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ORIGINAL ARTICLE

Hypoglycemic Effect of Ethyl Acetate Fraction of Methanol Extract from *Campylandra aurantiaca* Rhizome on High-fat Diet and Low-dose Streptozotocin-induced Diabetic Rats

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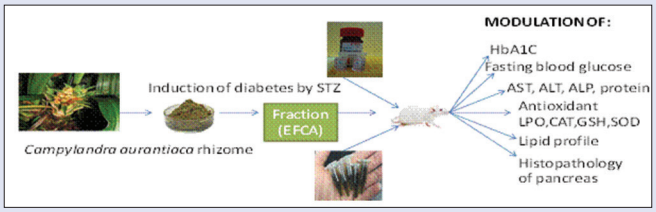
ABSTRACT

Background: *Campylandra aurantiaca* (Asparagaceae), commonly known as Nakima in Sikkimese Tibetan, is a plant grown in South-Central China and Northeast India. **Objective:** To evaluate the hypoglycemic activity of ethyl acetate fraction of methanol extract from *C. aurantiaca* rhizome (EFCA) in high-fat diet (HFD) and low-dose streptozotocin (STZ)-induced diabetic Wistar rats. **Materials and Methods:** In rats fed with HFD for 4 weeks, hyperglycemia was induced by single intraperitoneal injection of STZ (35 mg/kg body weight). Seven days after STZ induction, the hyperglycemic rats were treated with EFCA orally at the doses of 100 and 200 mg/kg b.w. daily for 28 days. Glibenclamide (0.5 mg/kg, orally) was used as reference drug. The fasting blood glucose levels were measured on every 7th day during the 28 days of treatment. Serum and hepatorenal biochemical parameters were estimated. Histological study of the pancreas was also performed. **Results:** EFCA at the doses of 100 and 200 mg/kg orally significantly ($P < 0.05$) and dose dependently reduced and normalized blood glucose levels as compared to that of STZ control group. Serum and hepatorenal parameters were significantly ($P < 0.05$) restored toward normal levels in EFCA-treated rats as compared to HFD-STZ control animals. **Conclusion:** The present study concludes that *C. aurantiaca* rhizome demonstrated promising hypoglycemic action in HFD-STZ-induced diabetic rats.

Key words: Diabetes, glibenclamide, hypoglycemic, obesity, streptozotocin

SUMMARY

- The present study evaluated the hypoglycemic activity of ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome (EFCA) in high-fat diet (HFD) and low-dose streptozotocin (STZ)-induced diabetic Wistar rats. In rats fed with HFD for 4 weeks, hyperglycemia was induced by single intraperitoneal injection of STZ (35 mg/kg body weight). Seven days after STZ induction, the hyperglycemic rats were treated with EFCA orally at the doses of 100 and 200 mg/kg b.w daily for 28 days. Glibenclamide (0.5 mg/kg orally) was used as reference drug. The blood glucose levels were measured on every 7th day during the 28 days of treatment. Serum and hepatorenal biochemical parameters were estimated. Histological study of pancreas was also performed. EFCA at the doses of 100 and 200 mg/kg orally significantly and dose dependently reduced and normalized blood glucose levels as compared STZ control group. Serum and hepatorenal parameters were significantly restored toward normal levels in EFCA-treated rats as compared to HFD-STZ control animals. The present study concludes that *C. aurantiaca* rhizome demonstrated promising hypoglycemic action in HFD-STZ-induced diabetic rats.



Abbreviations used: IDDM: Insulin-dependent diabetes mellitus, T2DM: Type 2 diabetes mellitus, NIDDM: Noninsulin-dependent diabetes mellitus, T1DM: Type 1 diabetes mellitus, IDF: International Diabetes Federation, STZ: Streptozotocin, EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome, OGTT: Oral glucose tolerance test, HFD: High-fat diet, FBG: Fasting blood glucose, HDL: High-density lipoprotein cholesterol, HbA1c: Glycosylated hemoglobin, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, LPO: Lipid peroxidation, GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, SEM: Standard error of mean, ANOVA: Analysis of variance, HPLC: High-performance liquid chromatography.

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INTRODUCTION

Diabetes mellitus, a metabolic disorder in which a person has high blood sugar, has become a matter of serious challenge because of its worldwide prevalence. This may be because of the body's inability to produce enough insulin or because of lesser response of the cells to insulin that is being produced. Diabetes mellitus is of different types based on the causes. The majority of type 1 diabetes mellitus (T1DM or insulin-dependent diabetes mellitus [IDDM]) is related to immunity, where beta-cells are destroyed by T-cell-mediated autoimmune attack. Type 2 diabetes mellitus (T2DM or non-IDDM [NIDDM]) occurs

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from insulin resistance and sometimes involves deficiency in insulin production.[1]

The prevalence of diabetes is growing worldwide due to urbanization and improper lifestyle management. In the Western countries, diabetes mostly develops with age; however, in Asian countries, it attacks mostly young to middle-aged people. This scenario leaves a huge impact on health and economy of the developing countries. The International Diabetes Federation has an estimation of the total number diabetics in India to be around 50.8 million in 2010 and gradually rising to about 87.0 million by 2030. Recent reports collected from various parts of India have detected the invading nature of diabetes in urban areas. Moreover, the prevalence of diabetes is also simultaneously increasing in rural areas as a result of the recent socioeconomic growth.[2]

Obesity is a complex, multifactorial, and largely preventable disease, affecting along with overweight, over one-third of the world's population today. Obesity increases risk of chronic diseases namely disability, depression, type 2 diabetes (T2D), cardiovascular diseases, certain cancers, and mortality.[3] Recent studies have identified links between obesity and T2D involving proinflammatory cytokines (tumor necrosis factor and interleukin-6), insulin resistance, deranged fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress.[4] Obesity and T2DM frequently occur together, and statistics show that 60–90% of all patients with T2DM are or have been obese. Obesity is generally considered to be a strong risk for the later development of T2DM.[5]

Marketed oral hypoglycemic agents exhibit a variety of adverse effects, including congestive heart failure with glitazones, gastrointestinal disturbances with glucosidase inhibitors, sulfonylureas, and meglitinides. Cardiac problems and weight gain are common adverse effects of sulfonylureas. Therefore, it is an urge to develop a less toxic therapeutic agent. Due to the multiple pathophysiological considerations, there is a need to develop multitarget therapeutic agent which would be helpful for the treatment of T2DM and its associated pathogenesis. Plant extract is a mixture of multiple compounds and shows their therapeutic value in a multiple way. Therefore, the present investigation has focused the antidiabetic potential of plant extract considering ethnopharmacological knowledge as reference.[6]

Campylandra aurantiaca (Asparagaceae), commonly known as Nakima or Thulo-Nakima in Sikkimese Tibetan, is an herbaceous plant grown in South-Central China including Tibet, Nepal, and Northeast India, including Sikkim. Different parts of *C. aurantiaca* have been used conventionally in India for certain medicinal purposes. Rhizome decoction is administered as antidiarrheic, antidysenteric, analgesic, antimalarial, antiarthritic, vermicidal, antipyretic, and stomachic. Flowers are made into curry and taken with staple food 2 times per week for 4–6 weeks to treat diabetes mellitus.[7,8] However, experimental studies on this plant are scanty. There is no experimental report demonstrating antidiabetic potential of this plant. This study therefore investigated the hypoglycemic effect of the ethyl acetate fraction of methanol extract of *C. aurantiaca* rhizome (EFCA) against streptozotocin (STZ)-induced diabetic Wistar rats to justify the traditional and folkloric attributes.

MATERIALS AND METHODS

Plant material

The mature rhizomes of *C. aurantiaca* were collected during June 2016 from Majhitar region of Sikkim state, India. The plant species were identified and authenticated by Dr. R. Gogoi, Taxonomist, Botanical Survey of India, Central National Herbarium (CNH), Howrah, India, and the voucher specimen (No. CNH/Tech.II/2016/38b) was deposited at the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India, for future reference. The collected rhizomes were thoroughly washed with running tap water, cut into small pieces, shade-dried (24°C–26°C) for 3–4 weeks, and ground mechanically into a coarse powder.

Drugs and chemicals

Trichloroacetic acid was obtained from Merck Ltd., Mumbai, India; thiobarbituric acid, STZ, 5,5'-dithiobis-2-nitrobenzoic acid, phenazonium methosulfate, nicotinamide adenine dinucleotide, and reduced glutathione (GSH) from Sisco Research Laboratory, Mumbai, India; potassium dichromate and glacial acetic acid from Ranbaxy, India; and glibenclamide from Hoechst, India. All the other reagents used were of analytical reagent grade obtained commercially.

Extraction and fractionation

The powdered material (500 g) was extracted successively by petroleum ether, chloroform, and methanol using Soxhlet extraction apparatus. The solvents were completely evaporated under reduced pressure. The dry methanol extract was used for further fractionation. Fractionation was performed successively using petroleum ether, chloroform, and ethyl acetate.[9] The EFCA was used for the present study. Preliminary phytochemical and high-performance liquid chromatography (HPLC) studies (in our previous work) were performed on EFCA.[10]

Experimental animals

Six to eight-week-old male Wistar albino rats (180 ± 20 g) were obtained from registered breeder, namely Chakraborty and Co., Kolkata, India. They were acclimatized to the laboratory conditions before the study for 7 days. The animals were kept at 25°C ± 2°C and a relative humidity of 40%–45% with alternative day and night cycles of 12 h each. The animals had free access to dry pellet food (Hindustan Unilever, Mumbai, India) and water *ad libitum*. All animal experimental procedures described were reviewed and approved by the University Animal Ethical Committee, Jadavpur University (Reg. no. 367001/C/CPCSEA).

Acute toxicity

EFCA was administered orally to male Swiss albino mice to evaluate the acute toxicity as per the reported method.[11]

Induction of diabetes with high-fat diet and streptozotocin

Male rats were fed with high-fat diet (HFD) comprising of 22% fat, 48% carbohydrate, and 20% protein in blend with standard laboratory chow consisting of 5% fat, 53% carbohydrate, and 23% protein for 4 weeks. After the period of dietary manipulation, rats were injected intraperitoneally (i.p.) with low dose of STZ (35 mg/kg body weight). Then, animals had free access to water and standard food.[12] One week after STZ injection, the fasting blood glucose (FBG) levels of overnight fasted rats were appraised and the animals exhibiting FBG levels of 170 ± 30 mg/dl were considered to be T2D rats and included for the further experiments.[6]

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed in overnight-fasted normal rats. Rats were divided into three groups ($n = 6$). Group I served as normal control and received distilled water (5 ml/kg b.w., p.o), and Groups II and III received EFCA at doses of 100 and 200 mg/kg b.w., respectively. After these treatments, all groups received glucose

(2 g/kg b.w.) orally. Blood was withdrawn from the tail vein just before and 30, 60, and 120 min after oral glucose administration.[13] Blood glucose levels were measured using single touch glucometer (Accu-Chek, Roche Diagnostics, USA).

Experimental design

Hyperglycemia was induced by HFDs *ad libitum* and low-dose of STZ as per recently reported method.[6,12] Briefly, the rats were fed HFDs *ad libitum* for 4 weeks and then treated with single dose of STZ (35 mg/kg b.w., i.p.). After 7 days STZ injection, the FBG levels of overnight-fasted rats were appraised, and the animals exhibiting FBG levels of 170 ± 30 mg/dl were considered to be T2DM rats and included for the further experiments. The rats were continued with HFDs throughout the course of the study. The animals were divided into five groups (n = 6) and received the following treatment for 28 days:

- Group I: Normal control rats were administered normal saline (0.5 ml/kg orally by oral gavages) daily
- Group II: Diabetic control rats were administered HFDs + normal saline (0.5 ml/kg daily)
- Group III: Diabetic rats were administered HFDs + EFCA (100 mg/kg b.w.) orally daily
- Group IV: Diabetic rats were administered HFDs + EFCA (200 mg/kg b.w.) orally daily
- Group V: Diabetic rats were administered HFDs + glibenclamide (0.5 mg/kg b.w.) orally daily.

Estimation of fasting blood glucose level

The rats were divided into five groups (n = 6). Except for Group I, which served as normal control, all other groups were comprised of diabetic rats. Group II served as diabetic (HFD-STZ) control. Groups III and IV received EFCA (100 and 200 mg/kg b.w., p.o., respectively) and Group V received reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 28 days.[14] FBG was measured on day 0, 7, 14, 21 and 28 using a one touch glucometer (Accu-Chek®).

Determination of serum biochemical parameters

Twenty-four hours after the last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, glycosylated hemoglobin (HbA1c), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total protein were estimated using commercially available reagent kits (ERBA Diagnostics, Andheri (E), Mumbai 400072, India, and Span Diagnostics Ltd., Surat, Gujarat 394230, India).[15]

Estimation of liver and kidney biochemical parameters

Livers and kidneys collected from the sacrificed animals were homogenized separately in 10 ml of phosphate buffer (20 mM, pH: 7.4) and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatants were collected and lipid peroxidation (LPO), GSH, superoxide dismutase (SOD), and catalase (CAT) were estimated.[11,16]

Histopathological study

A portion of pancreatic tissue was dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation, tissues were embedded in paraffin. Fixed tissues were cut and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.[17]

Statistical analysis

All the results are shown as mean ± standard error of mean. The results were analyzed for statistically significance by one-way analysis of variance, followed by *post hoc* Dunnett's test using GraphPad Prism 5.0 software (GraphPad Software Inc, La Jolla, CA, USA). $P < 0.05$ was considered as statistically significant.

RESULTS

Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, and tannins in EFCA. Our previous work on HPLC analysis revealed the presence of two flavonoids, namely myricetin and apigenin, in EFCA.[10] While performing the acute toxicity study, EFCA did not show any toxic effect or death up to the dose of 2000 mg/kg, b.w., p.o. in mice. The oral blood glucose tolerance test was done in normal rats. Glucose administration to the normal rats increased blood glucose levels in first 30 min and gradually decreased in 30 and 60 min and returned near to normal at 120 min [Table 1]. There was significantly ($P < 0.05$) elevated FBG level in HFD and STZ-induced diabetic rats as compared to normal control group. Administration of EFCA in diabetic rats at the doses of 100 and 200 mg/kg significantly ($P < 0.05$) reduced the FBG level toward normal as compared to the diabetic control group [Table 2]. HbA1c has been found to be reduced in EFCA-treated animals as compared to HFD and STZ control animals [Table 3].

Similarly, in case of serum biochemical parameters, EFCA showed a significant ($P < 0.05$) lowering when compared to the HFD and STZ control group. Level of total protein increased in treated group when compared to the HFD and STZ control [Figure 1]. Serum lipid profiles such as total cholesterol and triglyceride in HFD and STZ-induced diabetic rats were significantly ($P < 0.05$) elevated and the HDL cholesterol level significantly ($P < 0.05$) decreased compared to normal control group. Treatment with EFCA at the doses of 100 and 200 mg/kg significantly ($P < 0.05$) reduced the total cholesterol and triglyceride level and significantly ($P < 0.05$) increased the HDL cholesterol level when compared to the diabetic control group [Figure 2]. In liver and kidney tissue antioxidant studies, LPO level decreased ($P < 0.05$) and SOD, GSH, and CAT level increased ($P < 0.05$) in treated animals [Figure 3]. The histopathological studies on pancreas showed gradual improvement in pancreatic beta cells when treated with EFCA [Figure 4].

DISCUSSION

The present study aimed to investigate the hypoglycemic activity of EFCA in HFD and low-dose STZ (35 mg/kg)-induced diabetic rats. The results of the study revealed that EFCA at doses of 100 and 200 mg/kg significantly normalized elevated blood glucose level and restored serum, liver, and kidney biochemical parameters toward normal values.

The HFD-STZ-induced diabetic rat is one of the animal models of human NIDDM or T2DM. As in human T2DM, diet has a great influence on the development of diabetes as well as hypertension, hyperlipidemia,

Table 1: Effect of ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome on oral glucose tolerance test

Groups	0 min	30 min	60 min	120 min
Normal control	83.67±2.33	135.7±1.76	125±1.15	106.3±1.76
EFCA (100 mg/kg)	88.67±0.88	138.7±1.52	132.7±1.45*	126.3±0.88*
EFCA (200 mg/kg)	87.33±1.45	125.7±1.76*	119±1.15*	115.7±1.76*
Glibenclamide (0.5 mg/kg)	81±2.08	120±1.00*	115±1.15*	110.3±2.40*

*P<0.05 when compared to normal control. Values are represented as mean±SEM (n=6). EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome; SEM: Standard error of mean

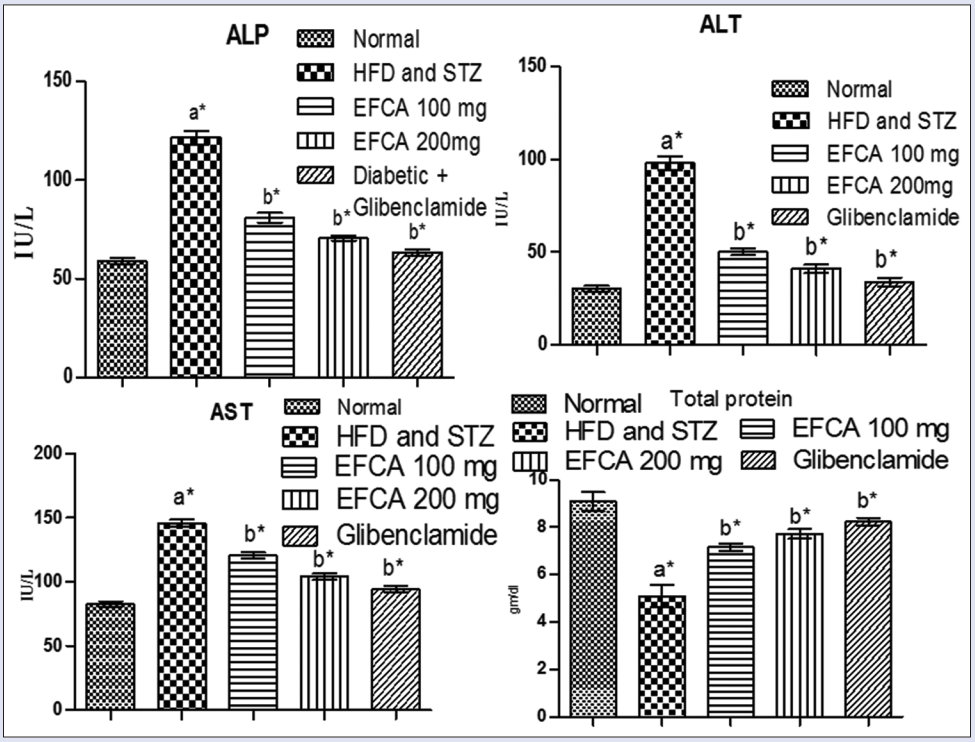

Figure 1: Effect of EFCA on ALP, ALT, AST, and total protein. Each value is expressed as mean ± standard error of mean (n = 6). a*: P <0.05 when compared to normal and b*: P <0.05 when compared to diabetic control. EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome, ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, STZ: Streptozotocin, HFD: High-fat diet\

Table 2: Effect of ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome on fasting blood glucose (mg/dl)

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control (5 ml/kg)	75.33±2.72	80.33±1.76	82±1.52	81±1.15	79±3.15
HFD and STZ control (35 mg/kg)	176.3±4.37*	187.3±3.18*	200±1.73*	207.7±4.66*	211.7±3.66*
STZ + EFCA (100 mg/kg)	177.7±3.52	172±2.74**	163±2.51**	153.7±1.76**	139±2.30**
STZ + EFCA (200 mg/kg)	172.7±3.28	155±3.21**	143±2.02**	135.7±2.40**	127.7±1.45**
STZ + glibenclamide (0.5 mg/kg)	176.3±1.76	155±2.64**	143±2.30**	116±3.21**	87.33±2.18**

*Normal control group versus diabetic control group, **All treated group versus diabetic control group on corresponding day, P<0.05. Each volume expressed as mean±SEM (n=6). HFD: High-fat diet; STZ: Streptozotocin; EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome; SEM: Standard error of mean

Table 3: Effect of ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome on glycosylated hemoglobin

Groups	HbA1c (%)
Normal control	6.4±0.20
STZ control (diabetic)	8.99±0.24*
Diabetic + EFCA (100 mg/kg)	7.33±0.14
Diabetic + EFCA (200 mg/kg)	6.5±0.14
Diabetic + glibenclamide (0.5 mg/kg)	6.02±0.26

Values are represented as mean±SEM (n=6). *P<0.05 when compared to normal control. STZ: Streptozotocin; EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome; SEM: Standard error of mean; HbA1c: Glycosylated hemoglobin

and eventually nephropathy in the experimental model.[18] According to the data from previous studies, feeding rats an HFD can promote the development of insulin resistance. Injections of high doses of STZ have been shown to critically damage pancreatic β-cell functioning, leading to insulin secretion, which is considered to resemble T1DM. Recently, HFD and low-dose injections of STZ have been reported to induce a gradual impairment of insulin secretion, which is similar to the natural progression of T2DM in humans. Therefore, in the current study, an HFD and low-dose of STZ (35 mg/kg) were adopted to develop T2D in rats.[19]

From the OGTT data, it is clear that administration EFCA at the dose 100 mg/kg and 200 mg/kg effectively prevented the increase in serum glucose level without causing a hypoglycemia as efficiently as the reference drug glibenclamide. This result confirms the reduction of intestinal glucose transporter and is similar to the finding.[15] Hyperglycemia was observed after 7 days of low-dose STZ induction. Treatment with HFD and low dose of STZ (35 mg/kg)-induced diabetic rats started reducing FBG levels in a dose-dependent manner after 7, 14, and 21 days and made them normoglycemic after 28 days. The antihyperglycemic effect of EFCA at the dose of 100 mg/kg and 200 mg/kg was found to be comparable to the effect exerted by the reference drug glibenclamide at a dose of 0.5 mg/kg.

STZ when injected i.p. causes pancreatic beta-cell destruction by generating free radicals resulting in gradual depletion of insulin production and elevated blood glucose level. Increased glucose level also hampers the lipid metabolism resulting in hypercholesteremia and hypertriglyceridemia, which are considered to be the primary factors

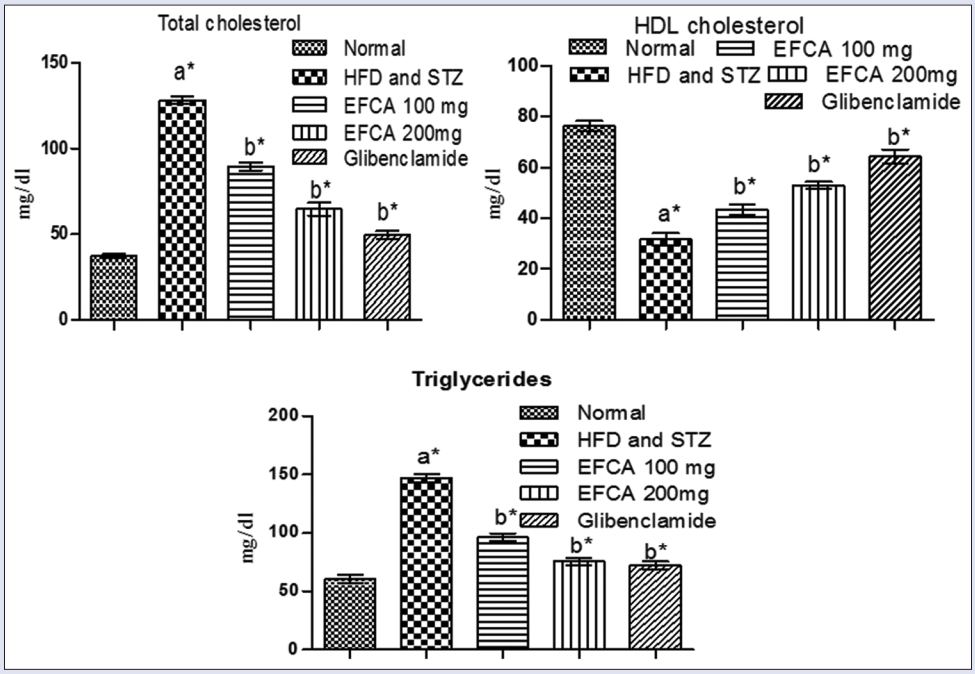

Figure 2: Effect of EFCA on total cholesterol, HDL cholesterol, and triglycerides. Each value is expressed as mean ± standard error of mean ($n = 6$). a*: $P < 0.05$ when compared to normal and b*: $P < 0.05$ when compared to diabetic control. EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome, HDL: High-density lipoprotein, HFD: High-fat diet, STZ: Streptozotocin

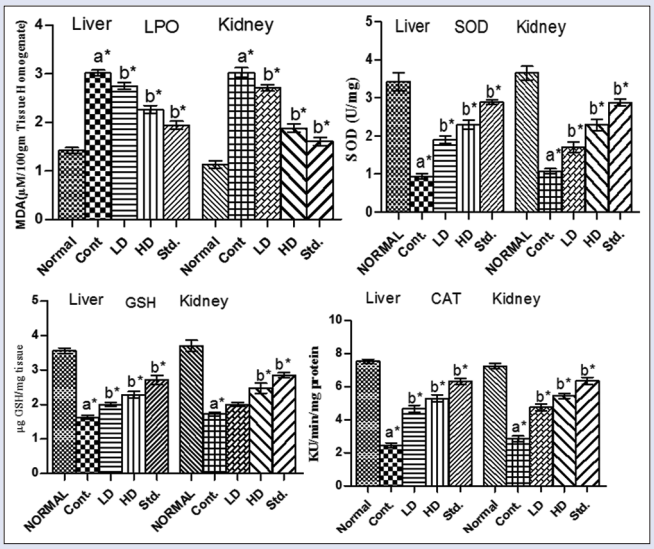

Figure 3: Effect of EFCA on SOD, LPO, GSH, and CAT. Each value is expressed as mean ± standard error of mean ($n = 6$). a*: $P < 0.05$ when compared to normal and b*: $P < 0.05$ when compared to diabetic control. EFCA: Ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome, SOD: Superoxide dismutase, LPO: Lipid peroxidation, GSH: Reduced glutathione, CAT: Catalase

involved in the development of atherosclerosis and coronary heart disease, which are the secondary complications of diabetes.[20] Treatment with EFCA remarkably restores all the parameter toward normal.

The role of dyslipidemia in the development of diabetic macrovascular complications has long been well established. The treatment with EFCA at a dose of 100 and 200 mg/kg was able to improve the serum lipid profile in diabetic rats. Treatment of diabetic rats with EFCA at the doses of 100 and 200 mg/kg shows considerable reduction in hepatic lipid accumulation. Lipase functions as a lipolytic enzyme that hydrolyzes triglycerides and phospholipids in circulating plasma lipoproteins. Reduction of fat absorption by the inhibition of pancreatic lipase is known to be beneficial for the regulation of obesity and related metabolic disorders.[21]

The increase in the activities of plasma AST, ALT, ALP and decreased level of total protein indicated that diabetes may be induced hepatic dysfunction that liver was necrotized in diabetic patients. Therefore, the increment of the activities of AST, ALT, and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream.[22] AST and ALT were used as markers to assess the extent of liver damage in STZ-induced diabetic rats.[23] On the other hand, treatment of the diabetic rats with EFCA 100 and 200 mg/kg caused reduction in the activity of these enzymes in plasma compared to the mean values of diabetic group. These results are in agreement with that the plant having good protective effect on liver.

Chronic hyperglycemia has shown to play a role in the development of diabetic microvascular and macrovascular complications. Four seemingly independent mechanisms are involved in the pathogenesis of diabetic complications: glucose-induced activation of protein kinase C isoforms, increased formation of glucose-derived advanced glycation endproducts, increased polyol pathway, and increased production of reactive oxygen species (ROS).[24]

Diabetes is a chronic metabolic disease associated with hyperglycemia and oxidative stress which generally causes several tissue damage and subsequently degenerative complications in many organs such as the kidney and liver.[25] Lipid peroxide-mediated tissue damage has been observed in the development of both T1DM and T2DM. Insulin secretion is impaired during diabetes and this may evoke LPO in biological systems. Enhanced levels of LPO observed in the liver and kidney of diabetic rats indicated excessive formation of free radicals and activation of lipid peroxidative system. The present study shows that administration of

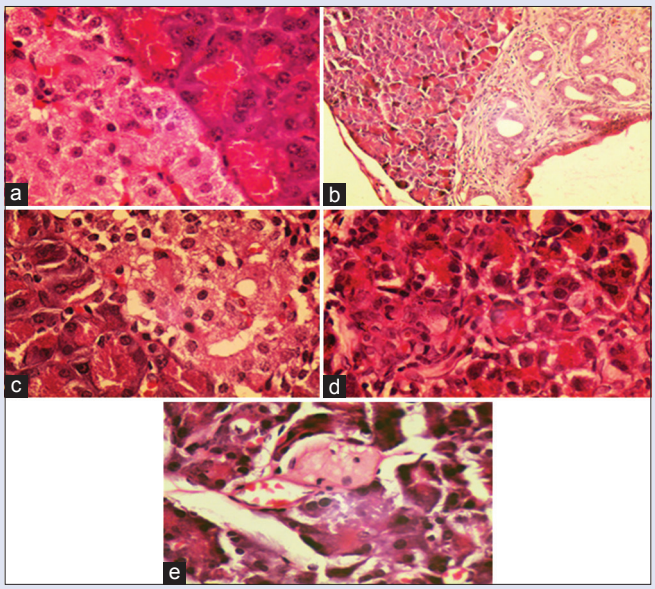

Figure 4: (a) Beta cells in normal control rats. (b) Total destruction of beta cells in high-fat diet and streptozotocin control rats. (c) Remnants of beta cells in ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome (100 mg/kg) treated rats. (d) Gradual regeneration of beta cells in ethyl acetate fraction of methanol extract from *Campylandra aurantiaca* rhizome (200 mg/kg) treated rats. (e) Functioning secretory granules in the islets of beta cells as seen in glibenclamide (0.5 mg/kg) treated rats

EFCA 100 and 200 mg/kg and glibenclamide inhibits production of lipid peroxides. This indicates the anti-lipid peroxidative potential of EFCA.

SOD and CAT are the two major endogenous scavenging enzymes that remove toxic-free radicals *in vivo* and are thought to play an important role in protecting the cell against the potentially deleterious effects of ROS. Reduced activity of SOD and CAT may result in a number of deleterious effects due to the accumulation of superoxide radicals ($O2^-$) and hydrogen peroxide.[26] Administration of EFCA 100 and 200 mg/kg and glibenclamide results in the activation of SOD and CAT to near normal levels in diabetic rats. The result of the SOD and CAT activity clearly shows that EFCA 100 and 200 mg/kg exhibited free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of $O2^-$ and OH^-.

Endogenous nonenzymatic antioxidant system glutathione plays an important role. Primarily acting as a reducing agent, it detoxifies hydrogen peroxide with the help of enzyme, glutathione peroxidase.[27] The depleted GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress in HFD and STZ-induced hyperglycemic animals but after treatment with EFCA increased the GSH level in both liver and kidney tissues.

Preliminary phytochemical studies revealed the presence of polyphenolic compounds, namely flavonoids and tannins in EFCA. In our previous HPLC study, EFCA revealed the presence of two putative flavonoids, viz., myricetin and apigenin. Polyphenols are believed to be responsible for several important biological activities of plants.[28] The hypoglycemic activity of EFCA may be attributed to its polyphenols, especially flavonoids content.

CONCLUSION

The current study clearly demonstrates that daily administration of EFCA exhibited a pronounced hypoglycemic effect (FBG) and also improved the antioxidant defense system such as LPO, SOD, GSH, and CAT in the liver and kidney of diabetic rats. These results suggest a promising effect in intestinal glucose transport, HbA1c, enzymatic liver, and kidney biochemical parameters and also improve the histology of pancreas. However, investigation of secondary metabolites principally flavonoids of this fraction, responsible for hypoglycemic and antioxidant effect, should be undertaken to confirm the compound which is responsible for these activities.

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Conflicts of interest

There are no conflicts of interest.

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