was responsible for different therapeutic actions and results were represented in Table 1.

Effect of extracts on body weights
Effect of EECC on BWs in all the groups. The BW of the control group increased significantly from 222 ± 2.24 to 259 ± 0.98. The diabetic rats showed a significant decrease in BW from 219 ± 0.76 to 168 ± 01.2. After treatment with the EECC and GLB, there was no statistical significance and slight change in the BWs was observed in Fig. 1.

Effect of extract on serum blood glucose
The effect of EECC was investigated for its hypoglycaemic potential in normal, diabetic, and extracts treated groups. The EECC at a dose of 200 mg/kg and 400 mg/kg for 21 days of administration showed a significant decrease in blood glucose compared to that of diabetic-induced rats in Fig. 2.

Table 1: Preliminary phytochemical analysis of ethanolic root extract of Commiphora caudata

<table>
<thead>
<tr>
<th>Chemical tests</th>
<th>Presence/ Absence</th>
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<tbody>
<tr>
<td>Test for carbohydrates</td>
<td>+</td>
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<tr>
<td>Test for alkaloids</td>
<td>−</td>
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<tr>
<td>Test for proteins</td>
<td>+</td>
</tr>
<tr>
<td>Test for glycosides</td>
<td>−</td>
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<tr>
<td>Test for flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>Test for terpenoids</td>
<td>+</td>
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<tr>
<td>Test for resins</td>
<td>−</td>
</tr>
<tr>
<td>Test for tannins</td>
<td>−</td>
</tr>
</tbody>
</table>

+: Presence, −: Absence

Fig. 1: Effect of extracts on body weights of rats

Fig. 2: Effect of extract on serum blood glucose levels
Effect of extract on lipid profile
In the present study, HFD + STZ significantly increased the TC, TG, LDL, VLDL levels and decreased the level of HDL compared to control groups. Flavonoids present in the extract have been reported to decrease LDL and increase HDL and it also helps in the removal of cholesterol from peripheral tissues to the liver for catabolism and excretion [16,17]. Hence, the decrease in levels of TC, TG, LDL, and increase the level of HDL depicted in the present study may be due to the presence of effects of chemical constituents in EECC. Standard treated group showed marked significantly decreased levels of lipid levels and increased level of HDL [18]. After administration of EECC extracts of 400 mg/kg b. w. and 200 mg/kg b. w for 21 days it reduced the TC, TG, LDL, VLDL levels and increased level of HDL in diabetic rats compared to control groups and thus showed significant hypoglycemic potential and the results were calculated as per the formula and depicted in Fig. 3.

**DISCUSSION**
In the present study, the initial attempts were directed toward finding the glucose lowering effect of ethanolic extracts of roots of *C. caudata* in HFD + STZ-induced diabetic rats and further sensitive for pharmacological testing. The HFD rat model with a dose of STZ (45 mg/kg) thus can be more considered to represent the path physiological state of type 2 diabetes and was accompanied by the characteristic of diabetic condition produced by high dose of STZ [19,20]. Hence, HFD in combination with low dose of STZ (45 mg/kg) was chosen for generating the rat model for further studies. The data obtained showed that the plasma levels of TG, TC, and LDL in HFD + STZ fed rats were significantly higher than those of control group; however, plasma HDL levels were significantly decreased, indicating that HFD + STZ feeding caused hyperglycaemic in rats [21,22]. After the treatment of extract in the experimental period, it significantly decreased these changes in plasma levels of TG, TC, and LDL in HFD + STZ-induced model. The EECC showed significant as the antidiabetic potential at 400 mg/kg rather than 200 mg/kg. So by this HFD + STZ induced model the ethanolic extract of roots of *C. caudata* has shown the significant changes in lipoprotein levels that depicts that the extract possesses the antidiabetic activity.

**CONCLUSION**
The EECC of two different doses 400 mg/kg and 200 mg/kg have shown the significant potential against the diabetic condition in the HFD + STZ-induced model. Compared to 200 mg/kg dose, 400 mg/kg showed the better significance of hypoglycemic potential. Further studies should keep an eye toward the characterization, isolation to know the exact mode of the mechanism of the hypoglycemic potential of roots of *C. caudata*.
ACKNOWLEDGMENT
Corresponding authors show gratitude toward the Chairman and Principal of Sree Vidyanikethan College of Pharmacy, A. Rangampet, Chittoor district of Andhra Pradesh for their Support and facilities provided to fulfil this research work.

AUTHORS' CONTRIBUTIONS
Performed the Experiment: Kuttiappan Anitha
Project guided: Dr. S. Mohana Lakshmi and Prof. S. V. Satyanarayana
Wrote the paper: Kuttiappan Anitha. Revised the Article: Kuttiappan Anitha.

CONFLICT OF INTEREST STATEMENT
We don't have the conflict of interest.

REFERENCES