Ind. J. Pharm. Edu. Res., 2025; 59(3s):s978-s988.
https://www.ijper.org

Original Article

Cassia auriculata Stem Bark Ameliorates the HFD+STZ-Induced Diabetes in both *in vitro* and *in vivo* Model

Saravanan Muthaiah[1,2,3], Seema Mudhol[1,2], Muthukumar Serva Peddha[1,2,*]

[1]Department of Biochemistry, CSIR-Central Food Technological Research Institute, Mysuru, Karnataka, INDIA.
[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, INDIA.
[3]Department of Toxicology, Vipragen Biosciences Private Limited, Hootagalli, Mysuru, Karnataka, INDIA.

ABSTRACT

Background: The secondary metabolites from natural herbs are exploited to treat various human ailments. The present study aimed to investigate the ameliorative effect of the methanolic extract of *Cassia auriculata* (*C. auriculata*) stem-bark in both *in vitro* and *in vivo* mice model. **Materials and Methods:** *In vitro* standard procedures were followed to determine the phytochemical profile. High-fat-diet+STZ was administered to 6-weeks old mice to induce type-2-diabetes. **Results:** *In vitro* study revealed that the highest total polyphenol and flavonoid contents with anti-oxidant and anti-diabetic property was observed in the methanolic extract of *C. auriculata* stem-bark. Type-2-diabetes rats showed significantly elevated fasting blood glucose levels with decreased serum insulin level. Further, the oxidative stress in the serum and muscle as well as altered activities of liver enzymes of carbohydrate metabolism in diabetic control mice compared to normal control mice was observed. The histomorphology of liver and pancreas was deleteriously altered in diabetic group mice. However, methanolic extract of *C. auriculata* stem-bark (200 mg/kg; b.wt) and metformin (200 mg/kg; b.wt) treated diabetic mice did not show the above-mentioned alterations suggesting its ameliorative effect in type-2-diabetes. **Conclusion:** The methanolic extract of *C. auriculata* is potent enough to be employed as antidiabetic agent.

Keywords: Type-2-diabetes, *Cassia auriculata*, High-fat diet, Glucose metabolism, Oxidative stress.

Correspondence:
Dr. Muthukumar Serva Peddha[1,2]
[1]Chief Scientist and Professor, Department of Biochemistry, CSIR-Central Food Technological Research Institute, Mysuru-570020, Karnataka, INDIA.
[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad -201002, Uttar Pradesh, INDIA.
Email: muthukumar@cftri.res.in

Received: 27-12-2024;
Revised: 19-02-2025;
Accepted: 02-05-2025.

INTRODUCTION

Diabetes is one of the major metabolic disorders affecting the population globally. The incidence and prevalence of diabetes are observed worldwide and is increasing at an alarming rate in low and middle-income countries compared to high-income countries. As per the International Diabetes Federation, it is expected that the number of individuals with diabetes may rise to 700 million by the year 2045.[1] Of the three forms of diabetes viz., type 1, type 2 and gestational diabetes; type-2 diabetes has been reported to affect humans the most widely, accounting for over 95% of cases.[2] In case of type-2 diabetes, the body is unable to utilize insulin produced by the islets of Langerhans of the pancreas effectively.[3] This is due to the body's established resistance to insulin, which prevents the cells from identifying or recognizing the insulin molecules even when they are present in the bloodstream. Hence, several insulin-sensitizing and insulin-secretory drugs such as metformin, thiazolidinedione, meglitinides, etc., have been introduced and are being used to treat type-2 diabetes.

DOI: 10.5530/ijper.20256700

Copyright Information :
Copyright Author (s) 2025 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

Since the body experiences various side-effect and adverse effects from the use of synthetic drugs, naturally available herbs would be a safer alternative owing to reduced adverse effects on the body.[4] Several naturally available herbs are used against type-2 diabetes. To cite a few, *Curcuma longa, Zingiber officinale, Piper longa, Moringa oliefera, Terminalia chebula, Asparagus racemosus, Withania somnifera, Acorus calamus, Garcinia cambogia*, etc have reported to be some of the widely used herbs for its anti-diabetic properties.[5]

Similarly, *Cassia auriculata* is one of the widely used naturally available herbs used for the treatment of various ailments. *C. auriculata* is an evergreen shrub with yellow flowers, belonging to the family Caesalpiniaceae. It is found in different parts of Asia including India.[6] Different parts of this herb have been reported for their varied beneficial properties. For instance, solvent extracts of *C. auriculata* leaves have reported to possess anthelmintic,[7]

anti-bacterial[8] and anti-cancer properties.[9] Similarly, the root extracts have hepatoprotective,[10] and anti-oxidant properties.[11] In addition, studies have reported the anti-diabetic property of various parts of *C. auriculata*, like the roots,[11,4] aqueous extract of the flower,[6,12,13] bud[14] and leaves.[11,15] Although several studies have shown various health-beneficial properties including anti-diabetic efficacy of different parts of *C. auriculata*, the anti-diabetic efficacy of *C. auriculata* stem bark has not been reported. The stem of this herb is used widely in the traditional Ayurveda system of medicine[13] and literature using other parts of the herb is available extensively, but the anti-diabetic property of stem-bark of *C. auriculata* is yet to be established. It is to be noted that the above-mentioned beneficial properties are primarily due to the various phytochemicals present in different parts of the herb. Hence, it is also necessary to understand the phytochemical content of stem-bark of *C. auriculata*. Since, the secondary metabolites i.e., the phytochemicals produced by the herbs have major health benefits, the present study aims to investigate the phytochemical profile and anti-diabetic property of *C. auriculata* stem bark in a mice model.

MATERIALS AND METHODS

Collection of plant material

Cassia auriculata stems were collected from a local botanical garden in Mysuru. The collected plant material was authenticated by a botanist with voucher number UOMBOT20CA17. The collected stems were freed from all adhering matters by washing under running water, its bark was removed, shade-dried and coarsely powdered. The powdered material was packed neatly and stored at room temperature until further use.

Extraction

The powdered *C. auriculata* stem bark was initially taken in conical flasks for defatting on a rotary shaken for 8 hr at room temperature (25±2ºC) using hexane (1:10 ratio).[16] The supernatant was discarded and the defatted material was thoroughly dried to free from the residual solvent. The dried defatted material was divided into three equal parts in a separate conical flasks and individually subjected to extraction following the same procedure as that of defatting with different solvents, viz., ethanol, methanol and water respectively. Three different extracts thus obtained. Care was taken to remove the impure and particulate materials by filtrating through filter paper and residual solvent was removed with the help of rotary evaporator. The extracts were air-dried followed by freeze dried and stored at -20ºC until further use.

Total polyphenols and total flavonoids content

The total polyphenols and flavonoids content was estimated in the three different solvent extracts of *C. auriculata* stem bark following the standard protocols as described by Kupina, *et al.*[17] and Chang *et al.*[18] The values of total polyphenols were expressed as gallic acid equivalents in milligram per 100 g dry weight of the sample (mg QE/g DW) and that of flavonoid as mg of quercetin equivalents per 100 g dry weight of the sample (mg QE/g DW).

In vitro antioxidant efficacy of different solvent extracts of *C. auriculata* stem bark

The different solvent extracts, i.e., ethanol, methanol and aqueous extracts of the defatted *C. auriculata* stem bark was evaluated for its anti-oxidant potential *in vitro* by conducting DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay following the method of Rakholiya *et al.*[19] and Bhalodia *et al.*,[20] respectively.

The IC_{50} values of each sample in both the assays were calculated using the percentage inhibition values obtained at each concentration of test samples.

FTIR analysis of methanolic extract

FTIR was used as a qualitative tool to get the fingerprint of methanolic extract by scanning in the range of 400-4000 cm^{-1} by recording at a resolution of 2 cm^{-1} using Bruker alpha.

HPLC analysis of methanolic extract

The methanolic extract was analysed for its phytochemical constituents using HPLC by following the method described by Singh *et al.*,[21] using Agilent 1260 Infinity (Gradient conditions with PDA) HPLC on a C18 phenomenex reversed phase column (250×4.6 mm^2). Identification and quantification of polyphenols present in the methanolic extract of defatted *C. auriculata* stem bark was carried out by comparing the retention time of the sample with respective reference standard peaks.

In vitro anti-diabetic efficacy of methanolic extracts of *C. auriculata* stem bark

To determine the anti-diabetic efficacy of methanolic extract of *C. auriculata* stem bark, the *in vitro* α-amylase and α-glucosidase inhibition assay was carried out as described by Hemalatha *et al.*[22]

The IC_{50} values of each sample in both the assays were calculated using the percentage inhibition values obtained at each concentration of test samples.

Experimental animals

Eight-weeks-old C57BL/6 male mice (20±2 g) were used for the studies. Animals were housed at the Animal House, Vipragen Biosciences, Mysuru, at 22±3ºC temperature, <55±5% humidity and 12 hr alternate light/dark cycle. The animals were given food and water *ad libitum* and acclimatized for 7 days before initiating the experiment. All the in-life protocols and methodologies were approved by the Institutional Animal Ethical Committee (IAEC) for the Care and Use of Laboratory Animals (VIP/

IAEC/206/2020) and experiments were conducted following the guidelines of CCSEA, India.

Acute oral toxicity study of methanolic extract

The methanolic extract of *C. auriculata* stem bark was tested for its safety in female C57BL/6 mice by following OECD 423 guideline, Acute Oral Toxicity-Acute Toxic Class Method. A total of 6 female mice, 5-6 weeks old were used in the study with 3 mice at each step. Mice were fasted, with only access to water, for 3-4 hr before dosing. The methanolic extract of *C. auriculata* stem bark were administered via oral route as a suspension in water with a maximum level set at a limit dose of 2000 mg/kg b.wt and dose volume of 10 mL/kg body weight. Doses were freshly prepared prior to administration. After dosing, mice were observed individually at least once during the 1st 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr and daily thereafter, for a total of 14 days. Animals were observed for mortality, clinical signs and body weight was recorded every 7 days.

In vivo anti-diabetic activity of methanolic extract in C57BL/6 mice-induced diabetes using high-fat diet and streptozotocin

Type-2 diabetes was induced in mice following the method reported by Srinivasan *et al.*,[23] 8-weeks-old, male C57BL/6 mice were acclimatized in the experimental room for a week before the start of the experiment. They were divided into control group having 10 animals and experimental group having 50 animals. Control group mice were fed with AIN-9 (normal) diet and experimental group mice were fed with High-Fat Diet (HFD) for 28 days. On day 29, all experimental animals were fasted for a period of 4-6 hr between 7 AM and 1 PM before administering a single low dose (35 mg/kg b.wt) of Streptozotocin (STZ) intraperitoneally. STZ was prepared by dissolving in citrate buffer (0.05 M, pH 4.5). Sucrose solution (5% in water) was administered within 24 hr after STZ injection. All mice had free access to water. Fasting Blood Glucose (FBG) levels were measured after 5 days of STZ injection.[4]

Animals with FBG≥200 mg/dL in the experimental group were selected as subjects for the anti-diabetic study with test interventions. Selected animals were divided into four experimental groups (groups 2-5) with a sample size of *n*=10. The experimental animal groups are as follows:

Group 1 mice were fed with normal diet and are considered as control,

Group 2 mice were untreated and were considered as diabetic control,

Groups 3 and 4 mice were administered methanolic extract of stem bark of *C. auriculata* at doses of 100 mg/kg and 200 mg/kg b.wt.p.o (doses were fixed based on the basis of 1/10th of safer dose from the acute toxicity experiment of the present study and from our previous study[4])respectively for 21 days,

Group 5 mice were administered metformin at a dose of 200 mg/kg b.wt.p.o for 21 days.

All the mice including the control group were maintained on the respective diets till the end of the experimentation period. At the end of experimental period (Day 22), blood samples were collected via retro-orbital plexus and serum was separated from blood samples via centrifugation at 2000 rpm at 4°C. Additionally, during the necropsy, gross examination was conducted to observe any visible abnormalities and target organs (liver, pancreas and muscle) were collected for further analysis. The remaining carcass were disposed according to standard protocols.

Weekly body weight and blood glucose were recorded. After necropsy, markers of carbohydrate metabolism, oxidative stress and histopathology were carried out. Glucose-6-phosphatase,[24] glycogen[25] and glucokinase contents[26] were quantified in liver tissue homogenate. Superoxide dismutase[27] was also measured in the liver tissue.

At the end of the treatment period, upon necropsy, a fraction of the liver, pancreas and muscle tissues were collected from all the experimental mice and washed with PBS to remove any residual blood. The organs were fixed in 10% buffered formalin for>48 hr, processed and embedded with paraffin and subsequently tissue sections of 4-5 micron thickness were stained using haematoxylin and eosin for further histopathological evaluations. Histological microscopic structures of pancreas and liver sections were examined using a light microscope (Olympus microscope CX33, China).

Statistical analysis

All results were expressed as mean±Standard Deviation (SD). The *in vitro* anti-diabetic efficacy of methanolic extract was determined by student's *t* test. For the *in vivo* studies, parametric One-way ANOVA was used for statistical analysis after subjecting to normality test, followed by *post-hoc* Duncan test to evaluate the significant differences ($p<0.05$) between the different experimental groups using GraphPad Prism 9.5.

RESULTS

Extraction yields

The yield of ethanol, methanol and water extracts obtained from the defatted stem bark of *C. auriculata* were found to be 13.3%, 14.3% and 11.6% (w/w), respectively (Table 1). The extracts obtained were properly stored in an airtight container at -20°C until further use for phytochemical and pharmacological evaluations.

Total polyphenols and total flavonoids content

The total polyphenol content was significantly higher in the methanolic extract (8013.41±82.69 mg/100 g) of defatted *C. auriculata* stem bark followed by the ethanolic (7328.39±81.73 mg/100 g) and aqueous (7960.12±129.70 mg/100 g) extracts (Table 1). However, the total flavonoid content was found to be more in the methanolic extract (1568.56±98.22 mg/100 g) followed by aqueous (901.88±38.28 mg/100 g) and ethanolic (1293.62±51.76 mg/100 g) extract of *C. auriculata* stembark (Table 1).

In vitro anti-oxidant activity of different solvent extracts of *C. auriculata* stem bark

The anti-oxidant activity of *C. auriculata* stem bark extracts was determined by DPPH and FRAP assay (Figure 1). The methanolic extract (57.71±4.12 µg/mL) was found to have highest anti-oxidant potential, as less concentration of the extract was required to scavenge 50% of DPPH radicals. As per the results obtained from FRAP assay, the methanolic extract had the highest (52.44±5.46 µg/mL) anti-oxidant potential as it could reduce Fe^{3+} to Fe^{2+} efficiently, followed by ethanolic (61.83±4.61 µg/mL, 95.68±6.5 µg/mL) and aqueous (71.34±5.68 µg/mL, 90.78±8.43 µg/mL) extracts.

FTIR analysis

The FTIR spectra of methanolic extract of *C. auriculata* extract is shown in Figure 2. The spectra showed the presence of hydroxyl group indicated by the band around 3320 and the bands around 2942 and 2831 could be due to C-H stretching.

HPLC analysis

The HPLC profile of methanolic extract of defatted *C. auriculata* stem bark revealed the presence of various polyphenols, viz., gallic acid, procatechuic acid, p-hydroxy benzoic acid, p-coumaric acid, vanillic acid, syringic acid, ferulic acid, trans-cinnamic acid and quercetin.

In vitro anti-diabetic activity of methanolic extract of *C. auriculata* stem bark

The anti-diabetic efficacy of methanolic extract of *C. auriculata* stem bark was determined by α-amylase and α-glucosidase inhibition assays. The results of both α-amylase and α-glucosidase inhibition assays revealed that the methanolic extract of *C. auriculata* stem bark was potent enough to inhibit the α-amylase (212.98±13.84 µg/mL) and α-glucosidase (94.83±7.30 µg/mL) enzymes significantly with least concentration compared to the standard acarbose (219.54±16.37 µg/mL, 120±8.28 µg/mL) respectively (Table 2).

Acute oral toxicity study

Methanolic extract of *C. auriculata* stem bark at single oral dose of 2000 mg/kg b.wt. did not result in mortality or any observed clinical signs of toxicity during the entire experiment. Further, no change in body weight was observed and no abnormalities were detected upon gross pathological examination. Therefore, based on the observed results, the median lethal dose of the extract after single oral dose administration to female rats, observed over a period of 14 days, the extract can be classified under Category 5 according to the GHS (Globally Harmonised System) Classification and Labelling of chemicals ("About the GHS | UNECE," n.d.), with LD_{50} greater than 2000 mg/kg body weight and LD_{50} cut off value of 5000 mg/kg body weight.

Anti-diabetic activity using HFD and STZ-induced type 2 diabetic C57BL/6 mice

Body weight

There was significant decrease in the body weight of animals in the diabetes control (17.80±1.60 g, 17.01±1.51 g) group (G2) on days 14 and 21 compared to normal control (22.22±1.04 g, 23.35±1.32 g) group (G1). The treatment of methanolic extract of *C. auriculata* at both 100 mg/kg b.wt. (21.91±1.26 g, 22.30±1.18 g) and 200 mg/kg b.wt. (21.42±1.61 g, 22.35±1.28 g) doses and metformin (200 mg/kg b.wt.) (21.92±1.07 g, 22.90±0.94 g) prevented decrease in the body weight induced by HFD-STZ diabetes indicating the potential protective effect of the methanolic extract of *C. auriculata* stem bark.

Fasting blood glucose level

A significant increase in the fasting blood glucose levels was observed in HFD+STZ administered mice (252.71±26.20 mg/dL) compared to normal control group mice (91.01±3.01 mg/dL) confirming the induction of diabetes (Figure 3a). However, the administration of methanolic extract of *C. auriculata* stembark

Table 1: Total polyphenols and total flavonoid content of different solvent extracts of *C. auriculata* stem bark.

Sl. No.	Extracts	Yield (%)	Total polyphenols (mg/100 g)	Total flavonoids (mg/100 g)
1	Ethanol	13.3[b]	7328.39±81.73[b]	1293.62±51.76[b]
2	Methanol	14.3[b]	8013.41±82.69[a]	1568.56±98.22[a]
3	Water	11.6[a]	7960.12±129.70[a]	901.88±38.28[c]

Note: All the values are mean±SEM.
Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly ($p<0.05$) different as judged by Duncan's *post-hoc* test.

Table 2: Alpha amylase and alpha glucosidase inhibition efficacy of methanolic extract of *C. auriculata* stem bark.

Samples	IC_{50} (µg/mL)	
	Alpha amylase inhibition assay	Alpha-glucosidase inhibition assay
Acarbose	219.54±16.37*	120±8.28*
Methanolic extract	212.98±13.84	94.83±7.30

All values are in mean±SD. Note * indicates significant difference.

Table 3: Efficacy of methanolic extract of *C. auriculata* stem bark on the activities of antioxidant enzymes and concentration of oxidative stress markers in the serum and muscle of HFD+STZ-induced diabetic mice.

Groups	Activities of Antioxidant Enzymes				Oxidative Stress markers			
	Superoxide Dismutase (SOD) activity (Unit/min/mg protein)		Catalase (CAT) activity (nmol/mg/min)		Reactive Oxygen Species (ROS) µmol of DCF formed/min/mg protein		Nitric Oxide (NO) ng/mg protein	
	Serum	Muscle	Serum	Muscle	Serum	Muscle	Serum	Muscle
G1	1766.98±147.67[b]	16.73±1.93[c]	0.63±0.08[b]	1.98±0.10[b]	2726.32±210.45[b]	2887.87±84.01[b]	4.53±0.74[b]	3.88±0.30[a]
G2	633.75±98.45[a]	7.45±1.80[a]	0.35±0.02[a]	0.56±0.04[a]	3234.65±102.36[c]	3944.93±183.15[c]	8.55±1.09[c]	6.72±0.44[b]
G3	1578.24±132.88[b]	12.46±1.99[b]	0.58±0.05[b]	1.68±0.14[b]	2464.76±120.78[b]	2794.16±201.94[b]	5.60±0.52[b]	4.23±0.21[a]
G4	2102.34±241.65[b,c]	13.91±0.92[b,c]	0.88±0.09[c]	2.93±0.17[b,c]	2144.98±195.09[a,b]	2276.06±98.44[a,b]	4.81±0.33[a,b]	3.24±0.12[a]
G5	2301.76±204.01[c]	14.98±1.55[c]	0.94±0.10[c]	3.02±0.19[c]	1955.34±101.10[a]	1955.84±78.43[a]	3.95±0.31[a]	3.05±0.78[a]

Note: All the values are mean±SD.
Mean values with same superscript letter in the given column are not significantly different, whereas those with different superscript letters are significantly (p<0.05) different as judged by Duncan's *post-hoc* test.

at both 100 mg/kgb.wt. (182.23±18.50 mg/dL) and 200 mg/kg b.wt (120.83±12.34 mg/dL) in HFD+STZ-induced diabetic mice reduced the blood glucose levels indicating its potential anti-hyperglycemic activity.

Fasting insulin level

There was a significant decrease in the serum insulin level in the HFD+STZ administered diabetic mice (0.34±0.01 µL U/mL), whereas the diabetic mice treated with the methanolic extract (200 mg/kg b.wt.) of *C. auriculata* stem bark (0.76±0.05 µL U/mL) showed normalised fasting insulin levels similar to that of normal control (0.73±0.02 µL U/mL) and standard metformin-treated (200 mg/kg b.wt.) diabetic mice (0.96±0.06 µL U/mL) (Figure 3b).

Activities of serum and muscle anti-oxidant enzymes

The activities of serum as well as muscle anti-oxidant enzymes, i.e., SOD and CAT were significantly decreased in the HFD+STZ-induced diabetic control mice (S 633.75±98.45, 0.35±0.02; M7.45±1.80, 0.56±0.04) compared to that of normal control group mice (S 1766.98±147.67, 0.63±0.08; M 16.73±1.93, 1.98±0.10) (Table 3). Whereas, the diabetic mice administered with the methanolic extract of both the 100 mg/kg b.wt. (S 1578.24±132.88, 0.58±0.05; M 12.46±1.99, 1.68±0.14) and 200 mg/kg b.wt. of *C. auriculata* stem bark (S 2102.34±241.65, 0.88±0.09; M 13.91±0.92, 2.93±0.17) elevated the anti-oxidant enzyme activities similar to that of normal control mice. However, the higher dose of methanolic extract treated mice showed better anti-oxidant enzyme activities comparable to that of metformin-treated (200 mg/kg b.wt.) mice (S 2301.76±204.01, 0.94±0.10; M 14.98±1.55, 3.02±0.19) (Table 3).

Oxidative stress marker levels in the serum and muscle

The HFD-STZ-induced diabetic mice (S 3234.65±102.36 µmol of DCF formed/min/mg protein, 8.55±1.09 ng/mg protein; M 3944.93±183.15 µmol of DCF formed/min/mg protein, 6.72±0.44 ng/mg protein) showed significant elevation in the concentration of Reactive Oxygen Species (ROS) and Nitric Oxide (NO) in the serum and muscle compared to normal control group mice (S 2726.32±210.45 µmol of DCF formed/min/mg protein, 4.53±0.74 ng/mg protein; M 2887.87±84.01 µmol of DCF formed/min/mg protein, 3.88±0.30 ng/mg protein) (Table 3). Further, the methanolic extract of 200 mg/kg b.wt. of *C. auriculata* stem bark treated diabetic mice (S 2144.98±195.09 µmol of DCF formed/min/mg protein, 4.81±0.33 ng/mg protein; M 2276.06±98.44 µmol of DCF formed/min/mg protein, 3.24±0.12 ng/mg protein) showed the normalised levels of ROS and NO comparable to that of normal control and metformin-treated (200 mg/kg b.wt.) diabetic mice (S 1955.34±101.10 µmol of DCF formed/min/mg protein, 3.95±0.31 ng/mg protein;1955.84±78.43 µmol of DCF formed/min/mg protein, 3.05±0.78 ng/mg protein).

Activities of glucose-6 phosphatase and glucokinase in the liver

The activities of key enzymes of carbohydrate metabolism were determined in the liver and found that there was a significant increase in the activity of glucose-6 phosphatase

Table 4: Efficacy of the methanolic extract of *C. auriculata* stem bark on the activities of glucokinase and glucose-6-phosphatase and the concentration of glycogen in the liver of HFD+STZ-induced diabetic mice.

Groups	Activities of		Glycogen content (mg/g tissue)
	Glucose-6-phosphatase (mg/min/mg protein)	Glucokinase (nM/min/mg protein)	
G1	16.56±2.97^{a}	6.28±1.70^{c}	2.13±0.41^{b}
G2	39.54±5.63^{c}	2.05±0.18^{a}	1.50±0.26^{a}
G3	32.56±3.12^{b}	2.41±0.32^{a}	1.73±0.57^{a,b}
G4	22.92±0.97^{a,b}	5.00±1.09^{b}	2.01±0.26^{b}
G5	23.75±3.03^{a,b}	6.09±0.49^{b,c}	2.26±0.16^{b}

Note: All the values are mean±SD.

Mean values with same superscript letter in the given column are not significantly different, whereas those with different superscript letters are significantly ($p<0.05$) different as judged by Duncan's *post-hoc* test.

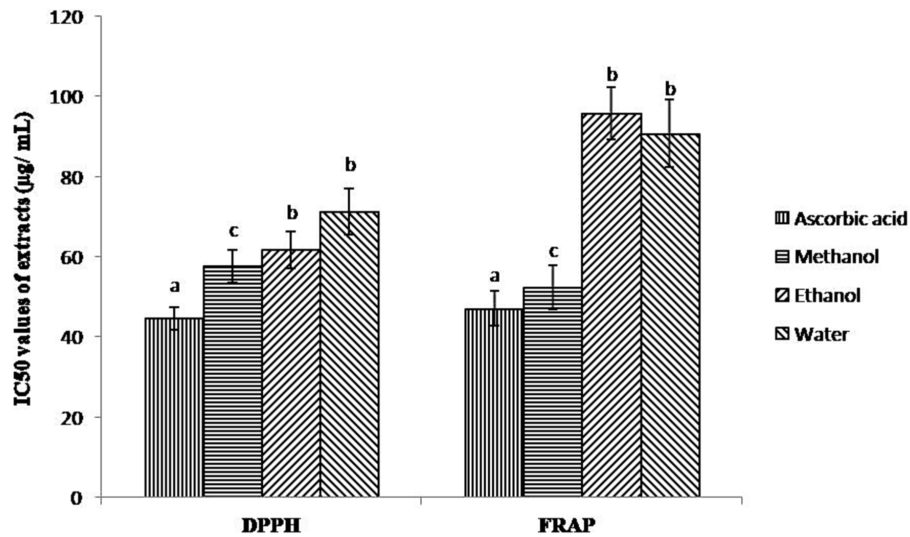


Figure 1: Vertical bar graphs showing the DPPH scavenging activity and FRAP potency of the methanolic extract of *C. auriculata* stem bark. Note: All the values are mean±SD.Mean values with same superscript letters for each assay are not significantly different, whereas those with different superscript letters are significantly ($p<0.05$) different as judged by Duncan's *post-hoc* test.

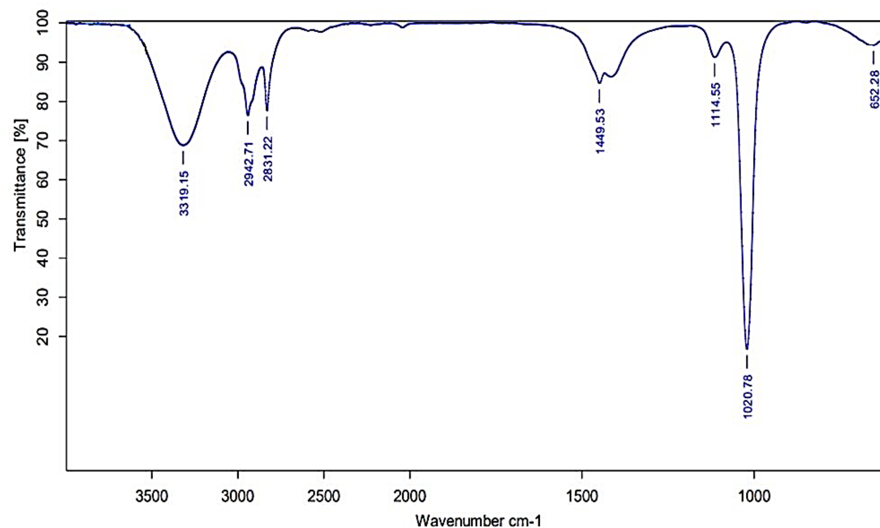


Figure 2: FTIR spectra of methanolic extract of *C. auriculata* stem bark showing the bands of different functional groups.

and a concomitant decrease in the activity of glucokinase in the diabetic control mice (39.54±5.63 mg/min/mg protein, 2.05±0.18 nm/min/mg protein) compared to that of normal control mice (16.56±2.97 mg/min/mg protein, 6.28±1.70 nm/min/mg protein) (Table 4). Conversely, the high dose of methanolic extractof *C. auriculata* stem bark treated diabetic mice (22.92±0.97 mg/min/mg protein, 5.00±1.09 nm/min/mg protein) showed the activities of these enzymes comparable to that of normal control and metformin-treated (200 mg/kg b.wt.) diabetic mice (23.75±3.03 mg/min/mg protein, 6.09±0.49 nm/min/mg protein).

Glycogen concentration in the liver

The concentration of glycogen was significantly lower in the HFD+STZ-induced diabetic mice (1.50±0.26 mg/g tissue) compared to normal control mice (2.13±0.41 mg/g tissue), whereas those administered with 200 mg/kg b.wt. of methanolic extract of *C. auriculata* stem bark(2.01±0.26 mg/g tissue) and metformin (200 mg/kg b.wt.) (2.26±0.16 mg/g tissue) showed higher concentration of glycogen in the liver similar to that of normal control mice (Table 4).

Histology

The histomorphology of the pancreas showed a reduction in the surface area of the islets of Langerhans evidently in HFD+STZ administered diabetic mice compared to that of normal control mice. However, the diabetic mice treated with 200 mg/kg b.wt. of methanolic extract of *C. auriculata* stem bark recovered the pancreatic histoarchitecture similar to that of normal control mice (Figures 4a-d).

Similarly, the histology of liver showed infiltration of inflammatory cells, sinusoidal haemorrhages in the diabetic control mice compared to normal control mice but not in those diabetic mice treated with the 200 mg/kg b.wt. methanolic extract of *C. auriculata* stem bark (Figures 4e-h).

DISCUSSION

Diabetes is one of the major metabolic disorders occuring worldwide. Several medicines are being used to treat diabetic condition which includes both synthetic and naturally available herbal supplements. Type-2 diabetes is one of the global metabolic disorders affecting all aged populations severely. The body fails to detect the insulin present leading to type-2 diabetic condition. Hence, several insulin sensitizing drugs have been developed and is being used to manage type-2 diabetes, viz., metformin, glibenclamide, etc. However, these drugs are known to have side-effects like flatulence, gastritis, etc. Therefore, there is a dire need for any alternate medicinal approach to manage type-2 diabetes. The naturally available herbs are some of the best alternative treatment approaches since they have minimum side-effects. The naturally available herbs exert beneficial effects owing to the secondary metabolites[28] synthesized by the plants to protect against the threat like harsh environmental conditions, chemicals, etc. These secondary metabolites and phytochemicals are exploited to exert similar health beneficial properties in humans. Therefore, it is necessary to understand the phytochemical profile of medicinal herbs. Indeed, several studies have reported the anti-diabetic efficacy due to the phytochemical present in the herbal supplement either in *in vitro* or *in vivo* models. For instance, resveratrol,[29] curcumin,[30,31] *Garcinia xanthochymus*,[32] etc, have been reported to ameliorate the diabetic condition either using *in vitro* or *in vivo* models. Similarly, *C. auriculata* is one such promising naturally available herb with several beneficial properties like, anthelminthic,[7] anti-bacterial,[8] anti-cancer,[9] hepatoprotective,[10] anti-oxidant,[11,13] etc. Further, studies have also reported the anti-diabetic activity of different parts of the herb, viz., root,[11] flower buds,[6,14,33] flower and leaves.[34] However, the phytochemical profile and anti-diabetic efficacy of *C. auriculata* stem-bark is not reported. Hence, the present study aimed to address the above mentioned lacunae.

In the present study, the *C. auriculata* stem bark was defatted using hexane and then subjected to extraction on rotary shaker with solvents, ethanol, methanol and water at room temperature. The extract obtained was freeze dried and analysed for concentration of total polyphenols and flavonoids. It was found that the concentration of total polyphenols and flavonoids was highest in methanolic extract followed by ethanol and aqueous extracts respectively. Further, the *in vitro* anti-oxidant property determined by DPPH and FRAP assay was found to be rich in the methanolic extract potentially due to its rich polyphenol content. These results were similar to that of our earlier study with *C. auriculata* roots.[4] Based on these data, the methanolic extract was selected for its further characterization. The methanolic extract of *C. auriculata* stem bark showed the highest total polyphenol and flavonoid content, potency in the *in vitro* anti-oxidant and anti-diabetic properties.

Initially, the chromatographic profile was determined by HPLC method and the presence of polyphenols, viz., gallic acid, procatechuic acid, p-hydroxy benzoic acid, p-coumaric acid, vanillic acid, syringic acid, ferulic acid, trans-cinnamic acid and quercetin were noted suggesting the rich polyphenol content in the methanolic extract of *C. auriculata* stembark. Further, the functional groups present were determined by FTIR analysis and the presence of major bands for -OH and C-H groups were observed. The anti-diabetic activity was estimated by *in vitro* α-amylase and α-glucosidase inhibition assays. The methanolic extractof *C. auriculata* stem bark inhibited the activity of α-amylase and α-glucosidase better than the standard acarbose, as least amount of the methanolic extract could inhibit the activities of these enzymes than that of the acarbose. Given that α-amylase and α-glucosidase are the two primary enzymes involved in the metabolism of glucose, inhibition of these enzymes slows

down the breakdown of carbohydrate in the small intestine and therefore, may reduce the post-prandial glucose elevation.[35]

Based on the results of the *in vitro* study, the *in vivo* anti-diabetic activity of the methanolic extraxt of *C. auriculata* stembark was investigated in the present study. The methanolic extract of *C. auriculata* stem bark ameliorated the HFD+STZ-induced diabetes in mice. About 5-6-week-old mice were administered with the HFD for 6 weeks and a dose of STZ (35 mg/kg b.wt) injection was given to induce type-2 diabetic condition. FBG levels were checked in all the experimental group mice to determine the induction of diabetes.The development of type-2 diabetes was confirmed by the elevated FBG level in HFD+STZ administered mice similar to those reported in other studies.[36] After the confirmation of type-2 diabetic condition, the ameliorative effect of the methanolic extract of *C. auriculata* stembark was investigated. For the same, 2 doses, i.e., 100 mg/kg b.wt. and 200 mg/kg b.wt. of methanolic extract was administered for 28 days and compared with normal control, diabetic control and diabetic mice treated with standard metformin (200 mg/kg b.wt.). At the end of treatment period, all experimental mice were euthanized, blood and tissue samples were collected and processed. The serum separated from the blood was used to analyze the anti-oxidant activity and serum insulin levels. The tissue samples were processed for histological analysis, anti-oxidant status and the activities of carbohydrate metabolism markers separately.

In addition to the hyperglycemic condition, the serum insulin level was reduced in the diabetic mice indicating the insulin resistance and type-2 diabetic condition. The decrease in the serum insulin level despite the administration of HFD may be either because of negative feedback mechanism to the pancreatic beta cells by the insulin resistance-induced compensatory hyperinsulinemia[4]or due to destruction of pancreatic beta cells by STZ injection. Though, serum insulin levels are reduced in the diabetic control mice, the insulin resistance is maintained in these mice to develop type-2 diabetic condition and these results are in line with earlier studies.[37]

Further, in the present study, type-2 diabetic control mice also showed a significant decrease in the activity of anti-oxidant enzymes with concomitant increase in the oxidative stress markers in the serum similar to previous reports.[38] Furthermore, oxidative stress was observed in the muscle tissue of diabetic control mice compared to the normal control mice. This is because, the activity of the anti-oxidant enzymes was reduced significantly in the muscle of diabetic control mice compared to normal control mice. In the present study, the oxidative stress in the serum and muscle tissue may be due to an increase in the generation of ROS and NO either by the hyperglycemic condition or because of HFD administered. This could resonate with studies that have reported that hyperglycemia[39] or HFD[40] induces oxidative stress.

Additionally, the carbohydrate metabolism was also altered in the type-2 diabetic mice. This is substantiated by the significant elevation in the activity of the liver glucose-6 phosphatase and decrease in the glucokinase activity with a significant reduction in the liver glycogen content in the type-2 diabetic control mice compared to normal control mice. The glucose 6-phosphatase is a hydrolytic enzyme involved in the production of hepatic glucose from glucose 6-phosphate. Increased hepatic glucose production is considered as one of the major contributors of hyperglycaemia in type-2 diabetes.[41] Similarly, glucokinase plays a vital role in the metabolism of glucose in the liver. To contribute to the elevated hepatic glucose levels, the activity of glucokinase was reduced in diabetic condition.[42] A significant increase in the activity of glucose-6-phosphatase and a decrease in the glucokinase activity in the liver of type-2 diabetic control mice in this study is in agreement with earlier studies.[42,43] This altered carbohydrate metabolism resulted in the elevated levels of glucose in the blood. Furthermore, the altered carbohydrate metabolism is also evident by a significant decrease in the concentration of liver glycogen in the HFD+STZ type-2 diabetic mice. While the type-2 diabetic mice showed a deleterious alteration in carbohydrate metabolism, the diabetic mice administered with 200 mg/kg b.wt of methanolic extract of *C. auriculata* stem bark showed an improvement in the glucose-6-phosphatase, glucokinase,

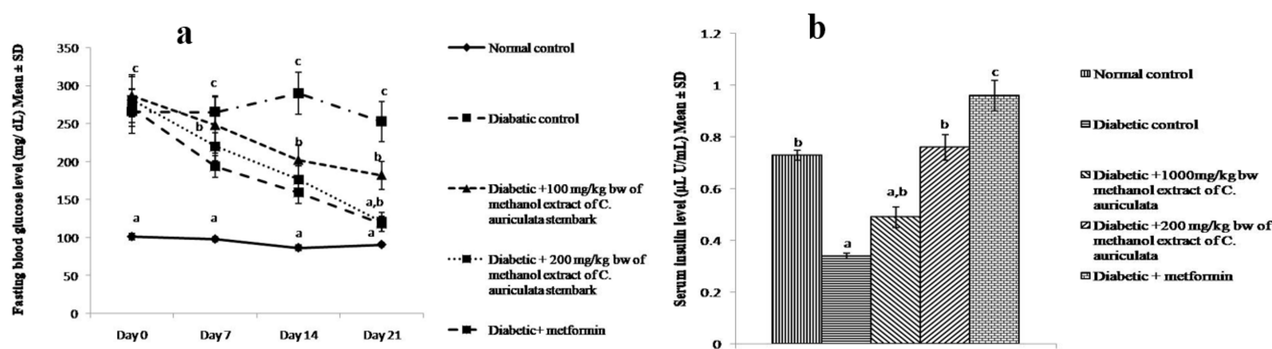
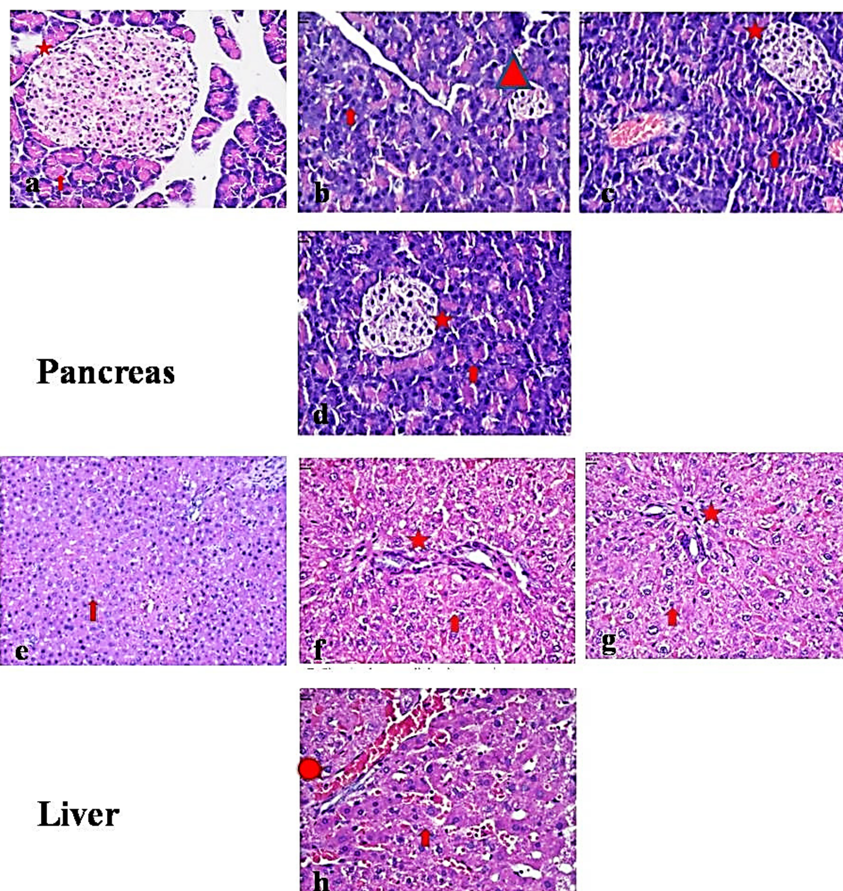


Figure 3a,b: a) Line graph showing the fasting blood glucose levels in different experiment group mice.Note: All the values are mean±SD. Mean values with same superscript letters for each assay are not significantly different, whereas those with different superscript letters are significantly ($p<0.05$) different as judged by Duncan's *post-hoc* test. b) Vertical bar graphs showing the serum insulin levels in different experimental group mice. Note: All the values are mean±SD. Mean values with same superscript letters for each assay are not significantly different, whereas those with different superscript letters are significantly ($p<0.05$) different as judged by Duncan's *post-hoc* test.



Figures 4a-h: Photomicrographs of pancreas of a) normal control, b) diabetic control, c) diabetic+methanolic extract of *C. auriculata* stem bark (200 mg/kg body weight) and d) diabetic+metformin.Note the reduction in the size of islet of Langerhans in the diabetic mice (triangle) compared to other groups (star) and also the normal pancreatic acini (arrow). 40X H & E stain. Photomicrographs of liver of e) normal control, f) diabetic control, g) diabetic+methanolic extract of C. auriculata (200 mg/kg body weight) and h) diabetic+metformin.Note the normal histomorphology of liver in the normal control group but infiltration of inflammatory cells (star) in the diabetic and reduction in the infiltration in methanolic extract treated group, sinusoidal haemorrhages (circle) in the metformin treated diabetic group and also find the normal hepatocyte parenchymal cells (arrow).

activities thereby establishing that the methanolic extract aids in normalizing the carbohydrate metabolism in type-2 diabetes.

Apart from the biochemical alterations, histoarchitectural alterations were found in the liver and pancreas of type-2 diabetic control mice than those of normal control mice where histoarchitecture were normal in both the tissues. The above-mentioned alterations were similar to earlier studies. While the type-2 diabetic mice showed the above-mentioned deleterious alterations, the diabetic mice administered with 200 mg/kg b.wt of methanolic extract of *C. auriculata* stem bark did not show these alterations. These results indicates the amelioration of diabetic condition by normalizing the fasting blood glucose levels and its associated alterations to the levels of normal control mice.

The probable mechanism of action of the methanolic extract of *C. auriculata* stem bark in the present study, may be as follows. The HFD+STZ-induced hyperglycemia may be because of destruction of islets of Langerhans, increased hepatic glucose production[44] and induction of insulin resistance in mice. This is evident from the histological architecture of pancreas, reduced serum insulin levels and alterations in the carbohydrate metabolism leading to hyperglycemia. The destruction of pancreatic beta cells and reduced serum insulin levels in the diabetic mice as evident by the histological images are because of STZ injection, whereas, the alterations in the carbohydrate metabolism in the diabetic mice is mainly by the HFD administered.[45]

Further, the hyperglycemic condition leads to an increase in oxidative stress, as observed in the serum and muscle with significant decrease in the activities of anti-oxidant enzymes and concomitant increase in the concentration of oxidative stress markers. This is because hyperglycemic condition is known to generate ROS resulting in the oxidative stress.[46] However, in the type-2 diabetic mice treated with 200 mg/kg b.wt of methanolic extract of *C. auriculata* stem-bark, the deleterious alterations were not observed and were similar to normal control mice.

Since, it is established that the HFD induces hyperglycemia by increased hepatic glucose production and reduced serum insulin levels, the inhibitory action of the methanolic extract of *C. auriculata* stem bark as shown by *in vitro* study, might have played a key role in lowering the blood glucose levels. The normalized blood glucose levels may have improved the insulin sensitization

and in turn serum insulin levels have been normalized to the levels of normal control mice. It is also evident by the normalized histomorphology of pancreas in the methanolic extraxt of *C. auriculata* stem bark treated diabetic mice. Further, the normalized FBG level by the methanolic extract of *C. auriculata* stem bark might have aided to reduce oxidative stress in addition to direct anti-oxidant activity of methanolic extract of *C. auriculata* stem bark as evident in our *in vitro* study.

Hence, the anti-diabetic property of the methanolic extract of *C. auriculata* stem bark in the present study can be established by targeting the blood glucose control mechanisms such as enhancing insulin sensitivity (indicated by increased glucokinase), reducing the hepatic glucose production (indicated by decreased glucose 6-phosphatase level), improving the storage of glucose (indicated by increased glycogen level) and by reducing the oxidative stress (indicated by increased activity SOD) in diabetic mice. The anti-diabetic effect of *C. auriculata* can be attributed to the presence of appreciable quantity of polyphenols and flavonoids identified in the methanolic extract. From the HPLC results it is evident that ferrulic and gallic acid are rich in the methanolic extract of *C. auriculata* stem-bark. Hence, it could be the action of ferrulic and gallic acid in the extract of our study that has improvised the insulin sensitivity, glycogenesis and reduced the glucose production as evident from earlier studies.[47-49]

CONCLUSION

The polyphenol rich methanolic extract of *C. auriculata* shows its anti-diabetic activity by decreasing the elevated FBG levels to normalcy by altering the activities of enzymes involved in the carbohydrate metabolism. Further, the products like 'chenna tea' obtained from the methanolic extract of *C. auriculata* can be analysed for its sensory applications and efficacy against diabetes as potential future perspective.

ACKNOWLEDGEMENT

The authors are grateful to the Director, CSIR-CFTRI, Mysuru for providing required facilities for the study.

FUNDING

The work was financially supported by Vipragen Biosciences Private Limited, Mysuru under Industry sponsored Ph.D. program.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALP: Alkaline phosphatase; **ALT:** Alanine transferase; **AST:** Aspartate transferase; **BUN:** Blood urea nitrogen; **b.wt:** Body weight; **DPPH:**2,2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferrous reducing antioxidant power; **FTIR:** Fourier-transform infrared spectroscopy; **HDL:** High-density lipoprotein; **HPLC:** High-pressure liquid chromatography; **LDL:** Low-density lipoprotein; **NO:** Nitric oxide; **p.o:** Peroral; **ROS:** Reactive oxygen species; **SOD:** Superoxide dismutase; **STZ:** Streptozotocin; **TC:** Total cholesterol; **TG:** Triglyceride; **T2DM:** Type-2 diabetic mellitus; **VLDL:** Very low-density lipoprotein.

ETHICAL APPROVAL

All the experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC) for the Care and Use of Laboratory Animals (VIP/IAEC/206/2020) and the CCSEA guidelines were followed throughout the experiment.

SUMMARY

The present study was aimed to investigate the anti-diabetic activity of *Cassia auriculata* stem-bark in both *in vitro* and *in vivo* models. The analysis using different solvent extracts of the *C. auriculata* stem-bark revealed that the methnol extract consisted of higher amount of polyphenols and showed highest anti-oxidant and anti-diabetic activity *in vitro*. Further, the methanolic extract were rich in polyphenols as inferred by HPLC analysis and strong -OH as observed by FTIR analysis. Further to check the anti-diabetic activity of methanolic extract of *C. auriculata* stem-bark, type-2 diabetic model was used. Male C57BL/6 mice were administered with HFD+STZ and the development of type-2 diabetes was confirmed by elevated glucose levels. The results revealed that the *C. auriculata* stem-bark methanolic extract administered diabetic mice showed normalised fasting blood glucose and insulin levels suggesting the amelioration of diabetic condition. Further, the extract-fed diabetic mice showed the restoration of all the alterations observed in diabetic mice, viz., oxidative stress in serum and muscle tissue, altered carbohydrate metabolism, histological alterations comparable to that of controls. Hence, the methanolic extract of *C. auriculata* stem-bark can be used in the treatment of type-2 diabetes and its associated alterations.

REFERENCES

1. Cheng Y, Sibusiso L, Hou L, Jiang H, Chen P, Zhang X, *et al.* Sargassum fusiforme fucoidan modifies the gut microbiota during alleviation of streptozotocin-induced hyperglycemia in mice. Int J Biol Macromol. 2019;131:1162-70.
2. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes-Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health.2020;10: 107-11.
3. Li X, Zhen M, Zhou C, Deng R, Yu T, Wu Y, *et al.* Gadofullerene nanoparticles reverse dysfunctions of pancreas and improve hepatic insulin resistance for type 2 diabetes mellitus treatment. Acs Nano. 2019;13:8597-608.
4. Salma B, Janhavi P, Muthaiah S, Veeresh P, SanthepeteNanjundaiah M, Divyashree S, *et al.* Ameliorative Efficacy of the *Cassia auriculata* Root Against High-Fat-Diet+STZ-Induced Type-2 Diabetes in C57BL/6 Mice. ACS Omega. 2020;6:492-504.
5. Pandey MM, Rastogi S, Rawat AKS. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary Altern Med. 2013; 2013:376327.
6. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J. 2002;43:617-21.
7. Joshi SG. Cesalpinaceae-*Cassia auriculata*. Textbook of Medicinal Plants, 3rd ed.: 2000; India Book House: Bangalore, India.

8. Samy PR, Ignacimithu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol. 2000;69:63-71.
9. Padmalochana K. Anti-cancer (liver cancer cell lines) and antioxidant activity of *Cassia auriculata* flower extract from acetone and methanol solvents. J Drug Discovery Ther. 2018;8:274-8.
10. Jaydeokar AV, Bandawane DD, Bibave KH, Patil TV. Hepatoprotective potential of *Cassia auriculata* roots on ethanol and antitubercular drug-induced hepatotoxicity in experimental models. Pharm Biol. 2014;52:344-55.
11. Gupta S, Sharma SB, Prabhu KM. Ameliorative effect of *Cassia auriculata* L. leaf extract on glycemic control and atherogenic lipid status in alloxan-induced diabetic rabbits. Indian J Exp Biol. 2009;47:974-80.
12. Latha M, Pari L. Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. Clin Exp Pharmacol Physiol. 2003a;30: 38-43.
13. Latha M, Pari L. Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Mol Cell Biochem. 2003b;243:23-8.
14. Nambirajan G, Karunanidhi K, Ganesan A, Rajendran R, Kandasamy R, Elangovan A, *et al.* Evaluation of antidiabetic activity of bud and flower of Avaram senna (*Cassia auriculata* L.) in high fat diet and streptozotocin induced diabetic rats. Biomed Pharmacother. 2018;108:1495-506.
15. Gupta S, Sharma SB, Singh UR, Bansal SK, Prabhu KM. Elucidation of mechanism of action of *Cassia auriculata* leaf extract for its antidiabetic activity in streptozotocin-induced diabetic rats. J Med Food. 2010;13(3):528-34.
16. Mudhol S, Serva Peddha M. Development of capsaicin loaded nanoparticles based microneedle patch for transdermal drug delivery. J Drug Deliv Sci Technol. 2023;80:104120.
17. Kupina S, Fields C, Roman MC, Brunelle SL. Determination of Total Phenolic Content Using the Folin-C Assay: Single-Laboratory Validation, First Action 2017. 13. J. AOAC Int. 2018;101:1466-72.
18. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-82.
19. Rakholiya K, Kaneria M, Sumitra C. Vegetable and fruit peels as a novel source of antioxidants. J Med Plant Res. 2011;5:63.
20. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. Ayu. 2013;34:209-14.
21. Singh GRS, Negi PS, Radha C. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour. J Funct Foods. 2013;5:1883-91.
22. Hemalatha P, Bomzan DP, Sathyendra Rao BV, Sreerama YN. Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chem. 2016;199:330-8.
23. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52:313-20.
24. Freedman R. The Enzymes of Biological Membranes, Volume 2: Biosynthesis of Cell Components. Biochem Soc Trans. 1976;4:1162-3.
25. Marulasiddaiah R, Kalkhambkar RG, Kulkarni MV. Synthesis and Biological Evaluation of Cyclic Imides with Coumarins and Azacoumarins. Open J Med Chem. 2012;2:89-97.
26. Newgard CB, Hirsch LJ, Foster DW, McGarry JD. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. A direct or an indirect pathway? J Biol Chem. 1983;258:8046-52.
27. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J BiochemBiophys. 1984;21:130-2.
28. Craig WJ. Health-promoting properties of common herbs. Am J Clin Nutr. 1999;70:491S-9S.
29. Bagul PK, Banerjee SK. Application of resveratrol in diabetes: rationale, strategies and challenges. Curr Mol Med. 2015;15(4):312-30.
30. Zagotta I, Dimova EY, Debatin KM, Wabitsch M, Kietzmann T, Fischer-Posovszky P. Obesity and inflammation: reduced cytokine expression due to resveratrol in a human *in vitro* model of inflamed adipose tissue. Front Pharmacol. 2015;6:79.
31. Sung MM, Kim TT, Denou E, Soltys CM, Hamza SM, Byrne NJ, *et al.* Improved glucose homeostasis in obese mice treated with resveratrol is associated with alterations in the gut microbiome. Diabetes. 2017;66(2):418-25.
32. Prakash J, Sallaram S, Martin A, Veeranna RP, Peddha MS. Phytochemical and functional characterization of different parts of the *Garcinia xanthochymus* fruit. ACS omega. 2022;7(24):21172-82.
33. Kalavani A, Umamaheshwari A, Vinayagam A, Kalavani K. Anti-hyperglycemic and antioxidant properties of *Cassia auriculata* leaves and flowers on alloxan induced diabetic rats. Pharmacologyonline. 2008;1:204-17.
34. Uma Devi P, Selvi PS, Suja S, Selvam K, Chinnaswamy P. Antidiabetic and hypolipidemic effect of *Cassia auriculata* in alloxan induced diabetic rats. Int J Pharmacol. 2006;2:601-7.
35. Kwon YI, Apostolidis E, Kim YC, Shetty K. Health benefits of traditional corn, beans and pumpkin: *in vitro* studies for hyperglycemia and hypertension management. J Med Food. 2007;10(2007):266-75.
36. Magalhaes DAD, Kume TW, Correia FS, Queirox TS, Allebrandt Neto EW, Santos MPD, *et al.* High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. An Acad Bras Cienc. 2019;91(1):e20180314.
37. Vatandoust N, Rami F, Salehi AR. Novel high-fat diet formulation and streptozotocin treatment for induction of prediabetes and type 2 diabetes in rats. Adv Biomed Res. 2018;7(107).
38. Zhao H, Lai Q, Zhang J, Huang C, Jia L. Antioxidant and hypoglycemic effects of acidic-extractable polysaccharides from *Cordyceps militaris* on type 2 diabetes mice. Oxid Med Cell Longev. 2018; 2018:9150807.
39. Gonzalez P, Gozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. International J Mol Sci. 2023;24(11):9352.
40. Tan BL, Norhaizan ME. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients. 2019;11(11):2579.
41. Solis-Herrera C, Triplitt C, Cersosimo E, DeFronzo RA. Pathogenesis of Type 2 Diabetes Mellitus, in: Feingold, K.R., Anawalt, B., Blackman, M.R., Boyce, A., Chrousos, G., Corpas, E., de Herder, W.W., Dhatariya, K., Dungan, K., Hofland, J., Kalra, S., Kaltsas, G., Kapoor, N., Koch, C., Kopp, P., Korbonits, M., Kovacs, C.S., Kuohung, W., Laferrère, B., Levy, M., McGee, E.A., McLachlan, R., New, M., Purnell, J., Sahay, R., Shah, A.S., Singer, F., Sperling, M.A., Stratakis, C.A., Trence, D.L., Wilson, D.P. (Eds.),2000; Endotext. MDText.com, Inc., South Dartmouth (MA).
42. Pozzo L, Vornoli A, Coppola I, Croce CMD, Giorgetti L, Gervasi PG, *et al.* Effect of HFD/STZ on expression of genes involved in lipid, cholesterol and glucose metabolism in rats. Life Sci. 2016;166:149-56.
43. Kim DY, Kim SR, Jung UJ. Myricitrin Ameliorates Hyperglycemia, Glucose Intolerance, Hepatic Steatosis and Inflammation in High-Fat Diet/Streptozotocin-Induced Diabetic Mice. Int J Mol Sci. 2020;21:1870.
44. Brons C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, *et al.* Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol. 2009;587(Pt10):2387-97.
45. El-Hamid Ibrahim IAA, El-Aziz MFMA, Ahmed AF, El-Aal Mohamed MA. Is the effect of high fat diet on lipid and carbohydrate metabolism related to inflammation? Mediterr J NutrMetab. 2011;4(3):203-9.
46. Florentino TV, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr Pharm Des. 2013;19(32):5695-703.
47. Purbowatiningrum, Ngadiwiyana, Ismiyarto, Fachriyah E, Eviana I, *et al.*, Antidiabetic activity from gallic acid encapsulated nanochitosan. IOP Conf Ser: Mater Sci Eng. 2017;172:012042.
48. Narasimhan A, Chinnaiyan M, Karundevi B. Ferulic acid exerts its antidiabetic effect by modulating insulin-signalling molecules in the liver of high-fat diet and fructose-induced type-2 diabetic adult male rat. Appl PhysiolNutrMetab. 2015;40(8):769-81.
49. Son MJ, Rico CW, Nam SH, Kang MYJJ. Effect of oryzanol and ferulic acid on the glucose metabolism of mice fed with a high-fat diet. J Food Sci. 2011;76:H7-10

Cite this article: Muthaiah S, Mudhol S, Peddha MS. *Cassia auriculata* Stem Bark Ameliorates the HFD+STZ-Induced Diabetes in both *in vitro* and *in vivo* Model. Indian J of Pharmaceutical Education and Research. 2025;59(3s):s978-s988.