Research Article

Evaluation of Antioxidant Effect of Ethanolic Root Extract of *Commiphora caudata* in High Fat Diet and Streptozotocin induced Diabetic Rats

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ABSTRACT

The Research work is to highlight the antioxidant potential of ethanolic root extract of *Commiphora caudata* in High Fat Diet and Streptozotocin (HFD+STZ) induced diabetic screening model. The dispute between free radicals and antioxidants plays a key role in disturbance of normal physiological conditions. Deficiency of antioxidant substances in body leads to `pathogenesis of multiple diseases. Ethanolic root extract of *Commiphora caudata* has been undergone fractionation to obtain flavonoid and terpenoid fractions and investigated for its antioxidant activity in HFD+STZ induced Diabetic model. The oxidative stress was determined like Serum glutamic oxaloacetic transaminases (SGOT), Serum glutamic pyruvic transaminases (SGOT) and Alkaline Phosphatase (ALP). Enzymatic markers include the Lipid Peroxidation (LPx), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione (GSH). Enzymatic parameters were observed in liver and kidney tissues homogenates by analyzing these observed biomarkers. SOD, GSH, CAT was decreased in diabetic group, however restoration of SOD, GSH, and CAT levels by supplementation with Ethanolic root extract of *Commiphora caudata* was observed and tabulated. Histopathological studies of pancreas of animals showed comparable regeneration of tissues with EECC. It was concluded that CCTF, CCTT showed significant antioxidant activity. Upcoming characterization studies will be carried out to know the exact mechanism of Antioxidant potential of *Canavalia species.*

Keywords: Antioxidant activity, *Commiphora caudata*, Glibenclamide, Histopathology, Streptozotocin.

INTRODUCTION

Commiphora caudata most abundant species of Commiphora in the family Burseraceae found mostly in southern India, Sri Lanka. The tree is usually grown in hilly dry zone areas that's the name hill Mango or green Commiphora.[1] It has a succulent green bark, smooth, that partly flakes off with age, giving rise to a characteristic patchwork of green and brown patches and Its sap has a strong resinous scent.[2] The flowers have a greenish to cream-yellow pedestal with pink to red petals, the fruit is a globose fleshy drupe with 2 to 6 valves and 1 seed that is black and root was brownish black. The tree is sometimes harvested from the wild for local medicinal use. [3] 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 two to three days to relieve stomach ache.[4] In Tamil it is called as Kiluvai, in Telugu Konda Mamidi. Examples of oxygen free radicals are hydroxyl radical, superoxide anion radical, hydroxide peroxide, nitric oxide radical and others.[5] The main cause for disease is due to imbalance created in between free radicals and antioxidants necessary for proper functioning of body.[6] Pathologenesis triggers free radical leading to disturbance in Carbohydrates, proteins, lipids and other substances.[7] The chronic metabolic disorders like hyperglycemia, hyperlipidemia and hyperinsulinemia play as triggers causing endothelial dysfunction through the influence of different mediator molecules. [8] There are several facts that oxidative stress caused by metabolic changes plays a key role in endothelial disturbance. [9] Reactive oxygen species (ROS) generated by elevated glucose is linked to increased glucose and other metabolic abnormalities important to the development of diabetic complications. [10] Prior report regarding antioxidant potential effect of *Commiphora caudata* has not been observed so this antioxidant study has been undertaken.

MATERIALS AND METHODS

Plant material Collection and authentication

Commiphora caudata roots were collected from Tirumala hills, Chittoor district of Andhra Pradesh and authentified by Professor N. Yasodamma, Department of botany, Sri Venkateswara University, Tirupati, India and compared to that of the standard Herbarium SVUTY, department of botany with specimen voucher.

Chemicals and reagents

For Analytical purpose Streptozotocin (STZ), Glibenclamide (GLB) and other SGOT, SGPT, ALP, antioxidant kits, chemicals were purchased from Sigma-Aldrich, Bangalore.

Plant Extraction

Collected roots were shade dried powdered and crude substance 500 gm was run in Soxhlet apparatus for 72 h using ethanol as a solvent by continuous hot percolation method.[11] The extract was evaporated in Rotary flask evaporator under reduced pressure at 65^{0}C; yield was

found to be 3.89% and undergone fractionation process using ethyl acetate and n-BnOH to obtain the terpenoids and flavonoids fractions of *Commiphora caudata* (CCTT, CCTF) and subjected to phytochemical tests[12] and indentified the presence of flavonoids and terpenoids, stored in the desiccators for the further use.

Experimental Animals

Albino wistar rats of 180-200g were selected for the study. Rats were procured from Sree Venkateswara enterprises Bangalore and housed in Sree Vidyanikethan College of Pharmacy. The rats were fed by Normal Standard Pellet (NSP) for the initial period of 2 Weeks and were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical Committee clearance was obtained from IAEC of Sree Vidyanikethan College of Pharmacy bearing 930/Po/Re/S/18/CPCSEA.

Acute Toxicity studies

As per OECD 423 guidelines the ethanolic root extract of *Commiphora caudata* (CCTT, CCTF) were administered to albino Wister rats starting from 5mg/kg to 2000mg/kg for 14 days. [13] The animals were monitored for any changes continuously and were not observed for any lethality hence 1/5th desired dose was selected for the study.

Treatment Design

The rats were fed by Normal Standard Pellet (NSP) for the initial period of 2 Weeks and separated into groups 6 rats in each batch. [14- 16] Group I: Control rats was administered citrate buffer daily (NC). Group II: Diabetic induced rats were administered HFD+STZ (40mg/kg). Group III: Diabetic positive control rats were administered HFD+STZ (40mg/kg) and glibenclamide (5mg/kg). Group IV: test I rats were administered HFD+STZ (40mg/kg) and ethanolic root extract of CCTT [400 mg/kg per body weight (BW). Group V: test II rats were administered HFD+STZ (40mg/kg) and ethanolic root extract of CCTF [400 mg/kg per body weight (BW)]. [17, 18]

Blood samples collection

At the end of experimental period of 45 days the diets were removed from the cages and Blood samples were collected by retro orbital method and sample was centrifuged to obtain serum. The rats were sacrificed pancreas was excised immediately, rinsed with phosphate buffer saline, and weighed. The samples were subjected for *in vivo* biochemical estimations and pancreas stored in 10% formalin solution for histopathological studies. [19]

Determination of serum biomarkers

Determination of the serum biochemical parameters such as SGOT, SGPT, ALP and liver glycogen content[20] were measured using the commercially available standard kits according to the instructions.

Determination of *in vivo* antioxidant parameters

After the experimental period organs were excised, washed with isotonic buffer saline and stored at -20^0 C. *In vivo* antioxidant parameters in 10% homogenate of both liver and kidney was measured for its antioxidant parameters such as LPx, GSH, CAT, SOD [21] sing the commercially available standard kits according to the instructions.

Histopathological Observations

Rats from each group were anesthetized and liver, pancreas, kidney was removed from each rat was excised quickly and fixed in 10% buffered- formaldehyde at room temperature. [22] After the tissue sections were stained with hematoxylin and eosin, mounted, viewed and then was examined.

Statistical analysis

The statistical analysis was carried out using latest Graph pad prism software. All values were expressed as Mean ± S.E.M. Data analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests. Difference level at P<0.05 was considered as statistically significant condition.

RESULTS AND DISCUSSION

Effect of Ethanolic root extracts of CCTT, CCTF on serum biomarkers

Serum biomarkers such as SGOT, SGPT and ALP were significantly elevated in diabetic induced rats and depict hepatic damage and were recovered by treatment with ethanolic root extract of *Commiphora caudata,* increased level of serum transaminases may be due to diabetic complications. As Serum biomarkers such as transaminases such as SGOT, SGPT and ALP were significantly increased in diabetic induced group (Figure 1). After supplementation with two fractions of CCTT, CCTF and glibenclamide the serum transaminases level was resettled compared to the diabetic control rats.

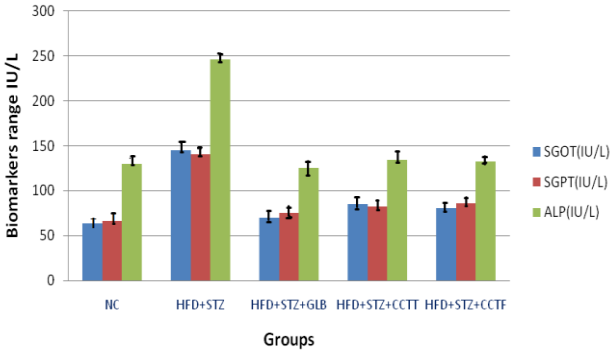

Figure 1: Effect of serum biomarkers in ethanolic root extracts of *Commiphora caudate* in HFD+STZ induced Diabetic model, #*p*< 0.001; [b]treated group Vs HFD+STZ induced diabetic group, **p*<0.001, ***p*<0.05

Status of In vivo antioxidant parameters in liver and kidney homogenates

As depicted measuring LPx value increased significantly in HFD+STZ induced diabetic rats as compared to normal group 19±0.892, 11.6±1.012 in liver and kidney, further reduced significantly in liver, kidney after treatment with Ethanolic root extract of *Commiphora caudata* Reduced GSH in liver, kidney was improved in liver and kidney after treatment with CCTT, CCTF fractions.

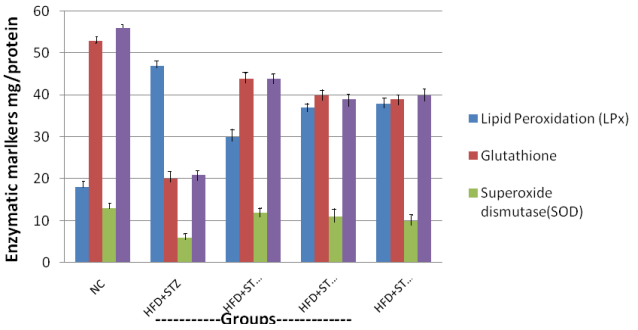

Figure 2: Effect of enzymatic markers in ethanolic root extract of *Commiphora caudate* in HFD+STZ induced Diabetic model on liver homogenates. #p< 0.001; [b]treated group Vs HFD+STZ induced diabetic group, *p<0.001, **p<0.05

Reduced SOD level in liver, kidney was resettled to some extent after supplementation with these fractions. Catalase level were restored in liver, kidney homogenates treating CCTT, CCTF fractions of flavonoids and terpenoids (Figure 2,3) represents the hepatic glycogen content was reduced in diabetic induced rats. After treatment with Ethanolic seed extracts of CETT, CETF, CGTT and CGTF significant increase in liver glycogen were observed. (Figure 4) depicts the Architecture of normal, induced and treated groups representing damaged structure in induced and significant recovery in treated groups.

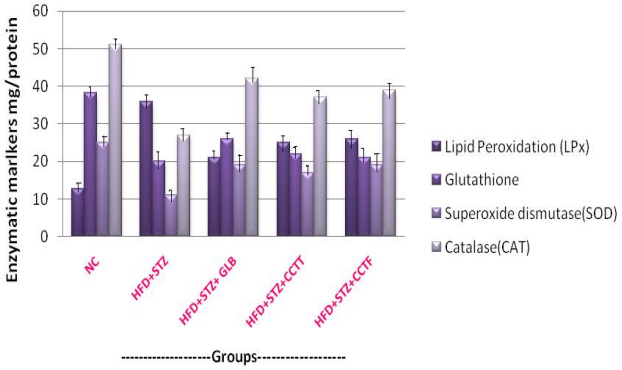

Figure 3: Effect of enzymatic markers in ethanolic root extract of *Commiphora caudate* in HFD+STZ induced Diabetic model on kidney homogenates. #p< 0.001; [b]treated group Vs HFD+STZ induced diabetic group, *p<0.001, **p<0.05

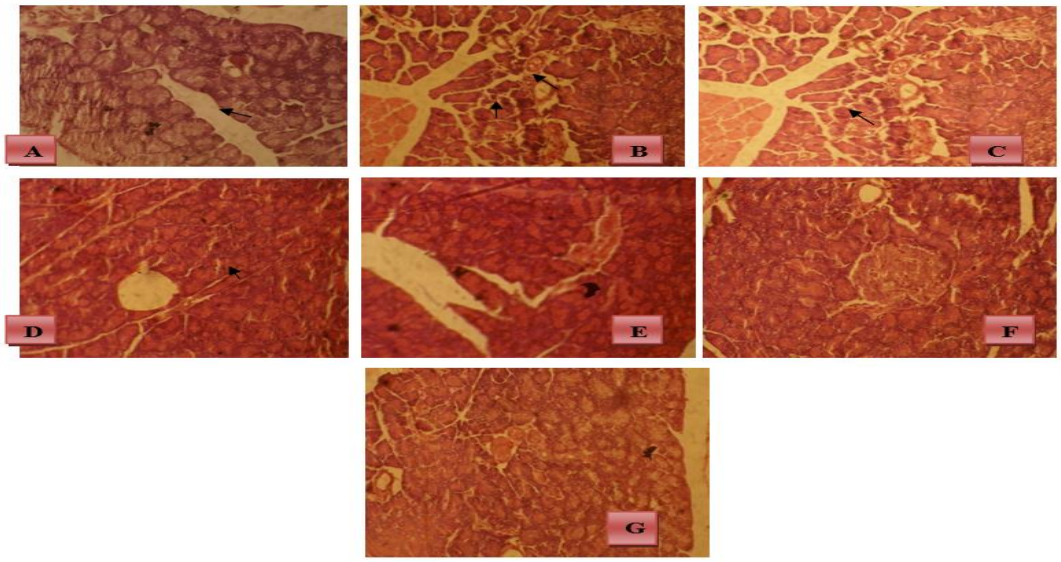

Figure 4: Photographs of the histopathological tissue sections:(A) Control group eliciting the normal pancreas architecture (B) Negative Control group showing the complete damage of pancreas architecture (c) Positive Control group showing the recovery of damaged pancreas architecture (D) CETT dose treated group showing the recovery of damaged pancreas architecture to some extent (E) CETF dose treated group showing the recovery of damaged pancreas architecture to a significant extent (F) CGTT dose treated group showing the recovery of damaged pancreas architecture to an extent (G) CGTF dose treated group showing the recovery of damaged pancreas architecture to a significant extent.

CONCLUSION

Free radicals play a significant role in pathogenesis of tissue damage, consequently having implications in many clinical conditions. Protective role will play by both endogenous and exogenous antioxidants that repair the damaged and injured cells. Scientifically till a greater number of new plants has to be indentified which have low side effects and high potent antioxidant activity. The results should be encouraged in future in characterizing and isolating the active principle molecules further it could ultimately lead to the development of product and that retain substantial antidiabetic and antioxidant capacity with minimal adverse effects.

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