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ORIGINAL ARTICLE

Antioxidant Potential of Ethanolic Extract of *Canavalia* Species in High-fat Diet and Streptozotocin-induced Diabetic Rats

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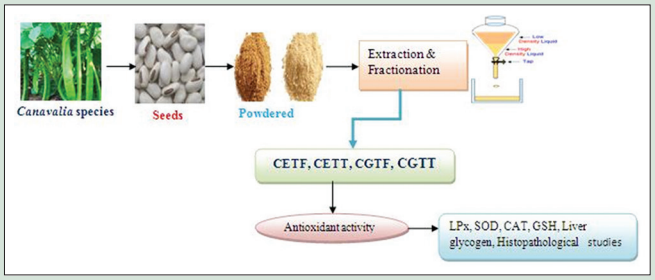
ABSTRACT

Background: The present study is to enlighten the antioxidant effect of ethanolic seed extracts of *Canavalia species* in high-fat diet and streptozotocin (HFD + STZ)-induced diabetic screening model. **Objectives:** The dispute between free radicals and antioxidants plays a key role in causing alteration of normal physiological conditions. Inadequate antioxidant in body leads to multiple pathological diseases. **Materials and Methods:** Ethanolic seed extracts of *Canavalia species* have been investigated for its antioxidant activity in HFD + STZ-induced screening models. The potential of antioxidants on oxidative stress is measured through certain serum biomarkers such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase. Enzymatic markers include lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). Enzymatic parameters were studied in liver and kidney homogenates by measuring these observed biomarkers. **Results:** SOD, GSH, and CAT were decreased in the diabetic group. However, restoration of SOD, GSH, and CAT levels by treatment with ethanolic seed extracts of *Canavalia* species was observed and tabulated. Histopathological studies of the pancreas of animals showed comparable regeneration of tissues with EECE. **Conclusion:** Further the characterization studies will be carried out to know the extract mechanism of antioxidant potential of *Canavalia* species.

Key words: Antioxidant activity, *Canavalia ensiformis*, *Canavalia gladiata*, glibenclamide, histopathology, streptozotocin

SUMMARY

- The Ethanolic seed extracts of *Canavalia* species showed a significant impact in reducing hyperglycemic condition and possess antioxidant property
- *In vivo* antioxidant parameters showed reduced levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) and increased level of lipid peroxidation (LPx) in high-fat diet and streptozotocin-induced rats further after supplementation of four fractions of *Canavalia ensiformis* total flavonoids, *Canavalia ensiformis* total terpenoids, *Canavalia gladiata* total flavonoids, *Canavalia gladiata* total terpenoids SOD, CAT, GSH levels increased and LPx level decreased
- Serum biomarkers such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase also supported the potential effect of ethanolic seed extracts of *Canavalia* species.



Abbreviations Used: *C. ensiformis: Canavalia ensiformis*, *C. gladiata: Canavalia gladiata*, HFD: High-fat diet, STZ: Streptozotocin, GLB: Glibenclamide, CETF: *Canavalia ensiformis* total flavonoids, CETT: *Canavalia ensiformis* total terpenoids, CGTF: *Canavalia gladiata* total flavonoids, CGTT: *Canavalia gladiata* total terpenoids, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase, LPx: Lipid peroxidation, CAT: Catalase, SOD: Superoxide dismutase, GSH: Reduced glutathione, ROS: Reactive oxygen species, OECD: Organization for Economic and Corporation Development, ANOVA: Analysis of variance.

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INTRODUCTION

The leguminous plant *Canavalia* species, though not eaten frequently, traditionally possess more medicinal properties yet to be scientifically proven.[1] The seeds, particularly the pink colored, were employed in traditional medicine.[2] Dietary fiber of both the species of *Canavalia ensiformis* and *Canavalia gladiata* was considered to be an important legume which contributes to health in various ways of reducing risks in coronary heart disease by lowering the serum cholesterol level, decreasing the absorption;[3] reduce the colon cancer risk, increasing the fecal bulk, decreasing the concentration of carcinogens; improve glucose tolerance in diabetic subjects by slowing the release of glucose into the bloodstream.[4]

The main cause is due to imbalance created between free radicals and antioxidants necessary for proper functioning of body.[5] Pathological states trigger free radical, thus leading to disturbance in proteins, lipids, and other chemical substances. Examples of oxygen free radicals are hydroxyl radical, superoxide anion radical, hydroxide peroxide, nitric oxide radical, and others [Figure 1].[6]

Imbalance between the free radical formation and elimination causes metabolic complications.[7] The complications of metabolic disorders of diabetes mellitus such as hyperglycemia, hyperlipidemia, and hyperinsulinemia triggers causing endothelial dysfunction through the influence of different mediator molecules.[8] There are several lines of impact on these facts that oxidative stress caused by these metabolic changes plays a key role in endothelial dysfunction.[9] Reactive oxygen species (ROS) generated by elevated glucose is linked to increased

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glucose and other metabolic abnormalities important to the development of diabetic complications.[10]

MATERIALS AND METHODS

Collection and authentication

The seeds of *Canavalia* species were collected from Tirumala hills, Chittoor district of Andhra Pradesh and authenticated by Professor N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India, and were compared to that of the standard Herbarium SVUTY, Department of Botany with specimen voucher No: 129 collected by A. Job Roger Binny on June 10, 2014 and preserved as Voucher Specimen No: KL-20.

Chemicals and reagents

For analytical purpose, streptozotocin (STZ), glibenclamide (GLB) and other serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), antioxidant kits, and chemicals were purchased from Sigma-Aldrich, Bengaluru.

Extraction, fractionation, and screening

The collected seeds were shade-dried and powdered and crude substance 500 g was run in a Soxhlet apparatus for 72 h using ethanol as a solvent by continuous hot percolation method.[10,11] The extract was evaporated in a rotary flask evaporator under reduced pressure at 65°C; yield was found to be 4.89% and undergone fractionation process using ethyl acetate and n-BnOH to obtain the terpenoid and flavonoid fractions of CE and CG (*C. ensiformis* total terpenoids [CETT], *C. ensiformis* total flavonoids [CETF], *Canavalia gladiata* total terpenoids [CGTT], and *Canavalia gladiata* total flavonoids [CGTF]) and subjected to phytochemical analysis[12] to reveal the presence of flavonoids and terpenoids and was stored in the desiccators for further use.

Ethical approval

Rats were acclimatized to laboratory conditions 1 week before initiation of experiments. Ethical committee clearance was obtained from IAEC of Sree Vidyanikethan College of Pharmacy bearing 930/Po/Re/S/18/CPCSEA.

Toxicity studies

As per the OECD 423 guidelines, the ethanolic extracts of *Canavalia* species (CETT, CETF, CGTT, and CGTF) were administered to albino Wister rats starting from 5 mg/kg to 2000 mg/kg for 14 days.[13] The animals were monitored for any changes continuously and were not observed for any lethality and hence desired dose was selected.

Induction of diabetes and experimental design

The albino Wister rats weighing between 180 and 200 g were selected for the study. The rats were fed by normal standard pellet for the initial period of 2 weeks and separated into seven groups with six in each batch.

- Group I: Normal control rats were administered citrate buffer daily (NC)
- Group II: Diabetic control rats were administered high-fat diet and STZ (HFD + STZ) (40 mg/kg)
- Group III: Diabetic control rats were administered HFD + STZ (40 mg/kg) and GLB (5 mg/kg)
- Group IV and V: Diabetic control rats were administered HFD + STZ (40 mg/kg) and ethanolic extract of CETT and CETF (400 mg/kg per body weight [BW])
- Group VI and VII: Diabetic control rats were administered HFD + STZ (40 mg/kg) and ethanolic extract of CGTT and CGTF (400 mg/kg per BW).

After 2 weeks of dietary manipulation, the group of rats fed by HFD except the control rats were given vehicle citrate buffer (pH 4.4) at a dose of 1 ml/kg, i.p.[14,15] The administration was continued for 3 weeks, and on 21st day, the overnight fasted rats were administered STZ (40 mg/kg, i.p)[16] and the fasting blood glucose was measured before and after 3 days of vehicle or STZ injection. 5% glucose for every 4 h of STZ injection was given to avoid hypoglycemic condition.[17] The rats with the fasting blood glucose ≥200 mg/dl were considered diabetic and selected for further comparative studies, and ethanolic extract of *Canavalia* species CETT, CETF, CGTT, and CGTF was continued for 21 days after STZ injection. BW was measured weekly.[18]

Collection of blood samples

At the end of 21 days of treatment of extracts, the diets were removed from the cages and blood samples were collected by cardiac puncture method and centrifuged to obtain serum after the collection of blood; the rats were sacrificed; and the liver, pancreas, and kidney were excised immediately, rinsed with phosphate buffer saline, and weighed. The samples were subjected to biochemical estimations, and the liver, pancreas, and kidney were stored in 10% formalin solution for histopathological studies.[19]

Measurement of serum biomarkers and liver glycogen content

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

Measurement of *in vivo* antioxidant parameters

After the experimental period, the organs were excised, washed with isotonic buffer saline, and stored at −20°C. *In vivo* antioxidant parameters in 10% homogenate of both liver and kidney were measured for its antioxidant parameters such as lipid peroxidation (LPx), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD)[21] using the commercially available standard kits according to the instructions.

Histopathology

After inducing diabetic condition, the rats from each group were anesthetized, and the liver, pancreas, and kidney were removed from each rat, were 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 examined.

Statistical analysis

The statistical analysis was carried out using GraphPad Prism software. All values were expressed as mean ± standard error of mean. 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

Effect of extracts on serum biomarkers

Serum biomarkers [Table 1] such as transaminases such as SGOT, SGPT, and ALP were significantly increased in the diabetic-induced group [Figures 2-4]. After supplementation with four fractions of CETT, CETF, CGTT, and CGTF and GLB, the serum transaminase level was recovered to some extent compared to the diabetic control.

In vivo antioxidant status and liver glycogen content

As depicted in Tables 2 and 3 measuring MDA content LPx value increased significantly in HFD + STZ-induced diabetic rats 49 ± 2.564, 32 ± as compared to normal group 19 ± 0.892, 11.6 ± 0.923 in liver and kidney, further reduced significantly 39 ± 2.541, 36 ± 1.564, 41 ± 1.008, 37 ± 2.015 in liver and 21 ± 3.024 19 ± 2.045, 16 ± 1.980, 20 ± 2.321 in kidney after treatment with ethanolic seed extracts of *Canavalia* species. Reduced GSH in liver 22 ± 3.125, 18 ± 1.980 in kidney was improved to 42 ± 2.521, 40 ± 2.054, 40 ± 3.016, 39 ± 2.136 in liver and as 26 ± 2.980, 25 ± 2.036, 27 ± 3.980, 25 ± 2.222 in kidney after treatment with CETT, CETF, CGTT, CGTF fractions. Reduced SOD level in liver 5 ± 1.360, 10 ± 2.456 in kidney was resettled to 11 ± 3.568, 10 ± 2.132, 11 ± 1.578, 8 ± 2.645 in liver and 21 ± 4.770, 18 ± 3.098, 19 ± 3.987, 17 ± 2.564 in kidney after supplementation with these four fractions. Catalase level were restored 43 ± 3.156, 41 ± 2.684, 42 ± 3.154, 40 ± 2.050 liver homogenates and 35 ± 2.654, 39 ± 3.154, 40 ± 3.061, 41 ± 4.987, in kidney homogenates treating with ethanolic *CE, CG* flavonoids and terpenoids fractions. Figure 5 represents that 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 was observed. Figure 6 depicts the architecture of normal, induced, and treated groups, representing damaged structure in induced and significant recovery in treated groups.

Table 1: Effect of serum biomarkers in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic model

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
NC	63.3±2.082	66.6±2.729	125.6±2.603
HFD + STZ	156±2.603$^{a,\#}$	148±3.180$^{a,\#}$	256±3.456$^{a,\#}$
HFD + STZ + GLB	72±0.881^{b,*}	75.6±1.333^{b,*}	138.6±1.856^{b,*}
HFD + STZ + CETT	86±1.856^{b,*}	89±2.856^{b,*}	145.2±2.881^{b,*}
HFD + STZ + CETF	81±2.333^{b,*}	86±1.785^{b,*}	142.1±2.33^{b,*}
HFD + STZ + CGTT	82±3.0188^{b,*}	80±2.0281^{b,*}	151±1.764^{b,*}
HFD + STZ + CGTF	79±2.856^{b,*}	77.6±1.453^{b,*}	153±2.603^{b,*}

Values are expressed as mean±SEM, (n=6); STZ (40 mg/kg) was injected to all groups except control. ^{a}HFD + STZ induced diabetic group versus normal group; btreated group versus HFD+STZ induced diabetic group. $^{\#}P$<0.001, $^{*}P$<0.001, $^{**}P$<0.05. HFD + STZ: High-fat diet and streptozotocin; SEM: Standard error of mean; GLB: Glibenclamide; CETF: *Canavalia ensiformis* total flavonoids; CETT: *Canavalia ensiformis* total terpenoids; CGTF: *Canavalia gladiata* total flavonoids; CGTT: *Canavalia gladiata* total terpenoids; SGOT: Serum glutamic oxaloacetic transaminases; NC: Normal Control

Table 2: Effect of antioxidant parameters in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic model in liver homogenates

Groups	LPx	Glutathione	SOD	CAT
NC	19±0.892	55±1.521	14±0.456	58±1.032
HFD + STZ	49±2.564	22±3.125	5±1.360	22±1.587
HFD + STZ + GLB	32±3.154$^{a,\#}$	31±1.574$^{a,\#}$	8±4.521$^{a,\#}$	46±2.526$^{a,\#}$
HFD + STZ + CETT	39±2.541^{b,*}	42±2.521^{b,*}	11±3.568^{b,*}	43±3.156^{b,*}
HFD + STZ + CETF	36±1.564^{b,*}	40±2.054^{b,*}	10±2.132^{b,*}	41±2.684^{b,*}
HFD + STZ + CGTT	41±1.008^{b,*}	40±3.016^{b,*}	11±1.578^{b,*}	42±3.154^{b,*}
HFD + STZ + CGTF	37±2.015^{b,*}	39±2.136^{b,*}	8±2.645^{b,*}	40±2.050^{b,*}

Values are expressed as mean±SEM, (n=6); STZ (40 mg/kg) was injected to all groups except control. bTreated group versus HFD + STZ induced diabetic group, ^{a}HFD + STZ induced diabetic group versus normal group. $^{\#}P$<0.001; $^{*}P$<0.001, $^{**}P$<0.05. LPx: Lipid peroxidation; CAT: Catalase; SOD: Superoxide dismutase; HFD + STZ: High-fat diet and streptozotocin; GLB: Glibenclamide; CETT: *Canavalia ensiformis* total terpenoids; CETF: *Canavalia ensiformis* total flavonoids; CGTT: *Canavalia gladiata* total terpenoids; CGTF: *Canavalia gladiata* total flavonoids

DISCUSSION

Free radicals are highly reactive because of the unpaired electron by which ATP molecules are released to enable the cell to carry out the physiological functions.[23] Mitochondria the vital source of radical derivatives of oxygen (ROS) production such as free radicals, antioxidants can be of endogenous and reduced glutathione can also be introduced to the biological system exogenous, usually through diet.[24] Antioxidants primarily function to balance out free radicals generated during metabolic processes including during mechanisms involved in protecting the gut from inflammation and injury. Increasing intake of natural antioxidants may help maintain a tolerable antioxidant status, thus preventing the oxidative stress that could lead to pathogenesis of diabetes mellitus.[25] Nowadays, diabetic micro- and macroapathy are considered to be main poly etiological multifactorial diseases. Many studies have evaluated the role of oxidative stress in the etiology of microvascular and macrovascular complications of diabetes mellitus.[26] Flavonoids are polyphenolic compounds which are present in most plants. Among the secondary metabolites flavonoids and terpenoids are the bioactive compounds[27,28] that have been revealed the presence in ethanolic seed extract of *Canavalia* species in our study and reported antioxidant and antidiabetic activities potential.

Main targets to manage diabetes mellitus are to reduce the absorption of glucose via inhibition of digestive enzymes like α-glucosidase and α-amylase.[29] Traditionally, naturally occurring flavonoids and synthetic analogs have been extensively studied as α-glucosidase inhibitors α-Glucosidase is a membrane-bound enzyme located at the epithelium of the small intestine that catalyzes the cleavage of glucose from disaccharides to monosaccharide.[30] Inhibitors of α-glucosidase are used to control the blood sugar levels for type 2 diabetes mellitus. DPP-4 is a serine exopeptidase hormone, known to degrade two major gut incretin hormones that stimulate insulin release, that is, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), leading to a very short half-life of the hormones.[31] Inhibition of DPP-4 will prolong the half-life of GLP-1 and GIP hormones, resulting in the elevation of plasma insulin levels in human body.[32]

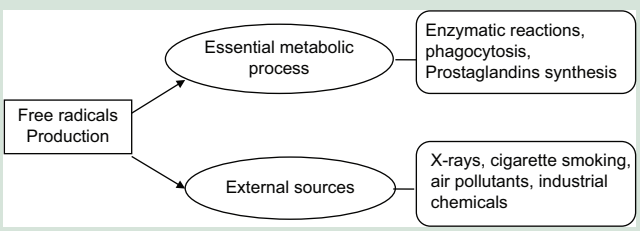

Figure 1: Schematic representation of free radical formation

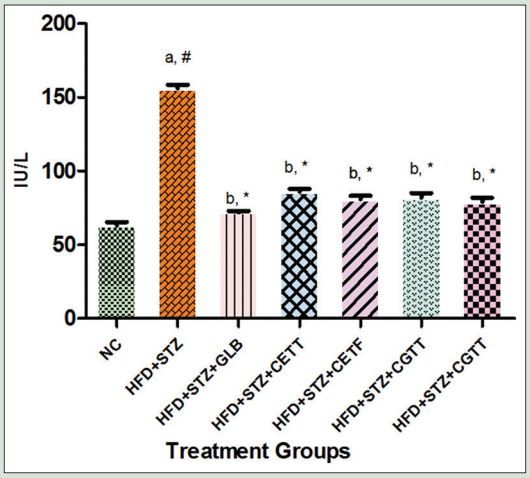

Figure 2: Effect of serum glutamic oxaloacetic transaminase in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic models

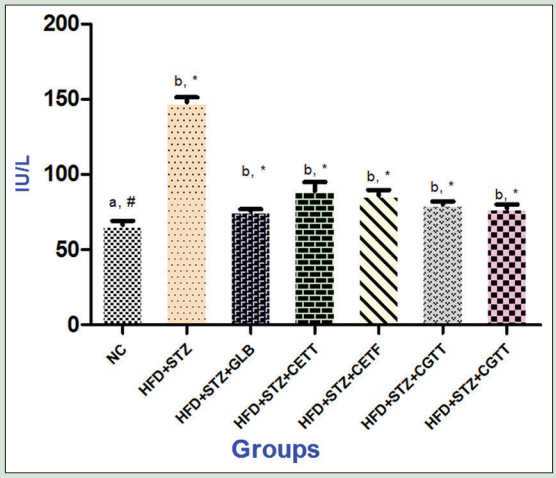

Figure 3: Effect of serum glutamic pyruvic transaminase in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic models

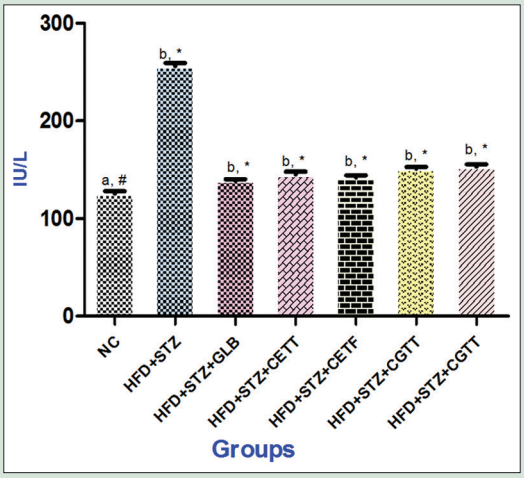

Figure 4: Effect of alkaline phosphatase in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic models

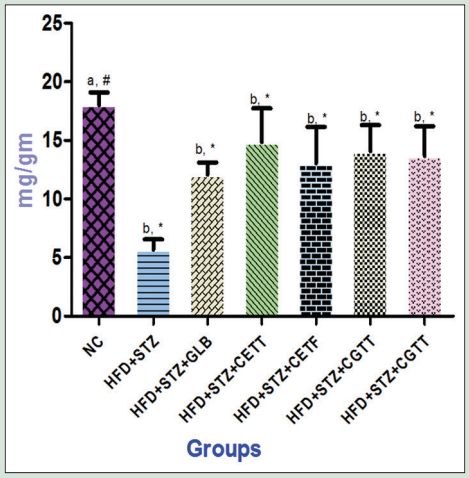

Figure 5: Effect of ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic models on liver glycogen content

Serum biomarkers such as SGOT, SGPT, and ALP were significantly increased in diabetic rats and depict hepatic damage. The damaged hepatocytes were restored by treatment with ethanolic seed extract of *Canavalia* species. Elevation of serum transaminases may be due to diabetic complications. Different possible ways of antidiabetogenic action one way will be increased insulin level, other ways by inactivating glycogen synthatase.[33] Reduced hepatic glycogen in diabetic rats shows impaired liver glycogen synthesis in diabetic condition. Significant increase in glycogen levels of ethanolic seed extract of *Canavalia* species-treated diabetic rats was observed.

There is considerable evidence that triggering of oxidative stress is a key process in the onset of diabetic complications, and precise mechanisms by which oxidative stress may accelerate the progression of complications in diabetes[34,35] are partly known. Evidence for the protective effect of antioxidants has been represented in experimental studies, which have demonstrated that antioxidants might be helpful in treating diabetes and its complications.

Table 3: Effect of antioxidant parameters in ethanolic seed extracts of *Canavalia* species in high-fat diet and streptozotocin-induced diabetic model in kidney homogenates

Groups	LPx	Glutathione	SOD	CAT
NC	11.6±0.923	35.7±0.882	25±0.674	55.2±1.076
HFD + STZ	32±2.654	18±1.980	10±2.456	25±2.064
HFD + STZ + GLB	25±1.876$^{a,\#}$	22±1.345$^{a,\#}$	14±1.765$^{a,\#}$	32±1.360$^{a,\#}$
HFD + STZ + CETT	21±3.024^{b,*}	26±2.980^{b,*}	21±4.770^{b,*}	35±2.654^{b,*}
HFD + STZ + CETF	19±2.045^{b,*}	25±2.036^{b,*}	19±3.987^{b,*}	39±3.154^{b,*}
HFD + STZ + CGTT	16±1.980^{b,*}	27±3.980^{b,*}	18±3.098^{b,*}	40±3.061^{b,*}
HFD + STZ + CGTF	20±2.321^{b,*}	25±2.222^{b,*}	17±2.564^{b,*}	41±4.987^{b,*}

Values are expressed as mean±SEM, (n=6); STZ (40 mg/kg) was injected to all groups except control; [a]HFD + STZ induced diabetic group versus normal group; [b]Treated group versus HFD + STZ induced diabetic group. *P<0.001, **P<0.05, #P<0.001. LPx: Lipid peroxidation; CAT: Catalase; SOD: Superoxide dismutase; HFD + STZ: High-fat diet and streptozotocin; GLB: Glibenclamide; CETT: *Canavalia ensiformis* total terpenoids; CETF: *Canavalia ensiformis* total flavonoids; CGTT: *Canavalia gladiata* total terpenoids; CGTF: *Canavalia gladiata* total flavonoids

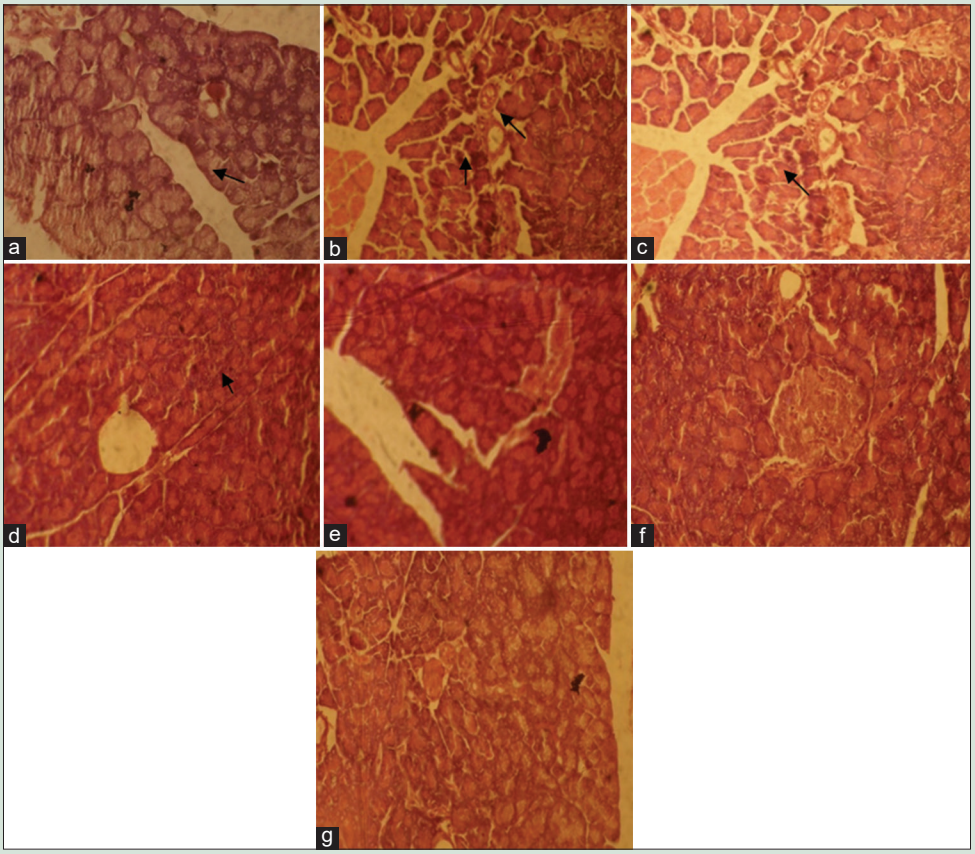

Figure 6: Photographs of the histopathological tissue sections. (a) Control group eliciting the normal pancreas architecture, (b) negative control group showing the complete damage of pancreas architecture, (c) positive control group showing the recovery of damaged pancreas architecture, (d) *Canavalia ensiformis* total terpenoids dose-treated group showing the recovery of damaged pancreas architecture to some extent, (e) *Canavalia ensiformis* total flavonoids dose-treated group showing the recovery of damaged pancreas architecture to a significant extent, (f) *Canavalia gladiata* total terpenoids dose-treated group showing the recovery of damaged pancreas architecture to an extent, (g) *Canavalia gladiata* total flavonoids 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 more number of new plants have to be identified 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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Nil.

Conflicts of interest

There are no conflicts of interest.

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