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Antioxidant and Antihyperglycemic Effects of *Ferula drudeana* and *Ferula huber-morathii* in Experimental Diabetic Rats

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ABSTRACT

In the present study, DPPH radical scavenging assay and ferric-reducing antioxidant assay were used to estimate the potential *in vitro* antioxidant effect of the methanol extracts of *Ferula drudeana* Korovin (*F. drudeana*) and *Ferula huber-morathii* Peşmen (*F. huber-morathii*). The antidiabetic activity of both extracts was evaluated in streptozotocin (STZ)-induced diabetic rats. Glibenclamide was taken as the standard drug. Both extracts showed considerable antioxidant potential in the DPPH radical scavenging assay and minimum reducing power in ferric-reducing antioxidant assay. Oral administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts to diabetic rats produced a marked reduction in Fasting Blood Glucose (FBG) and elevation in insulin levels after 14 and 28 days of treatment. A meaningful reduction in the concentrations of glycosylated hemoglobin (HbA1c), triglycerides (TG), Total Cholesterol (TC), Low Density Lipoprotein (LDL) in plasma and elevations in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione (GSH) in liver and pancreas homogenates were observed in diabetic animals medicated with *F. drudeana* (200 and 400 mg kg^{-1}) and *F. huber-morathii* (400 mg kg^{-1}) extracts. Levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, total protein and High Density Lipoprotein (HDL) in plasma and malondialdehyde (MDA) in liver and pancreas homogenates were recovered significantly in *F. drudeana* and *F. huber-morathii*-medicated diabetic rats. Besides, biochemical results were supported by histopathological findings. These findings showed the significant antioxidant and hypoglycemic activities of *F. drudeana* and *F. huber-morathii* extracts in diabetic rats.

Key words: Apiaceae, diabetes, streptozotocin, DPPH, insulin, blood glucose

INTRODUCTION

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia associated with absolute or relative deficiency in insulin secretion or insulin action (Saravanakumar *et al*., 2009). As a very common chronic disease, diabetes is becoming the third "killer" of the health of people along with cancer and cardiovascular diseases. There are two types of diabetes, namely type 1 and 2. In type 1 (insulin-dependent), the body does not produce any insulin. Type 1 diabetes accounts for 5-10% of diabetes. In type 2 (noninsulin-dependent), the body does not produce enough insulin. It is the most common form of the disease, accounting for 90-95% of diabetes. Drugs that are used to control diabetes can be mainly divided into insulin, insulin-secretagogues, insulin sensitivity improvement factor, aldose reductase inhibitor, α-glucosidase inhibitors and protein glycation inhibitor (Li *et al*., 2004).

Unfortunately, apart from having a number of side effects, none of the synthetic antidiabetic agents have been successful in controlling blood glucose levels (Belhekar *et al*., 2013). Therefore, the search for new antidiabetic agents with more effectiveness and lesser adverse effects has continued. More than 400 plant species having antihyperglycemic effect have been mentioned in the literature (Pattanayak *et al*., 2009). Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world (Hussain, 2002).

The genus *Ferula* L. (Apiaceae) contains about 180-185 species with the most diversity found in Central and Southwest Asia. The two species in Apiaceae, *F. drudeana* and *F. huber-morathii* were described and illustrated from central and East Anatolia, Turkey as endemic species and up till now, no biological data or investigations yet done on these herbs. Since, *Ferula* species are traditionally claimed for their anti-diabetic effects; hence, the present study was committed to investigate the potential antidiabetic activity of both *F. drudeana* and *F. huber-morathii* in STZ-diabetic rats.

MATERIALS AND METHODS

Plant material: Fresh roots of *F. drudeana* and *F. huber-morathii* were collected in summer 2013 from different locations. *Ferula drudeana* was collected from Kayseri, Yahyali, Çamlica kőyü, 1529 m, *F. huber-morathii* was collected from Muş-Varto road 40 km, 1280 m. Taxonomic identification was determined by Assistant Professor Dr. Şüra BAYKAN EREL and a voucher specimen from the plants (5520), (5524) were deposited at the Herbarium of Faculty of Pharmacy (IZEF), Ege University, Izmir, Turkey.

Preparation of the extracts: The collected plants *F. drudeana* and *F. huber-morathii* were shade dried and then grinded to fine powders. About 2360 and 870 g of the dried powders of each plant were extracted by percolation in methanol with occasional shaking for 48 h. Percolation was repeated three times and then the methanolic extracts of each plant was combined and concentrated under vacuum to give the total extract of 374.82 and 121.9 g, respectively.

Phytochemical screening: Preliminary phytochemical tests were carried out to identify the chemical constituents of the methanol extracts of *F. drudeana* and *F. huber-morathii* using standard procedures as described by Trease and Evans (1989).

***In vitro* anti-oxidant activity**

Antioxidant activity using DPPH radical scavenging assay: The method was carried out as described by Brand-Williams *et al*. (1995). Various concentrations of *F. drudeana* and *F. huber-morathii* extracts were used. The assay mixtures contained in total volume of 1 mL composed of 500 µL of each extract, 125 µL prepared DPPH and 375 µL solvent. Ascorbic acid used as, the positive control. After 30 min incubation at 25°C, the decrease in absorbance was measured at 517 nm on a UV visible light spectrophotometer. The radical scavenging activity was calculated from the equation:

$$\text{Radical scavenging acitivity (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Ferric-reducing antioxidant power assay: Reducing capacity was performed by using potassium ferricyanide ferric chloride system. One milliliter of the extract (0.2, 0.5, 0.8 and 1.0 mg mL^{-1}) was added to phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The content was heated at 50°C for 20 min then cooled. The 2.5 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3,000 rpm for 10 min. The 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%) and the absorbance was measured at 700 nm against a blank. Increasing absorbance of the reaction mixture indicates increasing reducing power. All the tests were performed in triplicate according to Rosalind *et al*. (2013).

Animals: Adult male albino rats weighing 160-175 g were used. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA. All animals were housed under standard conditions of natural 12 h light and dark cycle with free access to food and water and were allowed to adapt to the laboratory environment for one week before experimentation.

Acute toxicity study: Acute toxicity study for the methanol extracts of *F. drudeana* and *F. huber-morathii* was carried in adult male albino rats according to OECD-423 guidelines (OECD., 2001). Two groups of rats (n = 6) were fasted

overnight then received *F. drudeana* and *F. huber-morathii* extracts, respectively at a dose of 2000 mg kg^{-1} by the oral route. The control rats treated with the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions. Each animal was observed for symptoms of toxicity and/or mortalities for every 15 min in the first 4 h after medication, then every 30 min for the successive 6 h and then for 48 h. Since, there was no mortality at this level; the dose of both extracts was increased to 4000 mg kg^{-1} and animals were observed for another 48 h.

STZ-induced hyperglycemia: Hyperglycemia was induced in adult male albino rats by intraperitoneal injection of STZ (Sigma, Germany) dissolved in 0.1 M citrate buffer, pH 4.5, at 45 mg kg^{-1}. Seventy two hours later, blood samples were withdrawn from the tail vein. Rats with FBG levels $\geq$250 mg dL^{-1} were considered diabetic and used for further experimentation.

Experimental design: Hyperglycemic rats were randomly allocated into 7 groups (n = 6). The vehicle (5 mL kg^{-1}) was administered to the 1st (normal control) and 2nd (diabetic control) groups, Diabetic rats of the 3rd and 4th groups received *F. drudeana* extract at doses of 200 and 400 mg kg^{-1}, respectively. Diabetic rats of the 5th and 6th groups received *F. huber-morathii* extract at doses of 200 and 400 mg kg^{-1}, respectively. The 7th group (reference group) was diabetic rats given glibenclamide at a dose of 0.6 mg kg^{-1}. Vehicle, extracts and glibenclamide were given orally by gavage as single daily treatments, for 28 days.

Biochemical evaluation: Blood samples were withdrawn through the retro-orbital venous plexus under light ether anesthesia from the overnight fasted animals into sampling tubes containing sodium fluoride at days 14 and 28 post-medication. Blood samples were centrifuged at 3500 rpm for 15 min to separate plasma. The FBG and insulin levels were measured in plasma according to Trinder (1969) and Anderson *et al.* (1993), respectively.

At the end of the 28th day, two blood samples were withdrawn from the overnight fasted animals into heparinized tubes. The first blood sample was used for estimation of total Hb (Drabkin and Austin, 1932). The second sample was centrifuged at 3500 rpm for 15 min to separate plasma. Plasma levels of HbA1c (Nayak and Pattabiraman, 1981), TG (Foster and Dunn, 1973), TC (Zlatkis *et al*., 1953), HDL-C (Burstein *et al*., 1970) and LDL-C (Friedewald *et al*., 1972) were evaluated. The activities of ALT, AST and ALP and levels of bilirubin and total protein in plasma were estimated according to the instructor manual of commercially available kits. The SOD, GPx, CAT and GSH activities in hepatic and pancreatic tissues were estimated (Sun and Zigman, 1978; Mohandas *et al*., 1984; Chance and Maehley, 1955; Beutler *et al*., 1963, respectively). Lipid peroxidation products were estimated by determining MDA content in the hepatic and pancreatic tissue according to the procedure of Jain *et al*. (1990).

Histopathological study: Animals were euthanized; liver, kidneys, spleen and pancreas were collected and kept in 10% neutral buffered formalin for 48 h, then embedded within paraffin. Sections of 4 μm thickness were prepared using, a rotary microtome. The sections stained with haematoxylin and eosin (H and E) and then observed by light microscopy for histopathological examination.

Statistical analysis: Results are expressed as Mean±Standard Error (SE) of mean. Statistical analysis was performed, using a one-way analysis of variance (ANOVA). When, the F-value was found statistically significant ($p<0.05$), further comparisons among groups were made using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS software 17.0 (Released Aug. 23, 2008), Chicago, USA.

RESULTS

Phytochemical screening: Phytochemical screening of *F. drudeana* and *F. huber-morathii* (Table 1) indicated the presence of alkaloids, carbohydrates, glycosides, saponins, steroids, triterpenoids, phenolic, flavonoids, proteins and amino acids.

In vitro anti-oxidant activity

DPPH radical scavenging assay: The DPPH radical scavenging activity was recorded in terms of % inhibition as shown in Fig. 1a, *F. drudeana* and *F. huber-morathii* showed considerable antioxidant potential. Ascorbic acid revealed higher % inhibition indicating better scavenging activity or antioxidant potential.

Ferula drudeana at concentrations of 10, 50, 100, 500 and 1000 μg mL^{-1} showed scavenging activities of 1.4, 4.7, 7.1, 30.1 and 50.2%, respectively while those of *F. huber-morathii* were 7.5, 12.1, 19.9, 56.3 and 78.3%, respectively. The EC_{50} values for *F. drudeana* and *F. huber-morathii* were calculated to be 1019 and 353.8 μg mL^{-1}, respectively.

Ferric-reducing antioxidant power assay: As observed in Fig. 1b, *F. drudeana* and *F. huber-morathii* have minimum reducing power of 0.47 and 0.80%, respectively at concentration of 2000 μg mL^{-1}. While, ascorbic acid showed higher absorbance indicating more reducing power (2.34%) at concentration of 2000 μg mL^{-1}.

Acute toxicity test: Oral administration of *F. drudeana* and *F. huber-morathii* extracts did not cause death in the highest dose of 4 g kg^{-1} b.wt. No visible signs of toxicity were reported in the rats exposed to different doses of both

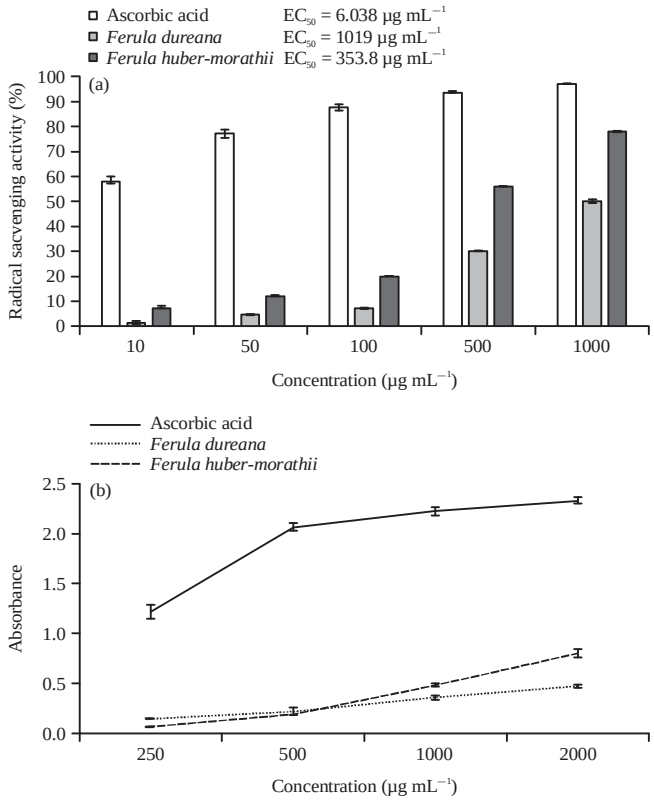

Fig. 1(a-b): Effect of *Ferula drudeana* and *Ferula huber-morathii* extracts on *in vitro* anti-oxidant activity, (a) Antioxidant activity using DPPH radical Scavenging assay and (b) Ferric-reducing antioxidant power assay

Table 1: Preliminary phytochemical screening of *Ferula drudeana* and *Ferula huber-morathii* extracts

Phyto-constituent	Test	*F. drudeana*	*F. huber-morathii*
Alkaloids	Mayer's test	+	+
	Dragendroff's test	+	+
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
Glycosides	Modified borntrager's test	+	+
	Modified fehling's test	+	+
Saponins	Froth test	+	+
Steroids and triterpenoids	Salkowski's test	+	+
Phenols and tannins	Ferric chloride test	+	+
Flavonoids	Alkaline reagent test	+	+
	Lead acetate test	+	+
Proteins and Amino acids	Ninhydrin test	+	+

extracts indicating their safety. Accordingly, the oral LD_{50} of *F. drudeana* and *F. huber-morathii* extracts was determined to be higher than 4 g kg^{-1} b.wt. which is the highest tested dose.

Effect on body weight, FBG and plasma insulin levels: Diabetic animals exhibited a significant increase in FBG at days 14 and 28 of treatment (325.5 and 321.6 mg dL^{-1}, respectively) as compared to normal control rats (106.4 and 110.7 mg dL^{-1}, respectively). The elevated FBG of diabetic rats showed a tendency toward normal levels after dosing of *F. drudeana* extract (400 mg kg^{-1}), *F. huber-morathii* extract (200 and 400 mg kg^{-1}) and glibenclamide (0.6 mg kg^{-1}) at days 14 and 28 of treatment (Table 2). Plasma insulin contents were decreased significantly in untreated diabetic animals at days 14 and 28 of treatment (8.6 and 8.8 U L^{-1}, respectively) compared with normal controls (19.2 and 19.3 U L^{-1}, respectively). Medication of diabetic animals with *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts increased the plasma insulin levels at days 14 (12.4, 13.5 and 14.1 U L^{-1}, respectively) and 28 (12.9, 13.7 and 15.5 U L^{-1}, respectively) of treatment compared with diabetic control animals. In addition, glibenclamide administration results in the restoration of plasma insulin to control levels. After 28 days of STZ-induced diabetes, body weight gain of diabetic control animals was greatly reduced compared with normal control rats. Diabetic rats treated with

Table 2: Levels of glucose and insulin in plasma of control and experimental rats after 14 and 28 days of treatment with *Ferula drudeana* and *Ferula huber-morathii* extracts

	Fasting insulin (U L^{-1})		FBG (mg dL^{-1})	
Groups	14 days	28 days	14 days	28 days
Normal control	106.4±2.33#	110.7±2.52#	19.2±0.19#	19.3±0.12#
DC	325.5±3.38*	321.6±4.17*	8.6±0.16*	8.8±0.14*
DC+*F. drudeana* (200 mg kg^{-1})	331.8±3.67*	316.5±3.63*	9.2±0.15*	9.3±0.16*
DC+*F. drudeana* (400 mg kg^{-1})	236.8±3.70*#	229.0±3.55*#	12.4±0.11*#	12.9±0.13*#
DC+*F. huber-morathii* (200 mg kg^{-1})	201.0±3.30*#	197.5±2.63*#	13.5±0.14*#	13.7±0.13*#
DC+*F. huber-morathii* (400 mg kg^{-1})	186.5±2.77*#	177.1±2.19*#	14.1±0.12*#	15.5±0.10*#
DC+Glibenclamide	168.6±2.57*#	161.5±2.25*#	17.5±0.15*#	17.7±0.14*#

Values are Mean±SE of six rats for each group. *Significantly different from the values of the normal control rats at p<0.05, #Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, FBG: Fasting blood glucose

Table 3: Levels of total hemoglobin and HbA1c of control and experimental rats after 28 days of treatment with *Ferula drudeana* and *Ferula huber-morathii* extracts

Groups	Total hemoglobin (mg dL^{-1})	HbA1c (Hb%)
Normal control	14.1±0.32#	3.6±0.10#
DC	10.3±0.28*	9.8±0.16*
DC+*F. drudeana* (200 mg kg^{-1})	10.9±0.28*	9.3±0.19*
DC+*F. drudeana* (400 mg kg^{-1})	12.2±0.31*#	7.4±0.12*#
DC+*F. huber-morathii* (200 mg kg^{-1})	12.4±0.33*#	5.7±0.12*#
DC+*F. huber-morathii* (400 mg kg^{-1})	12.6±0.35*#	5.0±0.10*#
DC+*Glibenclamide*	13.5±0.37*#	4.2±0.11*#

Values are Mean±SE of six rats for each group, *Significantly different from the values of the normal control rats at p<0.05, #Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, HbAlc: Glycosylated hemoglobin

Table 4: Levels of TG, TC, HDL-C and LDL-C in plasma of control and experimental rats after 28 days of treatment with *Ferula drudeana* and *Ferula huber-morathii* extracts

Groups	TG (mg dL^{-1})	TC (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})
Normal control	45.8±2.16#	54.9±2.51#	28.6±0.94#	17.00±0.32#
DC	88.6±2.38*	76.6±3.83*	19.2±0.25*	34.4±0.53*
DC+*F. drudeana* (200 mg kg^{-1})	84.7±3.61*	73.0±3.14*	20.5±0.77*	31.6±0.93*
DC+*F. drudeana* (400 mg kg^{-1})	67.5±3.22*#	65.4±2.88*#	22.8±0.79*#	29.4±0.85*#
DC+*F. huber-morathii* (200 mg kg^{-1})	63.3±2.37*#	63.3±2.75*#	25.5±0.80*#	23.7±0.69*#
DC+*F. huber-morathii* (400 mg kg^{-1})	58.5±2.55*#	59.2±2.26*#	26.7±0.84*#	21.8±0.73*#
DC+Glibenclamide	82.5±3.59*	60.6±2.53*#	27.1±0.94*#	19.4±0.72*#

Values are Mean±SE of six rats for each group, *Significantly different from the values of the normal control rats at p<0.05, #Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, TG: Total glyceride, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

F. drudeana (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts showed an amelioration in body weight in comparison to the diabetic control group (data not shown).

Effect on total hemoglobin and HbA1c: The contents of total hemoglobin and HbA1c in normal and diabetic rats are shown in Table 3. Control diabetic animals showed a considerable reduction in the level of total hemoglobin (10.3 mg dL^{-1}) and a significant elevation in HbA1c content (9.8% Hb) in comparison with normal controls (14.1 mg dL^{-1} and 3.6% Hb, respectively). Administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts to diabetic rats revert back the levels of total hemoglobin and HbA1c to normal conditions (Table 3). Similarly, total hemoglobin and HbA1c contents were restored toward their normal values by the administration of the standard drug glibenclamide.

Effect on plasma lipid profile: The concentrations of lipoproteins in plasma of normal control and diabetic groups are illustrated in Table 4. Diabetic animals had increased contents of plasma TG, TC and LDL-C and decreased level of HDL-C compared to normal control group. Oral administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts reversed the changes in plasma lipoproteins of diabetic rats and significantly improved their values towards near normal levels. Glibenclamide did not significantly change the blood level of TG when compared to diabetic controls.

Effect on markers of liver injury: The plasma levels of markers of liver injury (ALT, AST, ALP and bilirubin) were significantly increased in diabetic control rats in comparison with non-diabetic controls. After administration of *F. drudeana* extract (400 mg kg^{-1}), *F. huber-morathii* extract (200 and 400 mg kg^{-1}) and glibenclamide (0.6 mg kg^{-1}), the enzyme activities and bilirubin levels were brought back toward the typical levels (Table 5). The levels of plasma protein in normal control and diabetic rats were shown in Table 5. In diabetic rats, there was a meaningful reduction in

Table 5: Activities of ALT, AST and ALP and levels of bilirubin and total protein in plasma of control and experimental rats after 28 days of treatment with *Ferula drudeana* and *Ferula huber-morathii* extracts

Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Bilirubin (mg dL^{-1})	Total protein (g dL^{-1})
Normal control	44.7±1.47$^{\#}$	74.9±2.62$^{\#}$	83.7±3.63$^{\#}$	0.55±0.04$^{\#}$	8.2±0.18$^{\#}$
DC	127.3±2.38*	165.8±4.85*	172.3±4.25*	1.43±0.09*	4.7±0.11*
DC+*F. drudeana* (200 mg kg^{-1})	118.7±3.73*	158.3±4.15*	167.2±4.78*	1.35 ±0.06*	4.9±0.12*
DC+*F. drudeana* (400 mg kg^{-1})	110.4±3.51*$^{\#}$	155.0±4.53*$^{\#}$	162.3±4.55*$^{\#}$	1.17±0.04*$^{\#}$	5.3±0.15*$^{\#}$
DC+*F. huber-morathii* (200 mg kg^{-1})	84.3±2.83*$^{\#}$	110.2±3.68*$^{\#}$	126.4±3.51*$^{\#}$	0.80 ±0.03*$^{\#}$	6.5±0.12*$^{\#}$
DC+*F. huber-morathii* (400 mg kg^{-1})	80.3±2.87*$^{\#}$	96.6±3.27*$^{\#}$	106.7±3.55*$^{\#}$	0.76±0.04*$^{\#}$	6.9±0.11*$^{\#}$
DC+Glibenclamide	62.5±1.73*$^{\#}$	88.3±2.75*$^{\#}$	105.4±3.75*$^{\#}$	0.63±0.03*$^{\#}$	7.6±0.14*$^{\#}$

Values are Mean±SE of six rats for each group, *Significantly different from the values of the normal control rats at p<0.05, $^{\#}$Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

Table 6: Activities of SOD, GPx and CAT and levels of GSH and MDA in liver homogenate of control and experimental rats after 28 days of treatment with *Ferula drudeana* and *Ferula huber-morathii* extracts

Groups	SOD (U mg^{-1} protein)	Gpx (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH (µmol g^{-1} tissue)	MDA (nmol g^{-1} tissue)
Normal control	51.6±1.84$^{\#}$	3.5±0.12$^{\#}$	15.3±0.22$^{\#}$	10.5±0.17$^{\#}$	47.8±1.16$^{\#}$
DC	30.3±1.66*	1.3±0.10*	8.9±0.18*	6.0±0.13*	78.4±2.11*
DC+*F. drudeana* (200 mg kg^{-1})	32.6±1.54*	1.5±0.10*	9.2±0.14*	6.5±0.16*	72.3±1.73*
DC+*F. drudeana* (400 mg kg^{-1})	35.0±1.39*$^{\#}$	1.6±0.10*$^{\#}$	9.8±0.13*$^{\#}$	8.0±0.13*$^{\#}$	70.6±1.27*$^{\#}$
DC+*F. huber-morathii* (200 mg kg^{-1})	39.2±1.85*$^{\#}$	2.5±0.14*$^{\#}$	11.4±0.18*$^{\#}$	8.6±0.16*$^{\#}$	62.7±2.75*$^{\#}$
DC+*F. huber-morathii* (400 mg kg^{-1})	40.9±1.36*$^{\#}$	2.9±0.15*$^{\#}$	12.8±0.19*$^{\#}$	8.9±0.18*$^{\#}$	59.5±2.44*$^{\#}$
DC+Glibenclamide	46.8±1.67*$^{\#}$	3.1±0.11*$^{\#}$	13.6±0.15*$^{\#}$	9.8±0.11*$^{\#}$	51.5±1.66*$^{\#}$

Values are Mean±SE of six rats for each group, *Significantly different from the values of the normal control rats at p<0.05, $^{\#}$Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, SOD: Superoxide dismutase, Gpx: Glutathione peroxidase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde

Table 7: Activities of SOD, GPx and CAT and levels of GSH and MDA in pancreatic homogenate of control and experimental rats after 28 days of treatment with *F. drudeana* and *F. huber-morathii* extracts

Groups	SOD (U mg^{-1} protein)	Gpx (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH (µmol g^{-1} tissue)	MDA (nmol g^{-1} tissue)
Normal control	38.7±1.75$^{\#}$	3.7±0.15$^{\#}$	17.8±0.36$^{\#}$	9.9±0.29$^{\#}$	36.5±1.16$^{\#}$
DC	22.1±1.34*	1.8±0.11*	10.3±0.17*	5.7±0.25*	57.8±2.25*
DC+*F. drudeana* (200 mg kg^{-1})	24.7±1.22*	1.9±0.11*	11.1±0.35*	6.0±0.15*	54.2±1.26*
DC+*F. drudeana* (400 mg kg^{-1})	24.9±1.75*$^{\#}$	2.2±0.10*$^{\#}$	11.4±0.22*$^{\#}$	6.2±0.19*$^{\#}$	53.8±1.50*$^{\#}$
DC+*F. huber-morathii* (200 mg kg^{-1})	29.5±1.72*$^{\#}$	2.4±0.16*$^{\#}$	14.5±0.11*$^{\#}$	7.8±0.17*$^{\#}$	45.2±2.59*$^{\#}$
DC+*F. hubermorathi* (400 mg kg^{-1})	32.6±1.27*$^{\#}$	2.9±0.17*$^{\#}$	15.3±0.26*$^{\#}$	8.1±0.24*$^{\#}$	42.5±2.62*$^{\#}$
DC+Glibenclamide	34.4±1.38*$^{\#}$	3.2±0.13*$^{\#}$	16.5±0.29*$^{\#}$	8.8±0.20*$^{\#}$	39.2±1.42*$^{\#}$

Values are Mean±SE of six rats for each group, *Significantly different from the values of the normal control rats at p<0.05, $^{\#}$Significantly different from the values of the diabetic control rats at p<0.05, DC: Diabetic control, SOD: Superoxide dismutase, Gpx: Glutathione peroxidase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde

the protein level when compared with normal group. When *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts were dosed to diabetic animals for 28 days, the total protein levels reverted to near normal.

Effect on oxidative stress markers in hepatic and pancreatic tissues: Tables 6 and 7 clearly illustrate the effect of *F. drudeana* and *F. huber-morathii* extracts on the activities of antioxidant enzymes and levels of GSH and MDA in the hepatic and pancreatic homogenates of STZ-diabetic rats. A significant decrease was observed in the antioxidant activity of SOD, GPx and CAT and level of GSH in the liver and pancreas homogenates of diabetic animals along with elevation in the level of MDA. Administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts to diabetic rats for 28 days significantly increased SOD, GPx and CAT activities and GSH levels in the hepatic and pancreatic homogenates. The increased concentration of MDA was reversed following, *F. drudeana* and *F. huber-morathii* medication.

Histopathological findings: The liver of non-diabetic rats showed the normal histological structure of hepatic lobule (Fig. 2a). The injury to the hepatocytes including focal hepatic necrosis associated with inflammatory cells infiltration and apoptosis of hepatocytes can be observed in the liver of diabetic control animals (Fig. 2b). Livers of *F. drudeana* treated rats (200 mg kg^{-1}) showed focal hepatic necrosis associated with inflammatory cells infiltration and apoptosis of hepatocytes (Fig. 2c), while those medicated with 400 mg kg^{-1} showed congestion of hepatoportal blood vessel and cholangitis (Fig. 2d). The harm to the hepatocytes was reversed in *F. huber-morathii* treated groups. Livers of *F. huber-morathii* treated rats (200 mg kg^{-1}) showed Kupffer

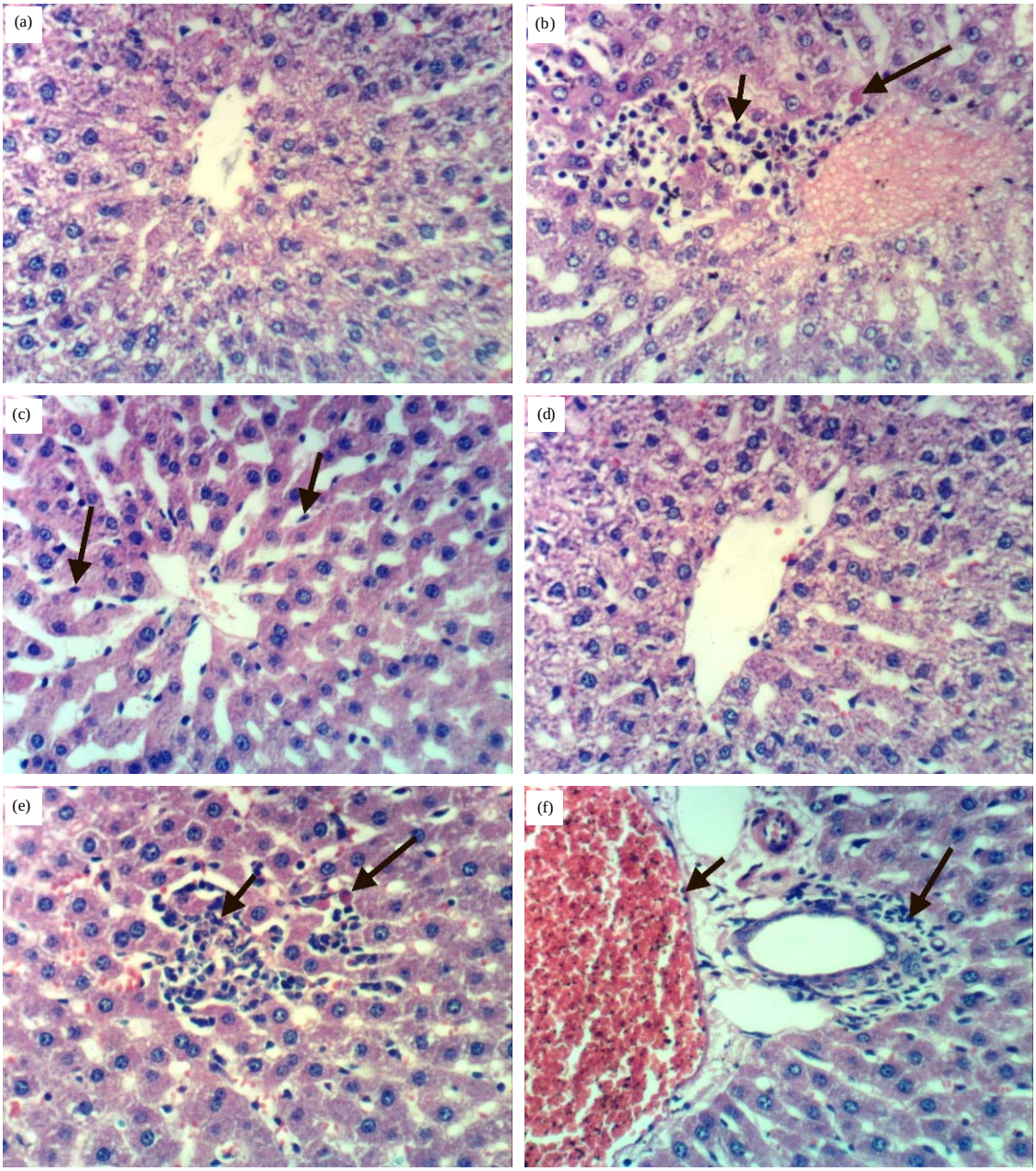

Fig. 2(a-f): Liver of rat from, (a) Normal control group showing the normal histological structure of hepatic lobule (H and E X400), (b) Diabetic control group showing focal hepatic necrosis associated with inflammatory cells infiltration and apoptosis of hepatocytes (H and E X400), (c) *Ferula drudeana* 200 mg kg^{-1} showing focal hepatic necrosis associated with inflammatory cells infiltration and apoptosis of hepatocytes (H and E X400), (d) *Ferula drudeana* 400 mg kg^{-1} showing congestion of hepatoportal blood vessel and cholangitis (H and E X400), (e) *Ferula huber-morathii* 200 mg kg^{-1} showing Kupffer cells activation (H and E X400), (f) *Ferula huber-morathii* 400 mg kg^{-1} showing no histopathological changes (H and E X400)

cells activation (Fig. 2e), while those received 400 mg kg^{-1} showed no histopathological changes (Fig. 2f). Non-diabetic rats showed no histopathological changes of pancreas (Fig. 3a). Pancreatic sections of the diabetic rats revealed vacuolations and necrosis of cells of islet's of Langerhan's (Fig. 3b). Pancreas of *F. drudeana* treated rats (200 mg kg^{-1}) showed vacuolation of cells of islet's of Langerhan's (Fig. 3c), while those medicated with 400 mg kg^{-1} showed necrosis of some cells of islet's of Langerhan's (Fig. 3d). Oral treatments of diabetic rats with *F. huber-morathii* extract (200 and 400 mg kg^{-1}) showed no histopathological changes (Fig. 3e-f, respectively).

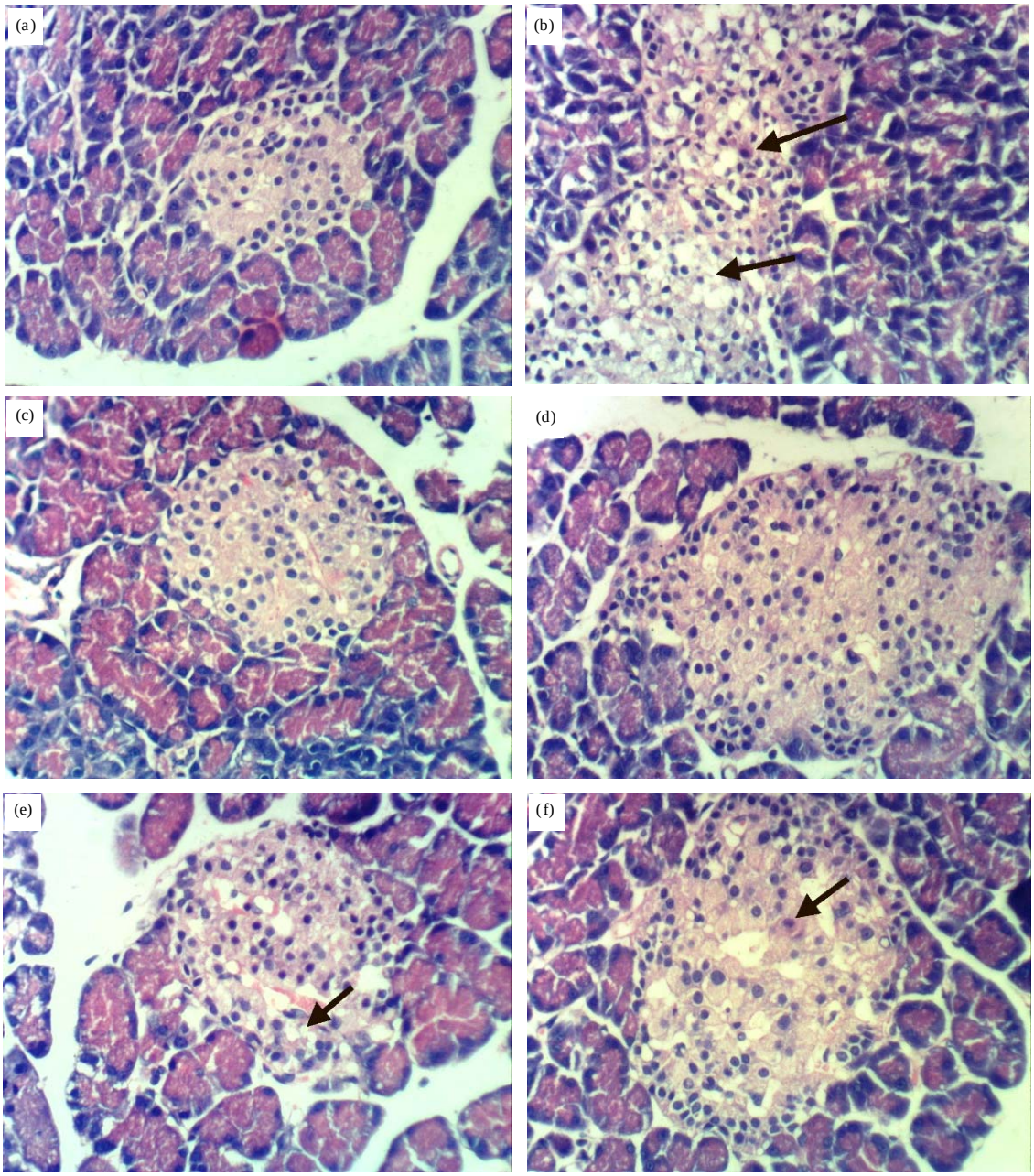

Fig. 3(a-f): Pancreas of rat from (a) Normal control group showing no histopathological changes (H and E X400), (b) Diabetic control group showing vacuolations and necrosis of cells of islet's of Langerhan's (H and E X400), (c) *Ferula drudeana* 200 mg kg^{-1} showing vacuolation of cells of islet's of Langerhan's (H and E X400), (d) *Ferula drudeana* 400 mg kg^{-1} showing necrosis of some cells of islet's of Langerhan's (H and E X400), (e) *Ferula huber-morathii* 200 mg kg^{-1} showing no histopathological changes (H and E X400), (f) *Ferula huber-morathii* 400 mg kg^{-1} showing no histopathological changes (H and E X400)

DISCUSSION

It is not constantly simple to determine if the consuming plant extracts are safe. In fact there are a large number of plant extracts with a wide range of adverse effect. In this study, oral administration of *F. drudeana* and *F. huber-morathii* extracts at doses up to 4000 mg kg^{-1} did not produce any sign of acute toxicity and none of animals died during 48 h of observation. Accordingly, it suggested that oral median lethal doses (LD_{50}) of the tested extracts were higher than 4000 mg kg^{-1}. Therefore, *F. drudeana* and *F. huber-morathii* plants can be categorized as quietly safe since substances possessing LD_{50} higher than 50 mg kg^{-1} are non-toxic (Buck *et al*., 1976).

Type 2 diabetes is the most common form of the disease, accounting for 90-95% of diabetes. To induce diabetes in animals, STZ is commonly used which produces moderate hyperglycemia with clinical symptoms similar to type 2 diabetes (Srinivasan and Ramarao, 2007). The STZ causes selective damage to pancreatic β-cell through the release of nitric oxide (Szkudelski, 2001). Level of FBG in diabetic animals is an important parameter for monitoring diabetes (Maiti *et al*., 2005). In this study, induction of type 2 diabetes showed significant increased FBG and decreased insulin levels compared to control rats which confirm the induction of diabetes. Glibenclamide is a standard hypoglycemic drug, used to compare the hypoglycemic property in experimental rats. Glibenclamide have been activating insulin release from pancreatic β cells mainly by inhibiting ATP sensitive KATP channels in the plasma membrane (Proks *et al*., 2002). Oral administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts resulted in a marked decrease in FBG and elevation in insulin levels in diabetic animals. Body weights of STZ-induced diabetic rats were significantly reduced when compared to normal rats. Loss of body weight is due to increased muscle destruction and loss of proteins contents in the tissue (Salahuddin and Jalalpure, 2010). Diabetic rats treated with *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts showed an improvement in their body weights in comparison to the diabetic control group, which signifies the protective effect of both extracts in controlling muscle wasting. Moreover, the ability to protect body weight loss seems to be as a result of their ability to reduce hyperglycemia. Qualitative phytochemical assay indicated the presence of flavonoids in the methanol extracts of *F. drudeana* and *F. huber-morathii*. Flavonoids are reported to possess antidiabetic activity as they can preserve β-cell function and prevent diabetes induced ROS formation (Aslan *et al*., 2007). Moreover, Hii and Howell (1985) mentioned that certain flavonoids enhanced insulin release from the isolated rat islets. Thus, the hypoglycemic effect of *F. drudeana* and *F. huber-morathii* may be due to the presence of flavonoids. Moreover, the antihyperglycemic effect may also be attributed to the presence of saponin, resins and triterpenes. Such compounds have been reported to be responsible for hypoglycemic action due to their ability to regenerate the pancreatic-cell (Chika and Bello, 2010).

The level of HbA1c has been shown to be an important parameter of glycaemic control in patients with diabetes. During diabetes, the excess glucose of the blood reacts with hemoglobin and form HbA1c. The rate of glycosylation is directly proportional to that of FBG level. These results showed that, the high level of HbA1c in diabetic rats was significantly reduced by the administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) extracts. Reduced HbA1c contents in the medicated diabetic rats could be related to the improvement in insulin release from the remnant pancreatic β-cells that resulting in improvement in glycemic state (Kondeti *et al*., 2010). In addition, substances with antioxidant activity may inhibit oxidative reactions associated with protein glycation (Elgawish *et al*., 1996). Therefore, *F. drudeana* and *F. huber-morathii* extracts with their free radical scavenging activity efficiently reduced the formation of glycated proteins. This explanation was supported by the present study, indicating that both extracts have a strong antioxidant activity. The persistent hyperglycemia is accompanied with the dyslipidemia (Bierman *et al*., 1966), which is commonly observed in type-2 diabetic patients. The prevalence of dyslipidemia in diabetes mellitus is 95% and it is a major risk factor for the development of coronary heart disease (Uttra *et al*., 2011). Presence of hypertriglyceridemia and hypercholesterolemia and reduction of HDL-C are the most common lipid abnormalities that were reported in diabetic condition (Uttra *et al*., 2011). Under normal condition, TG is being hydrolyzed by lipoprotein lipase which was activated by insulin (Ananthan *et al*., 2003). Moreover, insulin reduces elevated cholesterol by different mechanisms, such as; increasing uptake of fatty acids into peripheral tissue and inhibiting lipolysis. In case of insulin resistance circumstances, lipolysis is not inhibited and leads to hyperlipidemia (Ananthan *et al*., 2003). These results showed that *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) medication brought back the increased levels of TG, TC and LDL-C of STZ-diabetic rats near to their normal levels. The ability of both extracts to reduce plasma TG and TC in diabetic animals could be explained by the insulin releasing capacity of *F. drudeana* and *F. huber-morathii*. A marked increase in HDL-C was also observed in diabetic rats treated with both extracts. HDL-C is beneficial, as it prevent hardening of the arteries, which causes atherosclerosis by transporting cholesterol from peripheral tissues into the liver. Our finding showed that *F. drudeana* and *F. huber-morathii* could lower the TC/HDL-C ratio, which is directly proportional in lowering the risk of heart attack. Thus, it is reasonable to conclude that *F. drudeana* and *F. huber-morathii* could modulate blood lipid abnormalities and could offer protection against hyperlipidemia.

In this study, the marked increase in plasma ALT, AST and ALP levels that was observed in STZ-induced diabetic rats represents liver damage. Oral administration of *F. drudeana* (400 mg kg^{-1}) and *F. huber-morathii* (200 and 400 mg kg^{-1}) showed their protective nature on liver tissue by reducing the elevated levels of ALT, AST and ALP. Our study was also undertaken to explore the antioxidant effect of *F. drudeana* and *F. huber-morathii* in STZ-induced diabetic rats. STZ has been found to induce Reactive Oxygen Species (ROS). The ROS include free radicals like superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), in addition to nonradical molecules, such as, hydrogen peroxide (H_2O_2). Continual production of free radicals may lead to tissue damage by attacking

membranes through peroxidation of unsaturated fatty acids (Ravi *et al*., 2003). Lipid peroxidation aggravates the oxidative stress through production of lipid-derived radicals that can damage proteins and DNA. Lipid peroxidation has been widely used as, an indicator of ROS mediated damage to plasma membranes. The MDA is one of the final products of unsaturated fatty acids peroxidation and is responsible for damage of plasma membrane (Halliwell and Gutteridge, 1989). In present study, elevated MDA levels were induced in diabetic rats. Decreased levels of MDA following *F. drudeana* and *F. huber-morathii* medication indicated a reduction in free radical formation in tissues such as the liver and pancreas of diabetic rats. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of nonenzymic as well as enzymic antioxidants (Noctor and Foyer, 1998). The enzymic antioxidants include SOD, CAT and GPx. Reduction in the activity of these enzymes results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. In present study, reduced SOD, CAT and GPx levels were observed in STZ-induced diabetic rats. The action of *F. drudeana* and *F. huber-morathii* extracts to restore the altered antioxidant enzymes in STZ-induced diabetic rats indicates their free radical scavenging potential. The GSH serve as potent nonenzymic antioxidant within the cell. Decreased GSH levels in diabetes have been considered to be an indicator of increased oxidative stress (McLennan *et al*., 1991). A decrease was observed in GSH in liver and pancreas during diabetes. Administration of the extracts and glibenclamide increased the content of GSH in the liver and pancreas of diabetic rats. Decreased the level of lipid peroxidation and improved antioxidant status may be one of the mechanism by which extracts could contribute to the prevention of diabetic complications (Kamalakkannan and Prince, 2006). Results obtained from histopathological investigations of liver and pancreas of rats is in accordance with the obtained biochemical investigations.

CONCLUSION

The present study reveals that, *F. drudeana* and *F. huber-morathii* has antioxidant and hypoglycemic activity. Biological effects may be attributed, at least in part, to the presence of flavonoids, saponin, resins and triterpenes in the extracts. The probable mechanism of the antihyperglycemic activity may be through a stimulation of insulin release from the remnant pancreatic β-cells. Hence these plants may be considered as among the potential sources for the isolation of new oral anti hypoglycemic agents.

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REFERENCES

Ananthan, R., M. Latha, K.M. Ramkumar, L. Pari, C. Baskar and V.N. Bai, 2003. Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. Exp. Diabesity Res., 4: 183-189.

Anderson, L., B. Dinesen, P.N. Jorgesen, F. Poulsen and M.F. Roder, 1993. Enzyme immunoassay for intact human insulin in serum or plasma. Clin. Chim. Acta, 39: 578-582.

Aslan, M., D.D. Orhan, N. Orhan, E. Sezik and E. Yesilada, 2007. *In vivo* antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* capitulums in streptozotocin-induced-diabetic rats. J. Ethnopharmacol., 109: 54-59.

Belhekar, S.N., P.D. Chaudhari, J.S. Saryawanshi, K.K. Mali and R.B. Pandhare, 2013. Antidiabetic and antihyperlipidemic effects of *Thespesia populnea* fruit pulp extracts on alloxan-induced diabetic rats. Indian J. Pharm. Sci., 75: 217-221.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bierman, E.L., J.A.P. Amaral and B.H. Belknap, 1966. Hyperlipemia and diabetes mellitus. Diabetes, 15: 675-679.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Buck, W., G. Osweiter and A. Van Gelder, 1976. Clinical and Diagnostic Veterinary Toxicology. 2nd Edn., Kendall/Hunt Publishing Co., Iowa, USA., ISBN-13: 9780840307200, Pages: 380.

Burstein, M., H.R. Scholnick and R. Morfin, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11: 583-595.

Chance, B. and A.C. Maehly, 1955. Assay of catalases and peroxidases: Catalase: $2H_2O_2 \rightarrow 2H_2O+O_2$ (1), Catalase and peroxidase: $ROOH+AH_2 \rightarrow H_2O+ROH+A$ (2). Methods Enzymol., 2: 764-775.

Chika, A. and S.O. Bello, 2010. Antihyperglycaemic activity of aqueous leaf extract of *Combretum micranthum* (Combretaceae) in normal and alloxan-induced diabetic rats. J. Ethnopharmacol., 129: 34-37.

Drabkin, D.L. and J.M. Austin, 1932. Spectrophotometric studies: I. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J. Biol. Chem., 98: 719-733.

Elgawish, A., M. Glomb, M. Freelander and V.M. Monnier, 1996. Involvement of hydrogen peroxide in collagen cross-linking by high glucose *in vitro* and *in vivo*. J. Biol. Chem., 271: 12964-12971.

Foster, L.B. and R.T. Dunn, 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin. Chim. Acta, 19: 338-340.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Halliwell, B. and J.M. Gutteridge, 1989. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, UK.

Hii, C.S. and S.L. Howell, 1985. Effects of flavonoids on insulin secretion and $^{45}Ca^{2+}$ handling in rat islets of Langerhans. J. Endocrinol., 107: 1-8.

Hussain, H.E.M.A., 2002. Hypoglycemic, hypolipidemic and antioxidant properties of combination of *Curcumin* from *Curcuma longa*, Linn and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes. Indian J. Clin. Biochem., 17: 33-43.

Jain, S.K., S.N. Levine, J. Deutt and B. Hollier, 1990. Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. Metabolism, 39: 971-975.

Kamalakkannan, N. and P.S. Prince, 2006. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats. Basic Clin. Pharmacol. Toxicol., 98: 97-103.

Kondeti, V.K., K.R. Badri, D.R. Maddirala, S.K.M. Thur, S.S. Fatima, R.B. Kasetti and C.A. Rao, 2010. Effect of *Pterocarpus santalinus* bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. Food Chem. Toxicol., 48: 1281-1287.

Li, W.L., H.C. Zheng, J. Bukuru and N. de Kimpe, 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J. Ethnopharmacol., 92: 1-21.

Maiti, R., U.K. Das and D. Ghosh, 2005. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biol. Pharmacol., 28: 1172-1176.

McLennan, S.V., S. Heffernan, L. Wright, C. Rae, E. Fisher, D.K. Yue and J.R. Turtle, 1991. Changes in hepatic glutathione metabolism in diabetes. Diabetes, 40: 344-348.

Mohandas, J., J.J. Marshall, G.G. Duggin, J.S. Horvath and D.J. Tiller, 1984. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res., 44: 5086-5091.

Nayak, S.S. and T.N. Pattabiraman, 1981. A new colorimetric method for the estimation of glycosylated hemoglobin. Clinica Chimica Acta, 109: 267-274.

Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Mol. Biol., 49: 249-279.

OECD., 2001. OECD guideline for testing of chemicals: Acute oral toxicity-acute toxic class method. Guideline No. 423, Organization for Economic Co-operation and Development, December 17, 2001, pp: 1-14. http://ntp.niehs.nih.gov/iccvam/SuppDocs/FedDocs/OECD/OECD_GL423.pdf

Pattanayak, S., S.S. Nayak, D. Panda and V. Shende, 2009. Hypoglycemic of *Cajanus scarabaeoides* in glucose overloaded and streptozotocin-induced diabetic rats. Bangladesh J. Pharmacol., 4: 131-135.

Proks, P., F. Reimann, N. Green, F. Gribble and F. Ashcroft, 2002. Sulfonylurea stimulation of insulin secretion. Diabetes, 51: S368-S376.

Ravi, K., S. Rajasekaran and S. Subramanian, 2003. Hypoglycemic effect of *Eugenia jambolana* seed kernels on streptozotocin-induced diabetes in rats. Pharm. Biol., 41: 598-603.

Rosalind, T.H., B.K. Dutta and S.B. Paul, 2013. Evaluation of *In vitro* antioxidant activity, Estimation of total phenolic and flavonoid content of leaf extract of *Eurya japonica* Thunb. Asian J. Pharm. Clin. Res., 6: 152-155.

Salahuddin, M.D. and S.S. Jalalpure, 2010. Antidiabetic activity of aqueous fruit extract of *Cucumis trigonus* Roxb. in streptozotocin-induced-diabetic rats. J. Ethnopharmacol., 127: 565-567.

Saravanakumar, A., K. Venkateshwaran, J. Vanitha, M. Ganesh, M. Vasudevan and T. Sivakumar, 2009. Evaluation of antibacterial activity, phenol and flavonoid contents of *Thespesia populnea* flower extracts. Pak. J. Pharm. Sci., 22: 282-286.

Srinivasan, K. and P. Ramarao, 2007. Animal models in type 2 diabetes research: An overview. Indian J. Med. Res., 125: 451-472.

Sun, M. and S. Zigman, 1978. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal. Biochem., 90: 81-89.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., 50: 537-546.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 11th Edn., Macmillan Publishers, London, UK.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.

Uttra, K.M., B.R. Devrajani, S.Z.A. Shah, T. Devrajani, T. Das, S. Raza and Naseem, 2011. Lipid profile of patients with diabetes mellitus (A multidisciplinary study). World Applied Sci. J., 12: 1382-1384.

Zlatkis, A., B. Zak and A.J. Boyle, 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med., 41: 486-492.