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Hypoglycemic and antioxidant activities of *Caesalpinia ferrea* Martius leaf extract in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To evaluate the antidiabetic and antioxidant effects of aqueous ethanolic extract of *Caesalpinia ferrea* (*C. ferrea*) leaf in normal and streptozotocin (STZ) induced diabetic rats.

Methods: Male Sprague-Dawley rats divided into 6 groups of 6 rats each were assigned into diabetic and non-diabetic groups. Diabetes was induced in rats by single intraperitoneal administration of STZ (65 mg/kg body weight). *C. ferrea* extract at the doses of 250 and 500 mg/kg body weight was orally administered to both diabetic and non-diabetic animals for a period of 30 days. After completion of experimental duration serum, liver and pancreas were used for evaluating biochemical and histopathological changes.

Results: Oral administration of *C. ferrea* leaf extract significantly reduced elevated serum glucose, α-amylase, liver function levels and significantly increased serum insulin, total protein and body weight as well as improved lipid profile due to diabetes. Furthermore, the treatment resulted in a marked increase in glutathione peroxidase, superoxide dismutase, catalase and reduced glutathione, and diminished levels of lipid peroxidation in liver and pancreas of diabetic rats. Histopathological studies demonstrated the reduction in the pancreas and liver damage and confirmed the biochemical findings.

Conclusions: From the present study, it can be concluded that the *C. ferrea* leaf extract effectively improved hyperglycaemia while inhibiting the progression of oxidative stress in STZ-induced diabetic rats. Hence, it can be used in the management of diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by abnormal metabolism of carbohydrates, proteins, and fats resulting from inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action [1]. According to the American diabetes association, the chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [2]. DM is considered the most prevalent disease in the world affecting 25% of the population. It afflicts 150 million people and is predicted to rise to 300 million by 2025 [3]. It is likely to be the fifth leading cause of death worldwide [4].

Previous studies have demonstrated that DM exhibits enhanced oxidative stress and highly reactive oxygen species (ROS) production in pancreatic islets due to persistent and chronic hyperglycemia, thereby depletes the activity of the

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antioxidative defense system, and thus promotes free radical generation [5]. Oxygen free radicals have been suggested to be a contributory factor in complications of DM [6]. It seems to be an oxidative stress-related disorder and the antioxidants may be useful in preventing it [7]. Therefore supplementation of therapeutics with antioxidants may have a chemoprotective role in diabetes [8].

Many plant extracts and their products have been shown to have significant antioxidant effect in treating many kinds of diseases [9]. The use of medicinal plants for the treatment of human diseases has increased considerably worldwide [10]. Ethnopharmacological evidence has shown that the use of plants is also helpful in prophylaxis or treatment of diabetes. Given that, herbal medicine possesses significant efficacy, low incidence of side effects, low cost and relative safety [11], while synthetic anti-diabetic agents can produce serious side effects, as hypoglycemic coma and disturbances of the liver and kidneys [12].

The little studied genus *Caesalpinia* contains more than 500 species of worldwide distribution [13]. Previous studies of species of this genus reported remarkable biological activities such as antimicrobial (*Caesalpinia bonducella*) [14], antidiabetic (*C. bonducella*) [15], antimalarial (*Caesalpinia pluviosa*) [16], and anti-inflammatory (*Caesalpinia sappan*) [17]. To date, less than 30 species of this genus have been studied for their phytoconstituents. The metabolites described include predominantly flavonoid derivatives, steroids, triterpenoids, and cassane diterpenes [18].

Caesalpinia ferrea (*C. ferrea*) Martius (Leguminosae), popularly known as “pau-ferro” or “jucá”, is a large tree belonging to the Fabaceae family. It is found mainly in the north and northeast of Brazil. In folk medicine, the tea of the stem bark of *C. ferrea* has been used for the treatment of diabetes. In view of its ethnomedicinal importance, the Brazilian Ministry of Health has included this species on the national list of medicinal plants important to the health system [19].

The pharmacological properties of *C. ferrea* fruits or stem barks include antiulcerogenic [20], anti-inflammatory [21], analgesic [22], antibacterial [23], antihypertensive [24], antidiabetic [19], and cancer chemopreventive [25] activities. Recently a unique chalcone trimer (pauferrol A) and two chalcone dimers (pauferrol B and pauferrol C), were isolated from the stems of *C. ferrea*. These chalcones exhibited potent inhibitory activities against topoisomerase II [26,27]. The leaves contain three formerly unknown di-O-glycosyl-C-glucosyl flavones which were isolated, purified and identified namely: Isovitexin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β-xylopyranosyl]; Vitexin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β-xylopyranosyl]; Orientin 2″-O-β-[xylopyranosyl-(1‴→2″)-O-β- xylopyranosyl [28]. However, there is no experimental evidence proving biological activities of *C. ferrea* leaf up till now. Therefore, the present study was aimed to evaluate the possible hypoglycemic properties of *C. ferrea* leaf in streptozotocin (STZ) induced diabetic rats.

2. Materials and methods

2.1. Chemicals

STZ, reduced glutathione, 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB), 1-chloro 2,4-dinitrobenzene (CDNB) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Metaphosphoric acid (MPA) and nitroblue tetrazolium were purchased from Fluka (Switzerland), and pyrogallol from Merck (Germany). All chemicals were of analytical grade.

2.2. Plant material

Leaves of *C. ferrea* were collected from a tree cultivated in the Zoological Garden, Cairo, Egypt, in May 2012. The plant was identified by Prof. Salwa Quashti, National Research Centre (NRC), Cairo, Egypt. A voucher specimen (C253) has been deposited at the herbarium of the NRC.

2.3. Plant extraction and isolation

Leaves (2.5 kg), dried in the shadow, were crushed and exhaustively extracted with 70% (v/v) aqueous EtOH under reflux (three times, each extraction for 8 h with 2 L). The obtained eluent was dried under vacuum at 55–60 °C to give 200 g aqueous ethanolic extract that was used in the present study.

2.4. Phytochemical screening

This aqueous ethanol extract of *C. ferrea* was screened for the presence of various phytoconstituents such as steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, terpenoids, tannins, and phenolic compounds as described by Dawang & Datup and Mythili & Ravindhran [29,30].

2.5. Determination of the scavenging of DPPH radical

The quantitative DPPH assay was carried out according to the method of Kedare and Singh [31]. The extract was dissolved in a concentration of 1 mg/mL in ethanol. From this stock solution, concentrations of regular dilution were prepared. Then 500 μL of sample, 375 μL ethanol and 125 μL of 1 mmol/L prepared DPPH solution were placed together. The test was performed in triplicate. All samples were incubated in sequence for 30 min in the dark at room temperature and their absorbance was measured at a wavelength of 517 nm on UV–vis spectrophotometer (Shimadzu, Duisburg, Germany). Ascorbic acid was used as positive control. Percentage of radical scavenging activity (RSA) was calculated as follows:

$$RSA\% = [(Abs\ of\ control - Abs\ of\ sample)/Abs\ of\ blank] \times 100$$

2.6. Acute toxicity study

The mean lethal dose (LD_{50}) of the aqueous ethanolic extract of *C. ferrea* leaf was determined in rats (weighing 180–200 g) using the method described by Chinedu *et al*. [32].

2.7. Experimental animals

Male Sprague-Dawley rats weighting 180–200 g were purchased from the Animal House of National Research Centre, Egypt. Animals were acclimated for a period of 7 days in our

laboratory condition prior to the experiment. The animals were fed with standard laboratory diet and allowed to drink water *ad libitum* under well ventilated conditions of 12 h light/dark cycles. Experimental protocols for the animal studies were carried out in accordance with Institutional Ethical Guidelines for the care of laboratory animals of the National Research Centre.

2.8. Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal (*i.p.*) injection of a freshly buffered (0.1 mol/L citrate, pH 4.5) solution of STZ at a dosage of 65 mg/kg body weight. After 72 h of STZ administration, the tail vein blood was collected to determine fasting blood glucose level with an Accu-Chek sensor comfort glucometer (China). Only rats with hyperglycemia (glucose over 250 mg/dL) were considered diabetic and included in the experiment.

2.9. Experimental design

Rats were randomly divided into six groups, comprising six rats each. The treatment schedule was as follows:

Group I: normal control (NC); Group II: *C. ferrea* leaf extract (500 mg/kg body weight) treated normal rats (CF500-NC); Group III: *C. ferrea* leaf extract (250 mg/kg body weight) treated normal rats (CF250-NC); Group IV: diabetic control (DC); Group V: *C. ferrea* leaf extract (500 mg/kg body weight) treated diabetic rats (CF500-DC); Group VI: *C. ferrea* leaf extract (250 mg/kg body weight) treated diabetic rats (CF250-DC).

Different doses of *C. ferrea* aqueous ethanolic extract were administered orally using an intragastric tube daily to the respective group till the end of the experiment. All doses were started 72 h after STZ injection.

2.10. Blood and tissue sampling

At the end of the 30-day experiment (after diabetes induction), overnight fasting animals were ether anaesthetized. Venous retro orbital blood samples were collected using a glass capillary without anticoagulant. Serum was separated by centrifugation at 3000 r/min for 15 min. The resulting samples were stored at −20 °C until assayed. Liver and pancreas were removed and washed in ice-cold saline solution immediately, and then each organ was divided into two portions. A portion was homogenized in 0.1 mol/L potassium phosphate buffer (pH 7.4) using Tissue master TM125 (Omni International, USA). After centrifugation at 3000 r/min for 10 min, the clear supernatant was stored at −80 °C to be used for biochemical analysis. The other portion of the liver and pancreas was fixed in 10% formalin for histological analysis.

2.11. Biochemical analysis

2.11.1. Determination of serum glucose, insulin and α-amylase

Blood glucose was determined using Biodiagnostic kit, Egypt. Insulin level was estimated with sandwich immunoluminometric assay kit supplied from Snibe Co., Ltd., China using Maglumi 1000 fully automated chemiluminescence immunoassay analyzer (Snibe Co.,Ltd., China). Alpha-amylase activity was assayed using kits supplied by ELitech Clinical Systems, France.

2.11.2. Determination of serum lipid profile

Serum concentrations of triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using commercially available kits supplied by Reactivos GPL, Spain. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula [33]:

$$LDL = [(TC - HDL) - TG/5]$$

2.11.3. Determination of serum liver function

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed using kits provided by Biorexfars, UK. Serum alkaline phosphatase (ALP) was estimated using kits supplied by Stanbio, USA, whereas serum glutamyl transpeptidase (GGT) and serum total protein (TP) were measured using kits supplied by Reactivos GPL, Spain and Biodiagnostic, Egypt respectively.

2.11.4. Determination of oxidative stress markers in hepatic and pancreatic tissue

Glutathione peroxidase activity (GSH-Px) was measured according to the method of Necheles *et al*. [34]. Superoxide dismutase activity (SOD) was investigated utilizing the technique of Minami and Yoshikawa [35]. Catalase (CAT) activity was determined by the method of Aebi [36]. Lipid peroxidation was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) according to method of Lefèvre *et al*. [37]. Reduced glutathione (GSH) was estimated according to the method of Beutler *et al*. [38] after precipitating liver and pancreas proteins with 10% MPA.

2.11.5. Histopathological investigation

The histopathologic examination was performed by light microscopy on liver and pancreas specimens that were fixed in 10% formalin. After fixation, the samples were processed to obtain 5 μm thick paraffin sections. Pancreas and liver sections were stained with hematoxilin and eosin (H & E). Then the slides were observed under a Leica photomicroscope.

2.11.6. Image morphometry

The morphometric analysis was performed at the Pathology Department, National Research Center using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd., Cambridge, England) which consists of Leica DM-LB microscope with JVC color video camera attached to a computer system Leica Q 500IW. The morphometric analysis is carried out on H & E stained slides. The slides to be examined were placed on the stage of the microscope, and focused it at low power magnification (100×). We screen the slide to determine the boundaries of the tissue to be measured. The condenser is centered and focused, and the light source is set to the required level. Successful adjustment of illumination is checked for on the video monitor. The area of Langerhans islets were measured by drawing a line starting from one edge to the other and from one edge till the opposite, respectively. The results appear automatically on the monitor in the form of square micron (μm^2) with the mean and standard error.

2.12. Statistical analysis

Data were expressed as mean ± SEM. The statistical significance was evaluated by One-way analysis of variance (ANOVA) using SPSS-14 statistical software followed by LSD test to detect differences between groups. The differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Phytochemical screening of C. ferrea extract

The preliminary phytochemical screening of *C. ferrea* aqueous ethanolic extract indicated the presence of carbohydrates, glycosides, tannins, and phenolic compounds as shown in Table 1.

Table 1
Phytochemical screening of aqueous ethanolic extract of *C. ferrea* leaf.

Phytochemicals	Presence/absence
Carbohydrates and/or glycosides	Present
Tannins	Present
Saponins	Absent
Alkaloids	Absent
Anthraquinones	Absent
Unsaturated sterols or triterpenes	Absent
Phenolic compounds	Present

3.2. Radical scavenging activity of C. ferrea extract

During evaluation of the antioxidant activity, the aqueous ethanol extract of *C. ferrea* exhibited a remarkable radical scavenging activity in the DPPH assay. Figure 1 demonstrates this effect quantitatively in comparison to those of ascorbic acid. The antioxidant capacity of the extract (ED_{50}) was determined to be (12.45 ± 2.86) μg/mL.

3.3. Acute toxicity

Acute toxicity studies revealed the non-toxic nature of *C. ferrea* aqueous ethanolic extract as the treated rats appeared normal and did not display any significant changes in behavior or neurological responses up to 1500 mg/kg body weight of the extract. There was no mortality or toxicity reaction at any of the doses until the end of the study.

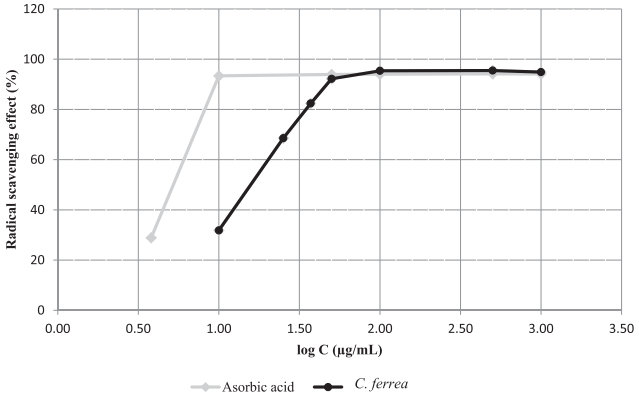

Figure 1. Antioxidant capacity of the aqueous ethanolic extract of *C. ferrea* leaf (DPPH assay).

3.4. Effect of C. ferrea extract on body weight

As shown in Table 2, body weights of rats in DC group were lower than those in other groups. STZ caused a significant weight loss of rats in DC and CF250-DC groups in comparing to NC group, while treatment with *C. ferrea* extract at 500 mg/kg body weight to diabetic rats significantly suppressed such decrease in the body weight. No significant difference was observed after treatment with *C. ferrea* extract in CF250-DC as compared to the DC group.

Table 2
Changes of the body weight of rats during the experimental period of 30 days.

Groups	Initial body weight (g)	Final body weight (g)
I) NC	185.00 ± 3.41	235.44 ± 6.24
II) CF500-NC	181.33 ± 1.74	230.92 ± 7.19[b]
III) CF250-NC	198.33 ± 4.01	235.50 ± 10.17[b]
IV) DC	190.00 ± 4.47	182.33 ± 5.67[a]
V) CF500-DC	183.66 ± 2.07	218.00 ± 4.53[b]
VI) CF250-DC	189.83 ± 3.74	198.70 ± 6.68[a]

Data are expressed as mean ± SEM ($n = 6$). Values with different superscripts down the column are significantly different at $P < 0.05$.
[a] Statistically different from NC group.
[b] Statistically different from DC group.

3.5. Effect of C. ferrea extract on blood glucose, insulin and α-amylase

As shown in Table 3, serum glucose levels of DC and CF-DC groups were significantly increased as compared to NC group. The administration of *C. ferrea* extract to STZ-induced diabetic rats in groups CF500-DC and CF250-DC significantly reduced serum glucose levels as compared to the DC group. Whereas, serum insulin levels of DC and CF250-DC groups significantly decreased as compared to NC group. The administration of 500 and 250 mg/kg of *C. ferrea* extract to diabetic rats significantly increased insulin level as compared to the DC group and nearly returned to the basal level in a dose-dependent manner. Moreover, α-amylase activity in the DC and CF-DC groups were significantly higher than those in the normal NC group. The administration of *C. ferrea* extract to diabetic rats in CF500-DC

Table 3
Serum glucose, insulin, and α-amylase values in all groups.

Groups	Glucose (mg/dL)	Insulin (μIU/mL)	α-amylase (IU/L)
I) NC	102.14 ± 3.87	2.80 ± 0.25	15.04 ± 0.68
II) CF500-NC	95.86 ± 2.99[b]	3.07 ± 0.20[b]	16.75 ± 0.97[b]
III) CF250-NC	96.84 ± 2.66[b]	2.92 ± 0.18[b]	14.85 ± 0.66[b]
IV) DC	388.49 ± 19.00[a]	1.23 ± 0.05[a]	249.50 ± 15.83[a]
V) CF500-DC	121.60 ± 5.32[a,b]	2.47 ± 0.13[b]	153.83 ± 4.98[a,b]
Change from DC (%)	68.60%	101.6%	38.34%
VI) CF250-DC	134.50 ± 8.76[a,b]	2.02 ± 0.10[a,b]	174.71 ± 6.63[a,b]
Change from DC (%)	65.37%	64.48%	29.97%

Data are expressed as mean ± SEM ($n = 6$). Values with different superscripts down the column are significantly different at $P < 0.05$.
[a] Statistically different from NC group.
[b] Statistically different from DC group.

Table 4
Effect of *C. ferrea* extract on serum lipid profile in all groups.

Groups	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
I) NC	48.39 ± 1.89	52.05 ± 1.57	28.71 ± 0.80	13.77 ± 1.23
II) CF500– NC	48.14 ± 1.93[b]	47.47 ± 1.43[b]	27.54 ± 0.96[b]	10.28 ± 0.98[b]
III) CF250– NC	28.46 ± 1.92[a,b]	46.74 ± 1.37[b]	29.62 ± 0.97[b]	11.43 ± 1.04[b]
IV) DC	108.17 ± 6.37[a]	99.59 ± 5.44[a]	18.36 ± 0.63[a]	59.60 ± 5.23[a]
V) CF500- DC	49.35 ± 1.36[b]	58.91 ± 2.63[b]	25.20 ± 1.40[a,b]	22.27 ± 2.28[a,b]
Change from DC (%)	54.37%	40.84%	37.25%	62.63%
VI) CF250- DC	61.14 ± 2.82[a,b]	56.38 ± 1.46[b]	24.41 ± 1.10[a,b]	21.32 ± 1.37[a,b]
Change from DC (%)	43.47%	43.38%	32.95%	64.22%

Data are expressed as mean ± SEM (n = 6). Values with different superscripts down the column are significantly different at $P < 0.05$.
[a] Statistically different from NC group.
[b] Statistically different from DC group.

and CF250-DC groups significantly decreased α-amylase activity as compared to the DC group.

3.6. Effect of C. ferrea extract on lipid profile

Table 4 shows the levels of serum lipid profile of rats in different experimental groups. Rats in DC group displayed a significant increase in the levels of TG, TC, and LDL-C in comparison with NC group. However, serum HDL-C level of rats in DC group was significantly lower than that of rats in NC group. Treatment with *C. ferrea* extract in CF500-DC and CF250-DC groups showed a significant decrease in the levels of serum TG, TC, and LDL-C and simultaneous significant increase in the level of HDL-C when compared with DC group. Although the serum HDL-C and LDL-C level did not return to the basal level of NC group, the serum TG level in CF500-DC group and TC level in both CF500-DC and CF250-DC groups were able to return.

3.7. Effect of C. ferrea extract on serum liver function

The data for serum liver function tests are presented in Table 5. Serum activities of AST, ALT, ALP, and GGT biomarkers of liver toxicity were significantly elevated in STZ induced diabetic rats when compared to normal controls. Treatment of diabetic rats with 500 and 250 mg/kg of *C. ferrea* extract significantly reduced the activity of these biomarkers with respect to diabetic control rats for both doses. Such reduction nearly returned to the basal normal level for AST and ALT activities but ALP and GGT could not return. On the contrary, serum level of TP was significantly decreased in DC group as compared to normal control rats. Administration of 500 and 250 mg/kg of *C. ferrea* extract for diabetic rats significantly increased TP level and adjusted to the normal level.

3.8. Effect of C. ferrea extract on hepatic and pancreatic oxidative stress markers

Table 6 reveals a significant decrease in antioxidant enzyme activities (GSH-Px, SOD, CAT) and antioxidant GSH level. A significant elevation in TBARS production was observed in the hepatic and pancreatic tissues of rats in DC group when compared with NC group. *C. ferrea* extract treatment of diabetic rats in CF500-DC and CF250-DC groups significantly raised GSH-Px, SOD & CAT enzyme activities and GSH level and inhibited the formation of TBARS as compared to DC group in a dose-dependent manner. Though, such improvement in GSH and TBARS levels did not restore to basal level of NC group, while GSH-Px, SOD and CAT enzyme activities returned to normal basal values in CF500-DC.

3.9. Effect of C. ferrea extract on pancreas and liver histopathological examination

The histological investigation of pancreas showed normal architecture in case of NC, CF500-NC and CF250-NC groups. The endocrine portions of pancreas or islets of Langerhans were present in the pancreatic tissue featured circular shapes with normal cell lining, while the exocrine components that included acini appeared well organized and with normal morphology. The interlobular duct was surrounded with the supporting tissue (Figure 2A–C). The image analyzer results showed that the

Table 5
Effect of *C. ferrea* extract on the activity of liver enzymes and total protein in all groups.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	TP (g/dL)
I) NC	44.00 ± 2.61	44.67 ± 1.93	23.24 ± 1.52	1.57 ± 0.16	5.55 ± 0.05
II) CF500- NC	45.66 ± 1.52[b]	43.92 ± 1.20[b]	25.36 ± 1.54[b]	1.40 ± 0.18[b]	5.70 ± 0.10[b]
III) CF250- NC	46.16 ± 1.82[b]	49.66 ± 1.91[b]	26.86 ± 1.17[b]	1.64 ± 0.05[b]	5.65 ± 0.18[b]
IV) DC	80.50 ± 3.33[a,b]	59.33 ± 1.89[a]	56.77 ± 2.03[a]	3.30 ± 0.38[a]	4.85 ± 0.18[a]
V) CF500- DC	50.16 ± 3.71[b]	46.50 ± 1.43[b]	36.77 ± 2.28[a,b]	2.54 ± 0.22[a,b]	5.73 ± 0.21[b]
Change from DC (%)	37.68%	21.62%	35.22%	23.03%	18.14%
VI) CF250- DC	51.66 ± 4.96[b]	47.83 ± 1.90[b]	37.63 ± 1.86[a,b]	2.57 ± 0.21[a,b]	5.53 ± 0.14[b]
Change from DC (%)	35.82%	19.38%	33.71%	22.12%	14.02%

Data are expressed as mean ± SEM (n = 6). Values with different superscripts down the column are significantly different at $P < 0.05$.
[a] Statistically different from NC group.
[b] Statistically different from DC group.

Table 6
Effect of *C. ferrea* extract on oxidative stress markers of liver and pancreas in all groups.

Groups	GSH-Px (IU/g tissue)	SOD (IU/g tissue)	CAT (IU/mg tissue)	GSH (mg%)	TBARS (nmol/mg tissue)
Liver					
I) NC	74.11 ± 1.54	99.37 ± 2.53	16.12 ± 0.67	12.09 ± 0.91	14.16 ± 0.92
II) CF500- NC	83.10 ± 1.93[b]	116.13 ± 1.67[b]	18.90 ± 0.78[b]	13.24 ± 0.87[b]	13.67 ± 0.74[b]
III) CF250- NC	76.94 ± 1.28[b]	102.53 ± 5.33[b]	17.89 ± 0.57[b]	11.70 ± 1.30[b]	14.78 ± 0.57[b]
IV) DC	59.70 ± 2.74[a]	73.40 ± 3.45[a]	10.72 ± 0.57[a]	1.20 ± 0.30[a]	25.74 ± 0.24[a]
V) CF500- DC	71.63 ± 1.12[b]	93.06 ± 2.93[b]	16.21 ± 0.65[b]	7.68 ± 2.26[a,b]	16.81 ± 0.45[a,b]
Change from DC (%)	19.98%	26.78%	51.21%	540%	34.69%
VI) CF250- DC	67.29 ± 1.13[a,b]	84.40 ± 2.27[a,b]	14.37 ± 0.61[b]	7.11 ± 1.68[a,b]	19.36 ± 0.28[a,b]
Change from DC (%)	12.71%	14.98%	34.04%	492.5%	24.78%
Pancreas					
I) NC	54.74 ± 1.84	67.28 ± 2.94	2.57 ± 0.17	4.29 ± 0.46	6.22 ± 0.47
II) CF500- NC	62.63 ± 1.87[b]	71.70 ± 3.19[b]	3.06 ± 0.22[b]	4.97 ± 0.47[b]	5.52 ± 0.54[b]
III) CF250- NC	58.53 ± 2.25[b]	64.69 ± 1.97[b]	2.89 ± 0.25[b]	4.44 ± 0.23[b]	4.72 ± 0.38[a,b]
IV) DC	41.85 ± 2.36[a]	43.63 ± 2.35[a]	0.68 ± 0.08[a]	0.37 ± 0.02[a]	13.22 ± 0.15[a]
V) CF500- DC	52.72 ± 1.98[b]	62.86 ± 2.54[b]	2.37 ± 0.16[b]	1.71 ± 0.16[a,b]	8.67 ± 0.43[a,b]
Change from DC (%)	25.97%	44.07%	284.52%	362.16%	34.41%
VI) CF250- DC	48.89 ± 2.14[b]	56.26 ± 1.99[a,b]	2.06 ± 0.17[b]	1.32 ± 0.13[a,b]	9.59 ± 0.32[a,b]
Change from DC (%)	16.82%	28.94%	202.94%	256.75%	27.45%

Data are expressed as mean ± SEM (n = 6). Values with different superscripts down the column are significantly different at $P < 0.05$.
[a] Statistically different from NC group.
[b] Statistically different from DC group.

mean islets area in non-diabetic rats was (163.30 ± 5.62) μm^2, whereas for CF500-NC and CF250-NC groups were (191.23 ± 1.05) and (171.17 ± 2.02) μm^2, respectively.

In case of pancreas of diabetic rats, histopathological examination of pancreas showed the acinar cells around the islets though seemed to be in normal proportion did not look classical. The cells of islets were in degenerative form with asymmetrical vacuoles. Intra islets hemorrhage was also seen (Figure 2D and E). A significant reduction in the number of β-cells and size of islet cells was detected. The mean islets area of the diabetic rats was (122.93 ± 15.13) μm^2. These indicate it appeared smaller in comparison with normal rats.

Microscopic investigation of pancreas sections of CF500-DC and CF250-DC groups revealed regeneration and restoration of size of Langerhans' islets along with β–cells repair (Figure 2F and G), suggesting a protective effect on the islets. This recovery of the β-cell was more evident at higher dose, whereas the mean of islets area for CF500-DC and CF250-DC groups were (196.33 ± 14.55) and (192.12 ± 5.22) μm^2, respectively.

The microscopic examinations of sections of liver of NC, CF500-NC and CF250-NC groups showed the normal structure of the hepatic lobule. The central vein is surrounded by the hepatocytes with eosinophilic cytoplasm and distinct nuclei. The hepatic sinusoids were shown between the hepatocytes (Figure 3A–C). Microscopic examination of liver of DC rats indicated congestion in the portal tract that was associated with necrosis of the hepatocytes that surrounded it and moderated inflammatory infiltration. Some of the nuclei of the hepatocytes revealed pyknotic form (Figure 3D). In CF500-DC rats, the hepatic lobule appeared more or less like control. The activated Kupffer cells in the sinusoids were seen (Figure 3E). In some rats congested portal tract associated with necrosis of the

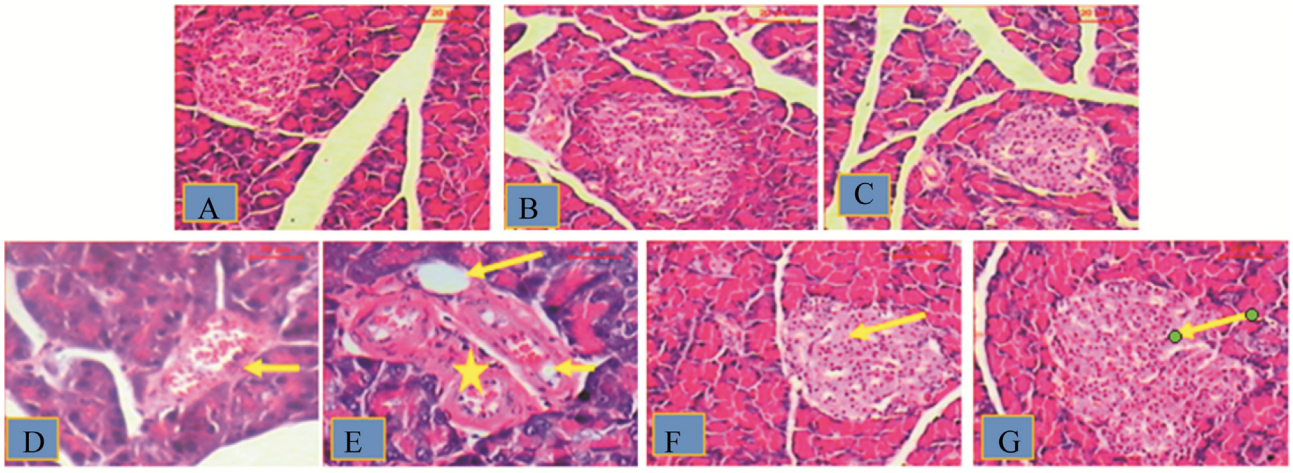

Figure 2. The histological investigation of pancreas. A, B, C: Pancreas sections of NC, CF500-NC and CF250-NC groups respectively, showing dense-staining acinar cells and a light-staining islet of Langerhans just right of the canter of the field; D: Diabetic rat showing the acinar cells around the islets though seemed to be in normal proportion did not look classical. The islet is shrunken and associated with intra islet hemorrhage (arrow). E: Diabetic rat showing degenerative islet of Langerhans (asterisk) associated with different size of vacuoles (long arrow) and hemorrhage (short arrow); F: CF500-DC rat showing the exocrine pancreas appearing more or less as control. Few degenerative cells are seen in the islet. G: CF250-DC rat showing islet of Langerhans that appeared relatively larger than the control one. Exocrine pancreas appeared more or less as control (H & E, Scale bar: 20 μm).

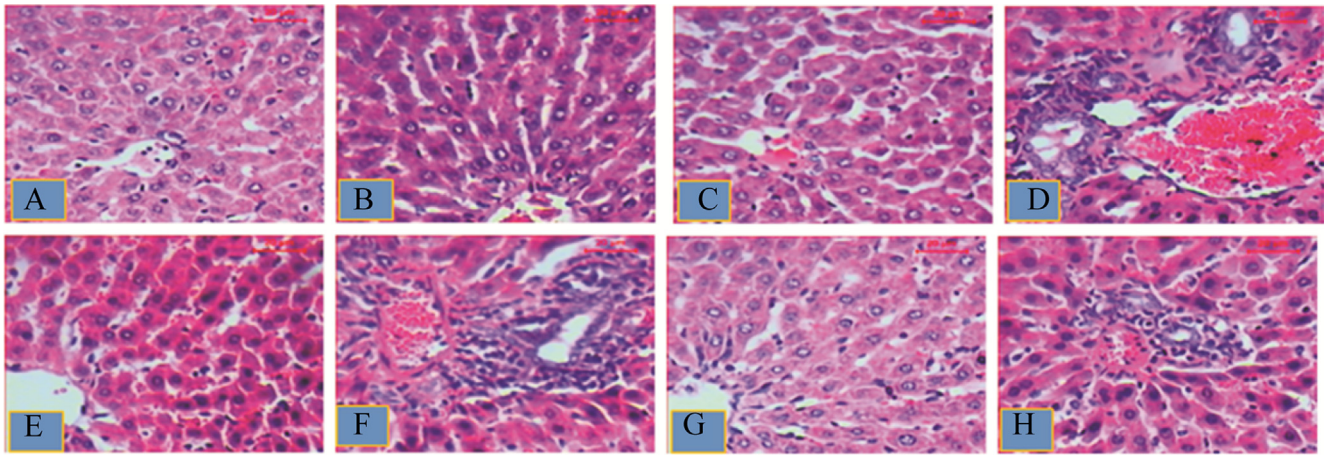

Figure 3. The microscopic examinations of sections of liver of NC, CF500-NC and CF250-NC groups. A, B, C: Liver sections of NC, CF500-NC and CF250-NC groups, respectively, showing the normal architecture of a hepatic lobule and hepatocytes; D: Diabetic group showing congested portal tract that is associated with necrosis of the hepatocytes that surround it and moderate inflammatory infiltration. Some of the nuclei of the hepatocytes are pyknotic. E: CF500-DC rat showing hepatic lobule that appears more or less like control. Notice the activated Kupffer cells. F: CF500-DC rat showing congested portal tract that is associated with necrosis of the hepatocytes that surround it and moderate inflammatory infiltration. G: CF250-DC rat showing the hepatocytes appearing more or less as the control; H: CF250-DC rat showing mild congestion of the portal tract that is associated with few inflammatory infiltrations (H & E, Scale bar: 20 μm).

hepatocytes that surrounded it and moderated inflammatory infiltration were found (Figure 3F). In CF250-DC rats, the liver examination showed that the hepatocytes appeared more or less as the control (Figure 3G), while in some cases mild congestion of the portal tract associated with few inflammatory infiltration was seen (Figure 3H).

4. Discussion

Diabetes mellitus is currently a major public health concern, because its incidence and prevalence are elevated and increasing, reaching epidemic proportions [39]. Cumulative evidence has shown that poorly and erratically controlled hyperglycemia produces abnormally high levels of ROS [40], and these reactive substances could react with essential molecules such as lipids, proteins and DNA, leading to histological changes as well as functional alterations [41]. STZ is a toxin frequently used to induce diabetes in experimental animals through its ability to induce selective destruction of pancreatic beta cells resulting in insulin deficiency and hyperglycemia [42]. To the best of our knowledge, this is the first report that analyzes hypoglycaemic effect of *C. ferrea* leaf aqueous ethanolic extract on STZ induced experimental diabetes.

In the present study, reduction in body weight in diabetic rats was observed which might be the result of degradation of structural proteins due to unavailability of carbohydrates for utilization as an energy source [43,44]. These results agree with previous observations that have also reported loss of body weight [45,46]. A significant increase was observed in body weight of diabetic rats treated with *C. ferrea* extract (CF500-DC group) as compared to diabetic group which indicates the preventive effect of the extract on degradation of structural proteins.

The diabetic rats were found to have higher glucose level and lower level of insulin when compared to normal control rats. From the results of the present experiment, it was observed that treatment with *C. ferrea* extract decreased the serum glucose and increased serum insulin in STZ induced diabetic rats (CF-DC groups). It is perhaps due to stimulation of insulin secretion from remnant pancreatic β-cells, which in turn enhances glucose utilization by peripheral tissues of diabetic rats either by promoting glucose uptake and metabolism, or by inhibiting hepatic gluconeogenesis [47]. This is confirmed by histopathological observations which show that the structural integrity of islets of Langerhans was restored towards normalization.

Alpha-amylase is one of the main enzymes in human body that is responsible for the breakdown of starch to more simple sugars. α-amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharides which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels [48]. In our investigation, a significant increase in α-amylase was observed in diabetic rats as compared to control. This result is in agreement with Adaramoye [49]. Treatment with *C. ferrea* extract in CF500-DC and CF250-DC groups moderately inhibited α-amylase. In our phytochemical screening, we have proved the presence of phenolic compounds in *C. ferrea* extract. Some phenolic compounds are known to inhibit the activity of carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase [50].

In diabetes, hyperglycemia is accompanied with dyslipidemia representing risk factor for coronary heart diseases. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyzes TGs. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia, and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [51]. TGs stimulate the secretion of very low-density lipoprotein cholesterol and such increase in very low-density lipoprotein cholesterol particles reduces the HDL-C level and increases the LDL-C particles [52]. The characteristic features of diabetic dyslipidemia are increase in serum TG, TC,

LDL-C, and fall in HDL-C levels [53]. In our study, the altered serum lipid profile was found in diabetic rats. This finding is in correlation with the findings of Pepato *et al*. and Sharma *et al*. [54,55]. This altered serum lipid profile was reversed towards normal after administration of *C. ferrea* extract with both doses. Thus, the extract could be helpful in improving lipid metabolism which will in turn help to prevent diabetic complications such as coronary heart diseases and atherosclerosis.

It has been well established that elevated levels of AST, ALT and ALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage [56]. In the present study, the injection of STZ induces hepatocellular damage, which is one of the characteristic changes in diabetes as evidenced by high serum levels of AST, ALT, ALP and GGT in diabetic group compared to the normal control, suggesting possible damage to the liver. Liver damage in diabetic rats was confirmed. However, diabetic groups treated with *C. ferrea* extract in CF500-DC and CF250-DC groups showed a significant reduction in the levels of these enzymes when compared to the diabetic untreated control, which consequently alleviated the damage caused by STZ as confirmed by hepatocytes morphology. This means that *C. ferrea* has some hepatoprotective potentials in diabetic rats by decreasing serum AST, ALT, ALP and GGT levels. Treatment of normal rats with *C. ferrea* in CF500-NC and CF250-NC groups maintained the levels of serum AST, ALT, ALP and GGT thereby showing its non-toxic nature.

Under condition of severe oxidative stress, free radical generation leads to protein modification. Proteins may be damaged directly by specific interactions of free radicals with particular susceptible amino acids [57]. The finding of our study revealed a significant decrease in the level of serum TP in diabetic rats. This could be due to increased peroxidation. On the other hand, *C. ferrea* treated rats showed increased level of TP, suggesting that *C. ferrea* extract has antioxidant capacity.

Oxidative stress is suggested as mechanism underlying diabetes and diabetic complications, which results from an imbalance between radical generating and radical scavenging systems [6]. Antioxidant enzymes as well as nonenzymatic antioxidants are first line of defense against ROS induced oxidative damage in a living organism [58]. SOD, CAT and GSH-Px are the three major scavenging enzymes that remove the toxic free radicals *in vivo* [7]. 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. CAT is a haemprotein, which is responsible for the detoxification of significant amounts of H_2O_2 [59]. GSH-Px plays a central role in the catabolism of H_2O_2 and the detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH [60]. Glutathione functions as a free radical scavenger and is an essential co-substrate for GSH-Px [61].

The decreased activity of antioxidant (GSH-Px, SOD and CAT) enzymes along with decreased GSH level was found in the liver and pancreatic tissues of diabetic rats. These results are in agreement with Cheng *et al*. [62]. It was suggested that decreased antioxidant enzyme activity in DC group could be due to glycation of these enzymes, which occurred at persistently elevated blood glucose levels [63]. However, administration of *C. ferrea* extract in CF500-DC, CF250-DC groups increased the GSH-Px, SOD and CAT activities and GSH level in the liver and pancreas of diabetic rats.

Lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals [64]. In the present study, it was observed that TBARS level in liver and pancreas of STZ-induced diabetes was significantly increased when compared to the control. 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 [7]. Diabetic rats treated with *C. ferrea* extract in CF500-DC, CF250-DC groups showed decreased level of TBARS.

Robertson *et al*. demonstrated that antioxidants have been shown to break the worsening of diabetes by improving β-cells function in animal models and suggested that enhancing antioxidant defense mechanisms in pancreatic islets may be a valuable pharmacologic approach to managing diabetes [65].

In the present study, the biochemical findings observed in diabetic rats are in conformity with histopathological alterations of β-cells of pancreas and hepatocytes. Such histopathological alterations were reduced by administration of *C. ferrea* extract at both doses.

It can be concluded that the aqueous ethanolic extract of *C. ferrea* leaf has potential antihyperglycemic activity in STZ induced diabetic rats. In this sense, the antidiabetic effect may be due to the presence of secondary metabolites like phenols and flavonoids in the *C. ferrea* leaf extract which are responsible for antioxidant actions and have been found to be beneficial in controlling diabetes as evident from earlier studies. The three new phenolic compounds (isovitexin, vitexin and orientin derivatives) isolated in a previous study showed high antioxidant properties (results not shown) and may contribute to the major antioxidant activity of the *C. ferrea* leaf extract [28]. Experimental evidence obtained from this study is encouraging enough to warrant further studies on the leaf extract of this plant to find out its mechanism of action and to establish its therapeutic potential in the prophylaxis and/or treatment of diabetes and diabetic complications.

Conflict of interest statement

We declare that we have no conflict of interest.

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