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ORIGINAL ARTICLE

Evaluation of Protective Effects of Hydroalcoholic Extract of *Cassia fistula* Linn. Pod on Pancreas in Streptozotocin-induced Diabetic Rats

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ABSTRACT

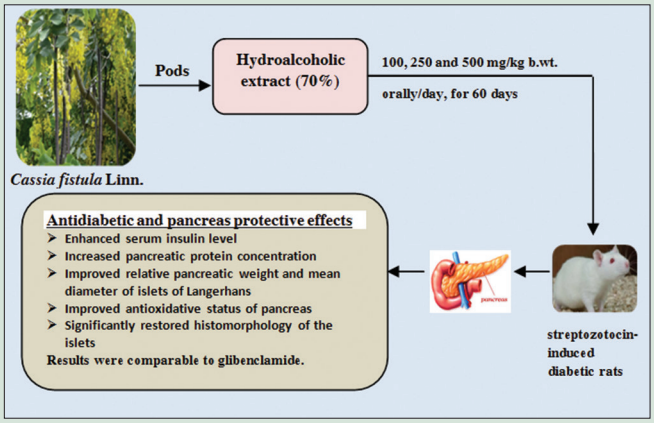
Background: Diabetes mellitus (DM) is associated with oxidative stress. Medicinal plants and herbs are the rich sources of antioxidants which ameliorate oxidative stress-induced diabetic complications and could play an important role in the management of diabetes. **Objective:** The present study aimed to evaluate the protective effects of 70% ethanolic extract of *Cassia fistula* pod on pancreas in streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** Diabetes was induced in male Wistar rats by single intraperitoneal injection of STZ (60 mg/kg b.wt.). The diabetic rats were administered orally with *C. fistula* pod extract at three different doses (100, 250, and 500 mg/kg b.wt./day) for 60 days. The results were compared with standard drug glibenclamide (5 mg/kg b.wt./day) treated rats. Relative pancreatic weight and serum insulin level were determined. Histopathological changes and oxidative stress parameters, i.e., lipid peroxidation (thiobarbituric acid reactive substance [TBARS]) and antioxidative defense markers (superoxide dismutase, catalase, glutathione, and ascorbic acid), in the pancreas were investigated. **Results:** Oral administration of *C. fistula* pod extract (100, 250, and 500 mg/kg b.wt./day) or glibenclamide in diabetic rats significantly improved serum insulin level, total protein concentration, relative pancreatic weight, and mean diameter of islets of Langerhans as compared to diabetic control rats. Furthermore, treatment with extract also reduced TBARS levels and improved the levels of antioxidant markers in the pancreas. The histomorphological picture of the pancreas showed marked restoration of islets morphology. These results were comparable with glibenclamide. **Conclusions:** The results of the present study showed that *C. fistula* pod extract possesses significant antidiabetic activity though enhanced insulin secretion, improvement of antioxidative status of pancreas, and preservation of the integrity of pancreatic islets.

Key words: Antioxidants, *Cassia fistula*, insulin, pancreas, streptozotocin

SUMMARY

Protective effects of hydroalcoholic extract of *Cassia fistula* Linn. pod on pancreas in streptozotocin (STZ)-induced diabetic rats were investigated.

- Extract treatment in STZ-induced diabetic rat significantly enhanced serum insulin level
- The diminished activities of pancreatic superoxide dismutase and catalase and the decreased levels of glutathione and ascorbic acid were significantly improved with concomitant decrease in lipid peroxide (thiobarbituric acid reactive substance) levels in extract-treated diabetic rats
- Extract treatment in diabetic rats also significantly improved relative pancreatic weight, restored pancreatic islets morphology, and diameter
- The antidiabetic effects of the extract were comparable with standard drug glibenclamide.



Abbreviations used: b.wt.: Body weight; CAT: Catalase; DM: Diabetes mellitus; DNA: Deoxyribonucleic acid; GSH: Glutathione; ROS: Reactive oxygen species; SOD: Superoxide dismutase; STZ: Streptozotocin; TBARS: Thiobarbituric acid reactive substances.

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INTRODUCTION

Diabetes mellitus (DM) is defined as a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion, insulin action, or both.[1] The incidence of DM is rising all over the world. According to the International Diabetes Federation estimation, around 415 million people had diabetes mellitus in 2015 and this number is expected to rise to 642 million by 2040. Nearly 75% of individuals with diabetes mellitus live in low- and middle-income countries.[2] The World Health Organization projects that diabetes will be the seventh leading cause of death in 2030.[3] Although many drugs are available for the treatment of the disease, they have limited efficacy, side effects, and high cost.[4,5] Therefore, there is increased demand for more effective, safe, and cheap hypoglycemic agents. Many Indian medicinal plants and herbs have been reported to be useful in the management of diabetes acting through a variety of mechanisms.[6,7]

Numerous studies demonstrated that chronic hyperglycemia continuously generates reactive oxygen species (ROS) and superoxide

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anions, which further aggravate the diabetic complications.[8,9] Oxidative stress could affect pancreatic β-cells which could be destroyed by direct insult by free radicals. Antioxidants play an important role in scavenging free radicals and protect from oxidative stress-induced cellular damages.[8,10] Hence, drug possessing both antioxidant and antihyperglycemic activities would be useful for the treatment of diabetes mellitus.[11,12] Medicinal plants and herbs are the rich sources of bioactive phytoconstituents, which cause lowering of blood glucose level and/or also act as antioxidants, resulting in the amelioration of oxidative stress-induced diabetic complications.[13-15]

Cassia fistula Linn (Hindi – Amaltas; English – Golden Shower or Indian Laburnum), a medium-sized tree belonging to the Family – *Caesalpiniaceae*, is widely cultivated throughout India as an ornamental plant and is used for its medicinal activities. Nearly every part of this plant including root, bark, leaf, fruits (pods), flowers, and seeds have been used for the treatment of various ailments in the indigenous system of medicine.[16-18] Different parts of this plant are reported to have a wide range of pharmacological activities such as anticancerous,[19] antifertility,[20] antifungal,[21] antihelmintic,[22] antihyperlipidemic,[23] anti-inflammatory,[24] antipyretic activity,[25] antimicrobial,[26] antiulcer activity,[27] central nervous system activity,[28] hepatoprotective,[29] immunomodulatory,[30] laxative effects,[31] and wound healing activity,[32] and almost all parts of the plant are reported to have antioxidative action.[33,34]

Phytochemical studies revealed that the pod of the Indian laburnum is an important source of ingredients. It is a rich source of potassium, calcium, iron, and manganese and also of aspartic acid, glutamic acid, and lysine amino acids.[35] The seeds of the plant are rich in glycerides with linoleic, oleic, stearic, and palmitic acids as major fatty acids together with traces of caprylic and myristic acids and carbohydrates such as galactomannan.[36,37] Oxyanthraquinones, chrysophanein, and chrysophanol were isolated by Kuo *et al*.,[38] and a new bioactive flavone glycoside 5,3′ ,4′ -tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1 → 2)-O-β-D-galactopyranoside was isolated by Yadav and Verma from the seeds of *C. fistula*.[39] Pods contained flavon-3-ol and proanthocyanidins such as catechin, epiafzelechin, epicatechin, procyanidin B-2,[40] rhein; 1,8-dihydroxy-3-anthraquinone carboxylic acid,[41] fistulic acid; an anthraquinone acid,[42] 3-formyl-1-hydroxy-8-methoxy anthraquinone,[43] diterpene; 3B-hydroxy-17-norpimar-8 (9)-en-15-one,[44] 5-nonatetracontanone, 2-hentriacontanone, triacontane, 16-hentriacontanone; and β-sitosterol,[45] kaempferol, dihydrokaempferol,[46] isoflavone: biochanin A,[47] and quercetin dehydrates.[26]

Antidiabetic activity of various extracts of *C. fistula* root,[48] bark,[49] flowers,[50,51] and leaves[52,53] has been reported in experimental diabetic rats. Recently, we have reported antihyperglycemic activity of 70% ethanol extract of *C. fistula* pods in streptozotocin (STZ)-induced diabetic rats.[54] However, the precise mechanism of antidiabetic activity and its effect on pancreas are not clear. Therefore, the present study aimed to investigate protective effects of the 70% ethanol extract of *C. fistula* mature pods on serum insulin level, lipid peroxidation (thiobarbituric acid reactive substance [TBARS]), antioxidant defense marker parameters, and histomorphology of the pancreas in STZ-induced diabetic rats.

MATERIALS AND METHODS

Plant material and preparation of extract

Mature pods of *C. fistula* were collected in April–June 2013, from the campus of University of Rajasthan, Jaipur, and authenticated by Prof. K.P. Sharma, Incharge, Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen (RUBL21057) was also deposited in the Herbarium. The pods were washed with distilled water, shade dried, and powdered in an electric grinder. The powder (300 g) was suspended in 70% ethanol (w/v 1:10) and allowed to stand for 24 h. The mixture was subjected to Soxhlet apparatus for extraction at 60°C–70°C for 35 h. It was then filtered using a filter paper and the filtrate was evaporated to dryness in an oven at 40°C. A brownish residue weighing 38.5 g (12.83% of dried powder) was obtained. This was kept in an airtight bottle in a refrigerator until used. The extract was suspended in water before administering to experimental animals.

Animals

Colony bred, adult, healthy, male rats of Wistar strain (*Rattus norvegicus*) weighing 170–200 g were used in the present study. The animals were housed in polypropylene cages under standard husbandry conditions (12-h-light/12-h-dark cycle; 25°C ± 3°C temperature). Rats were provided with water and nutritionally adequate pellet diet (Aashirwad Food Industries, Chandigarh, India) *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals were followed for maintenance of the animals. The study was approved by the Institutional Animal Ethical Committee.

Chemicals

STZ was obtained from HiMedia Laboratory limited, Mumbai, India. Glibenclamide tablets (Daonil; Aventis Pharma. Ltd., India) were purchased from the medical store. All other chemicals and reagents used were of analytical grade.

Experimental induction of diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of STZ dissolved in citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight in overnight-fasted rats. The STZ-treated animals were given 2% glucose solution for 24 h after 5 h of STZ injection to prevent initial drug-induced hypoglycemic mortality. Development of diabetes was verified after 1 week of STZ injection by measuring the blood glucose level in the blood samples obtained from the tail vein of overnight-fasted rats. The rats having blood glucose level above 250 mg/dL were considered to be diabetic and used in the study. This day was considered as the zero (0) day of the experiment.

Experimental design

The rats were divided into six different groups, each consisting of six animals and treated as follows.

- Group I: Control rats receiving vehicle (0.5 ml distilled water/rat/day) orally for 60 days
- Group II: Diabetic rats receiving vehicle (0.5 ml distilled water/rat/day) orally for 60 days
- Group III: Diabetic rats receiving *C. fistula* extract (100 mg/kg b.wt./day) orally for 60 days
- Group IV: Diabetic rats receiving *C. fistula* extract (250 mg/kg b.wt./day) orally for 60 days
- Group V: Diabetic rats receiving *C. fistula* extract (500 mg/kg b.wt./day) orally for 60 days
- Group VI: Diabetic rats receiving glibenclamide standard drug (5 mg/kg b.wt./day) orally for 60 days.

Autopsy

After 24 h of the last treatment, all the overnight-fasted animals of different groups were weighed and autopsied under mild ether anesthesia. Pancreatic tissue samples were carefully dissected out, washed in ice-cold saline, and weighed using a digital electronic balance and reported as

relative weights (organ weight/body weight × 100) and stored −20°C for further investigation. Blood was collected directly by cardiac puncture, of which 2 ml was added to an anticoagulant vial for the estimation of parameters in blood. Rest of the samples was allowed to clot at 37°C, and the serum was separated by centrifugation at 3000 rpm for 20 min and stored at −20°C until assayed.

Serum insulin

Serum insulin level was analyzed through chemiluminescence in fully automatic Advia Centaur ImmunoAssay System.

Tissue biochemistry

Frozen pancreatic tissue samples were used for biochemical analysis of total protein,[55] lipid peroxidation assay (TBARS),[56] superoxide dismutase (SOD) activity,[57] catalase (CAT) activity,[58] glutathione (GSH) levels,[59] and ascorbic acid levels.[60]

Histopathological study

Pancreatic tissues were collected after autopsy, fixed in Bouin's fixative, and processed through an ascending series of ethanol and cleared in xylene. The tissues were then embedded in paraffin wax; 5 µm thick sections were cut, stained with hematoxylin and eosin, and observed under light microscope for histopathological changes. Diameters of islets of pancreas were measured using a light microscope equipped with ocular micrometer calibrated with stage micrometer (at least 10 per animal). Two diameters perpendicular to each other were measured at ×100 magnification, averaged, and expressed as islets diameter.

Statistical analysis

All the data were calculated and statistically analyzed with SPSS 20.0 computer software package for Windows (SPSS INC., Chicago, IL, USA). The data were expressed as mean ± standard error of mean and tested for variance. All the data statistically were analyzed with one-way ANOVA followed by Tukey's as a *post hoc* test. Differences in means were considered significant at $P < 0.05$.

RESULTS

Serum insulin

The serum insulin levels in control and experimental rats have been shown in Figure 1. There was a significant ($P \leq 0.001$) decline in the levels of serum insulin in STZ-induced diabetic rats (Group II) as compared to normal control rats (Group I). Oral administration of *C. fistula* extract 250 and 500 mg/kg b.wt./day for 60 days in diabetic rats significantly ($P \leq 0.05$ and $P \leq 0.001$, respectively) enhanced the level of serum insulin as compared to diabetic control rats. Oral administration of glibenclamide in diabetic rats also improved serum insulin level significantly ($P \leq 0.001$) as compared to diabetic rats.

Pancreas weight

The relative weight of the pancreas in control and experimental rats has been depicted in Figure 2. A significant ($P \leq 0.01$) decrease in the relative weight of pancreas was observed in diabetic control rats (Group II) when compared with normal control rats (Group I). Treatment of diabetic rats with 500 mg/kg/day dose of *C. fistula* extract (Group V) or standard drug glibenclamide (Group VI) significantly ($P \leq 0.05$) increased relative weight of the pancreas as compared to diabetic control rats. However, there was nonsignificant increase in relative weight of pancreas in lowest dose (Group III) and medium dose (Group IV) of *C. fistula* extract-treated diabetic rats as compared to diabetic control rats.

Diameter of islets

The diameter of islets of Langerhans in the pancreas of control and experimental rats has been shown in Figure 3. The diameter of islets decreased significantly ($P \leq 0.001$) in diabetic control rats as compared with normal control rats. Treatment of *C. fistula* extract in diabetic rats at 250 and 500 mg/kg/day doses significantly ($P \leq 0.01$, $P \leq 0.001$; respectively) increased the diameter of islets as compared to diabetic control rats. Oral administration of glibenclamide also significantly ($P \leq 0.001$) increased the diameter of islets as compared with diabetic control rats.

Total protein

The level of total proteins in the pancreas of normal control and experimental rats is depicted in Table 1. The diabetic control

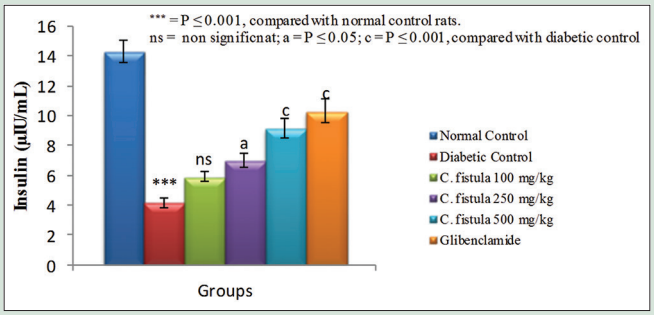

Figure 1: Effect of *Cassia fistula* extract on serum insulin level in streptozotocin-induced diabetic rats

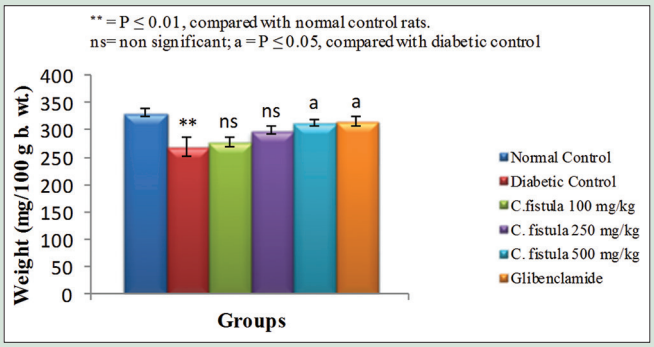

Figure 2: Effect of *Cassia fistula* extract on relative weight of pancreas in streptozotocin-induced diabetic rats

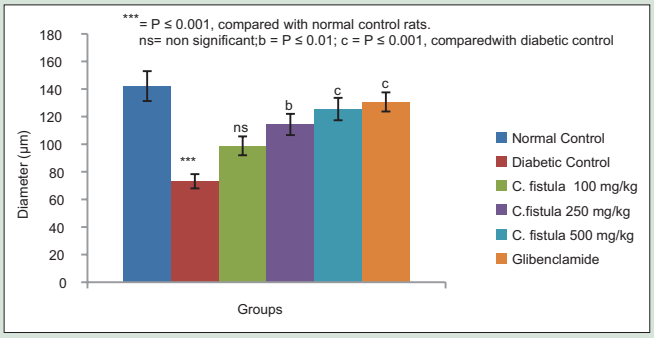

Figure 3: Effect of *Cassia fistula* extract on diameter of islets of pancreas in streptozotocin-induced diabetic rats

rats (Group II) showed a significant ($P \leq 0.001$) decrease in the concentration of total protein in pancreas as compared to normal control rats (Group I). Diabetic rats treated with different doses of *C. fistula* pod extract (100, 250, and 500 mg/kg b.wt./day) or glibenclamide showed significant increase in the levels of total protein in the pancreas of Group III ($P \leq 0.05$), Group IV ($P \leq 0.01$), Group V and Group VI ($P \leq 0.001$) as compared to diabetic control rats.

Lipid peroxidation and antioxidant defense markers

The changes in lipid peroxidation (TBARS) and antioxidant defense markers in the pancreas of control and experimental rats have been depicted in Table 1. Diabetic control rats (Group II) showed a significant ($P \leq 0.001$) elevation in TBARS concentration in the pancreas as compared with normal control rats (Group I). Diabetic rats treated with different doses (100, 250, and 500 mg/kg b.wt./day) of *C. fistula* pod extract showed significant dose-dependent ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively) decline in TBARS level as compared to diabetic control rats. Diabetic rats treated with glibenclamide also revealed significant ($P \leq 0.001$) decline in TBARS level as compared to diabetic control rats.

As compared to normal control rats, the activities of SOD and CAT and concentrations of GSH and ascorbic acid in the pancreas of diabetic control rats (Group II) were significantly ($P \leq 0.001$) declined. Diabetic rats treated with different doses of *C. fistula* pod extract or glibenclamide showed significant increase in the activities of SOD (Group IV [$P \leq 0.01$], Group V and Group VI [$P \leq 0.001$]), CAT (Group III [$P \leq 0.05$], Group IV [$P \leq 0.01$], Group V and Group VI [$P \leq 0.001$]), GSH content (Group III [$P \leq 0.05$], Group IV [$P \leq 0.01$], Group V and Group VI [$P \leq 0.001$]) and ascorbic acid levels (Group IV [$P \leq 0.01$], Group V and Group VI [$P \leq 0.01$]) as compared to diabetic control rats (Group II).

Histopathological study

Histomorphological picture of the pancreas of normal control rat (Group I) exhibited normal distribution of islet of Langerhans with the exocrine part, acini. The islets of Langerhans were regular with well-defined boundaries [Figure 4a]. Histomorphological picture of the pancreas of untreated diabetic control rat (Group II) revealed degenerative and atrophic changes in islets of Langerhans. Significant reduction in the size, cellular density, and granulation was observed in the islets [Figure 4b]. Histomorphological picture of the pancreas of diabetic rats treated with 100 mg/kg b.wt. of *C. fistula* pod extract (Group III) exhibited mild decrease in necrosis and vacuolization with slight increase in size and cellular density of islets [Figure 4c]. However, diabetic rats treated with 250 mg/kg b.wt. of *C. fistula* pod extract (Group IV) showed moderate amelioration of necrotic changes, reduction in vacuolization concomitantly with an increase in the diameter and cellular density of islets [Figure 4d]. Histomorphological picture of the pancreas of diabetic rats treated with 500 mg/kg b.wt. *C. fistula* pod extract (Group V) depicted significant improvement of histological alterations. The islets showed near-normal morphology with cells showing mild vacuolization, degeneration, and degranulation [Figure 4e]. Diabetic rats treated with glibenclamide (Group VI) also showed restoration of the pancreatic histoarchitecture near to normal control rat [Figure 4f].

DISCUSSION

Studies during the last few decades have shown that plant and plant-based therapies have a potential to control and manage diabetes

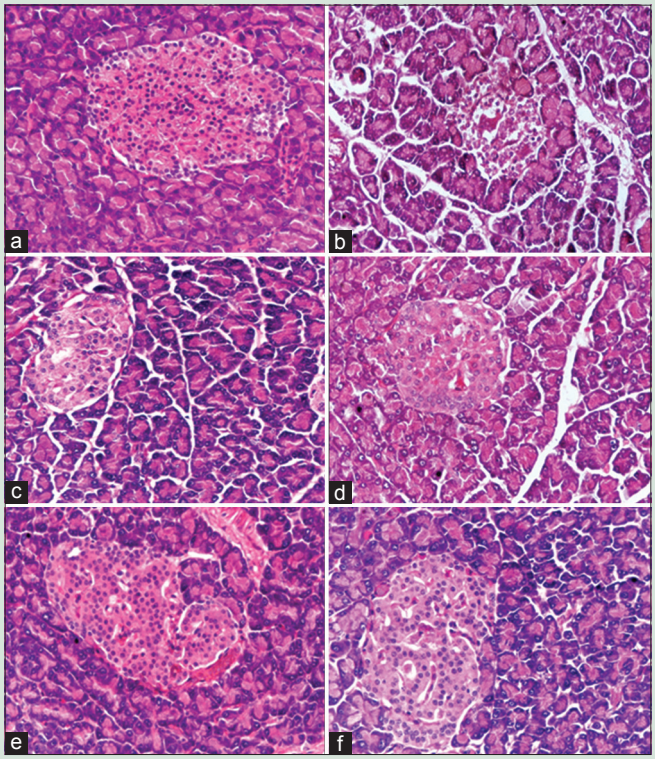

Figure 4: Photomicrograph of the pancreatic section (H and E, ×200). (a) Control rat (Group I) showing normal islets of Langerhans interspersed among the acini. (b) Diabetic control rat (Group II) showing shrunken islets of Langerhans, displaying degenerative and atrophic changes. (c) *Cassia fistula* extract (100 mg/kg b.wt.) treated diabetic rat (Group III) showing mild improvement of islet histoarchitecture. (d) *Cassia fistula* extract (250 mg/kg b.wt.) treated diabetic rat (Group IV) showing an increase in the size of islets and prevention of β-cell damage. (e) *Cassia fistula* extract (500 mg/kg b.wt.) treated diabetic rat (Group V) showing improvement in morphology, size, and cellular density of islets. (f) Glibenclamide (5 mg/kg b.wt.) treated diabetic rat (Group VI) showing prevention of cellular damage and restoration of pancreatic histology

Table 1: Effect of *Cassia fistula* pod extract on lipid peroxidation and antioxidant parameters of pancreas in streptozotocin - induded diabetic rats

Treatment	Pancreas protein (mg/g)	TBARS (nmole/ mg tissue)	SOD (U/mg protein)	CAT (nmole H_2O_2/ min/mg protein)	GSH (µ mole/g tissue)	Ascorbic acid (mg/g tissue)
Group I	54.73±2.17	2.87±0.19	17.78±0.74	63.22±2.31	2.80±0.11	1.42±0.14
Group II	22.02±1.58***	8.34±0.76***	10.26±0.71***	27.25±1.57***	1.13±0.10***	0.58±0.05***
Group III	30.28±1.09[a]	6.41±0.40[a]	12.46±0.79 (ns)	39.73±2.01[a]	1.73±0.11[a]	0.84±0.08 (ns)
Group IV	33.28±2.27[b]	5.57±0.46[b]	14.47±0.77[b]	42.57±1.91[b]	1.89±0.17[b]	1.02±0.08[a]
Group V	38.82±2.04[c]	3.98±0.41[c]	15.98±0.65[c]	49.28±4.21[c]	2.10±0.15[c]	1.12±0.10[b]
Group VI	42.33±2.09[c]	3.35±0.21[c]	16.15±1.05[c]	52.85±4.15[c]	2.18±0.17[c]	1.18±0.10[b]

Values represent mean ± SEM (n=6); Level of significance: *** = P ≤ 0.001, compared with normal control rats. NS = Nonsignificant; a = $P \leq 0.05$; b = $P \leq 0.01$; c = $P \leq 0.001$, compared with diabetic control rats

and its complications. They are better than allopathic drugs, which have a lot of adverse side effects.[15,61]

STZ is a widely used chemical for the induction of experimental diabetes in animals. STZ selectively destroys the pancreatic β-cells involving uptake by glucose transporter-2 and causes alkylation of DNA. It also generates ROS, which contribute to DNA fragmentation, and evokes other deleterious changes in the β cells of pancreas ultimately inducing beta-cell necrosis and depletion of insulin biosynthesis and secretion.[62-64] This was evident from the marked decrease in serum insulin level in STZ-treated diabetic control rats observed in the present study. These results are parallel with other reports which have also observed similar depletion of plasma/serum insulin level in STZ-treated diabetic rats.[49,50,53]

Diabetic rats treated with different doses of *C. fistula* pod extract or glibenclamide for 60 days showed an elevation of serum insulin. Our results are consistent with the previous reports which have also mentioned that subchronic treatment with ethanol extract of flowers[50] or ethanol extract of leaves[52] or hexane extract of bark[49] or methanol extract of various parts[53] of *C. fistula* in diabetic rats significantly lowered fasting blood glucose level and increased plasma insulin level. The elevation of plasma insulin level with reduction of fasting blood glucose level by *C. fistula* pod extract may be due to its many potential bioactive phytochemicals, especially, flavonoids, anthraquinone, β-sitosterol, kaempferol, quercetin dehydrates, and proanthocyanidins such as catechin and epicatechin which might show ameliorative effect on glycemic index by virtue of their synergistic action, resulting in an increased secretion of insulin by repair/regeneration of beta-cells of islets, as also evident through histopathological study. In this context, Daisy *et al*. did not observe any increase in insulin level in STZ-induced diabetic rats treated with catechin isolated from methanol extract of *C. fistula* bark. They reported ameliorative effects of catechin in diabetic rats by virtue of insulin mimetic activity.[65]

Glibenclamide is a standard drug widely used in diabetic animals for the comparison of antidiabetic activity of test substances. Administration of glibenclamide in diabetic rats significantly increased the serum insulin level, suggesting an insulinogenic action. These results are in accordance with earlier studies which also reported enhancement of insulin secretion in diabetic animals following glibenclamide treatment.[66,67]

In the present study, a significant reduction in the relative weight of pancreas and diameter of islets of Langerhans were observed in STZ-induced diabetic control rats. The reduction observed in relative weight of pancreas might be due to degenerative and atrophic changes in both endocrine and exocrine parts of the pancreas and also by virtue of reduction in the numbers of islets in pancreas. These results are in agreement with earlier reports where similar decline in mean pancreatic weight, mean number, and diameter of islets was observed in STZ-treated rats.[68-70] Treatment of diabetic rats with *C. fistula* pod extract/glibenclamide significantly increased the relative weight and diameter of islets of pancreas. Similar to our finding, many researchers also reported that several plant extracts having antihyperglycemic activity succeeded in restoring the mean weight and diameter of islets of pancreas in diabetic rats by prevention of degenerative and atrophic changes in the pancreas, increasing the number of islets, and/or regenerating β-cells in islets.[68-71]

A significant decline of protein content in the pancreas of STZ-induced diabetic rats indicates adverse impact on protein metabolism and secretory activities of the pancreas due to deficiency of insulin hormone and oxidative stress-induced cell toxicity. Similar to our finding, Changrani *et al.* also reported significant decline of protein concentration in pancreas of diabetic rats due to diminished protein and amylase secretion as a result of disturbances in cation homeostasis, pancreatic atrophy, altered intracellular signaling, derangement in gene expression for protein synthesis.[72] Treatment of diabetic rats with *C. fistula* extract/glibenclamide restored the protein content in the pancreas by scavenging free radicals and consequently improving antioxidants status, insulin secretion, and pancreatic morphology.

Prooxidants and antioxidants balance is vital for normal biological functions of the cell. Any disturbances that change this balance can provoke excessive production of ROS, which create a condition frequently known as oxidative stress. Oxidative stress is suggested as mechanism underlying diabetes and diabetic complications.[73,74]

Lipid peroxidation is frequently used as an indicator of increased oxidative stress and subsequent oxidative damage. The rate of lipid peroxidation was measured indirectly by estimating TBARS. The increased free radicals may react with polyunsaturated fatty acids in the cell membranes, leading to lipid peroxidation. Lipid peroxidation is highly destructive process that effects cellular organelles, enzymes, and other molecules and causes them to lose biochemical functions and/or structural integrity, leading to cell death.[11,75]

Antioxidant enzymes and nonenzymatic antioxidants are the first line of defense against ROS-induced oxidative stress.[76] However, pancreatic islets cells possess very low levels of free radical scavenging enzymes and are vulnerable to free radical-induced toxicity.[77] Moreover, diabetes also induces chronic oxidative stress and produces changes in the tissue content and activity of the antioxidant enzymes.[78,79]

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical, converting it into H_2O_2 and molecular oxygen, which both damage the cell membrane and other biological structures,[80] CAT is a hemprotein, which is responsible for the detoxification of significant amounts of H_2O_2 to water and oxygen,[81] GSH is a major endogenous antioxidant which functions as a free radical scavenger and is an essential co-substrate for glutathione peroxidase,[82,83] and ascorbic acid is the most powerful water-soluble extracellular antioxidant; under physiological conditions, it can directly scavenge superoxide, hydroxyl radicals, and single oxygen.[84]

In the present study, it was observed that level of TBARS in the pancreas of STZ-induced diabetic rats was significantly increased with concomitant decrease in the activities of SOD, CAT, GSH, and ascorbic acid levels when compared with normal control rats. The decreased activity of antioxidant molecules along with elevated TBARS level in diabetic rats could probably be associated with oxidative stress and decreased antioxidant defense potential.[78,85] Similar significant elevation of TBARs level and significant decline of antioxidant defense parameters (SOD, CAT, GSH, ascorbic acid, etc) have been reported in STZ-induced diabetic rats by various workers.[66,86,87] It was suggested that decreased antioxidant enzymes activity in diabetic state could be due to overutilization of these in scavenging excessive free radicals generated due to hyperglycemia, glucose autooxidation, and glycation of these enzymes.[88]

Treatment of diabetic rats with *C. fistula* pod extract showed significant dose-dependent decline of TBARS level with concomitant increase in antioxidant markers (SOD, CAT, GSH, and ascorbic acid) in the pancreas, suggesting potent antioxidant activity of the extract. It has been reported that *C. fistula* pods are rich in polyphenolic and flavonoid phytoconstituents which possess potent antioxidant activities.[33,89,90] Furthermore, improvement of antioxidant parameters and decline of lipid peroxidation have also been reported earlier by many researchers in diabetic rats by treatment with extracts from different parts of *C. fistula*.[50,91] These results are parallel with many other reports which have also reported decreased TBARS level with a concomitant increase in free radical scavenging antioxidant molecules in the pancreas of diabetic rats receiving various plant extract treatment having antihyperglycemic effect.[15,66,87,92,93]

The histopathological examination of islets of Langerhans in the pancreas of STZ diabetic rats showed severe degenerative and atrophic changes. The islets were shrunken showing decreased cell mass and granulation and cytoplasmic vacuolization. These results are in agreement with the previous reports which have also shown similar type of histopathological lesions in pancreas islets of STZ-induced diabetic rats.[70,94,95] Such pathological changes could be attributed to glucotoxicity, which arises from excessive uptake of glucose by β-cells in diabetes.[64] The excessive sugar glycation reactions and mitochondrial electron transport chain produce ROS at the level beyond the antioxidant capacities of the cell. The ensuing oxidative stress impairs insulin synthesis and secretion and initiates a cascade of cellular events that ultimately lead to beta-cells cytotoxicity and death.[96,97]

The histopathological study of the pancreas in *C. fistula* pod extract-treated diabetic rats showed significant dose-dependent restoration of histoarchitecture. The observed ameliorative effects of *C. fistula* pod extract may be due to the presence of secondary metabolites such as polyphenols and flavonoids which exert antioxidant-like effects, consequently alleviation of oxidative stress and enhancing insulin secretion possibly by virtue of regeneration or proliferation of beta-cells in diabetic rats.[50,53] This is probably because the pancreas contains stable (quiescent) cells which have the capacity of regeneration[98] or the self-duplication/self-proliferation consequently increasing the number of beta-cells in islets.[99-102] Similar to our finding, a number of phytochemicals present in different plants, especially polyphenols and flavonoids have shown potent antioxidant and free radical scavenging activity, consequently reducing β-cell damage and increasing their proliferation, resulting in restoration of islets morphology in diabetic rats.[70,72,97,103]

CONCLUSIONS

The results of the present study demonstrated that 70% hydroalcoholic extract of *C. fistula* pod possess significant antidiabetic activity through enhanced secretion of insulin via restoration of islets morphology by β-cell regeneration/proliferation and improvement of antioxidants status in the pancreas. The antidiabetic effects of the extract were comparable with glibenclamide. Further study is needed to isolate the bioactive phytoconstituent(s) responsible for this activity.

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Conflicts of interest

There are no conflicts of interest.

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