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Antihyperglycemic and Antihyperlipidemic Effects of *Ferula duranii* in Experimental Type 2 Diabetic Rats

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ABSTRACT

In the present study, DPPH radical scavenging assay and ferric-reducing antioxidant assay were used to evaluate *in vitro* antioxidant potential of the methanol extract of *Ferula duranii* (*F. duranii*). The antihyperglycemic and antihyperlipidemic activities of *F. duranii* extract were evaluated in streptozotocin (STZ)-induced diabetic rats. *F. duranii* showed considerable antioxidant potential in the DPPH radical scavenging assay and minimum reducing power in ferric-reducing antioxidant power assay. A meaningful reduction in the concentrations of Fasting Blood Glucose (FBG), glycosylated hemoglobin (Hb A1c), triglycerides (TG), Total Cholesterol (TC) and Low Density Lipoprotein (LDL-C) in plasma and an elevation in the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) in hepatic and pancreas homogenates were observed in diabetic animals medicated with *F. duranii* extract in comparison with diabetic control rats. The level of insulin raised significantly in plasma of diabetic groups received *F. duranii* in respect to diabetic control one. Levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, total protein and High Density Lipoprotein (HDL-C) in plasma and malondialdehyde (MDA) in liver and pancreas homogenates were recovered significantly in *F. duranii*-medicated diabetic rats in comparison with diabetic controls. The present data suggest that *F. duranii* has both antidiabetic and antihyperlipidemic effects.

Key words: *Ferula duranii*, diabetes, streptozotocin, hypolipidemic, rats

INTRODUCTION

Diabetes Mellitus (DM) is a chronic illness affecting approximately 5-10% of the world inhabitance. There is an intension in propagation of DM in industrialized and developing countries (Hamdan and Afifi, 2004). The disease can result in some complications that occur mainly in the tiny blood vessels leading to myocardial infarction and strokes (Watkins, 2003). The antidiabetic drugs are focused on controlling and reducing blood sugar to a normal level. Although several therapies are currently used in the treatment of diabetes, draw backs such as cost, hypoglycemia, weight

gain and liver toxicity are major concerns to search for alternative medicaments to treat or control hyperglycemia. Nowadays, there has been a growing need for obtaining more safe antidiabetic agents. Plants and other natural products have long been used in diverse traditional systems of medicine for their medicinal properties.

Ferula is a genus of about 170 different species that belongs to the Umbelliferae (Apiaceae) family. The plants grow mostly in arid climate in Mediterranean region, central Asia and Northern Africa. Many species of this genus are used in traditional medicine against some disorders (Pimenov and Leonov, 1993). A new species, *Ferula duranii* was described in East and South Anatolia, Turkey by Sagiroglu and Duman (2010) and up till now, no biological data or investigations yet done on this herb. Different ferula species have different names between the Turkish populations as "çakşır otu, çaşır otu, hiltik, hiltit, siyabo". In Hatay and Adana region of Turkey, roots of *F. elaeochytris* are grinded and mixed with honey then used as aphrodisiac. While, in East Anatolia some ferula species leaves like *F. communis* L., *F. orientalis* L. and *F. rigidula* are boiled with water and eaten as a food. In addition, *F. capsica* (Girmizi bolu) is used as stomachic while the over ground parts of *Ferula orientalis* (Heliz) is used for gynecological problems (Baytop, 1999).

So far, more than 70 Ferula species have been exposed to phytochemical analysis. Phytochemical studies revealed that plants of Ferula species are rich in bioactive compounds including aromatic resins (Chen *et al*., 2000), terpenoid derivatives and sesquiterpene coumarins (Iranshahi *et al*., 2009). Recent investigations have led to the discovery of some new biological activities of the plants of this genus. These include antimicrobial, antinociceptive, anti-inflammatory, anticonvulsant, antioxidant and hypotensive activities (Sahebkar and Iranshahi, 2010). At least, part of the biological activities of the plants of this genus can be attributed to their essential oils (Maggi *et al*., 2009). Since Ferula species are traditionally used in Asia to prevent diabetic complications; therefore, the current investigation was devoted to study the possible anti-diabetic potential of *F. duranii*.

MATERIAL AND METHODS

Plant material: Fresh roots of *F. duranii* L. was collected at summer 2013, from Antalya, Akseki, Çukurköy, 1500 m. Taxonomic identification was determined by Assistant Professor Dr. Şüra Baykan Erel and a voucher specimen from the plant (5521) was deposited at the Herbarium of Faculty of Pharmacy (IZEF), Ege University, Izmir, Turkey.

Preparation of the extracts: The collected plants were shade dried and then grinded to fine powder. About 1160 g of the dried powder of the plant was extracted by percolation in methanol with occasional shaking for 48 h. Percolation was repeated three times and then the methanolic extracts of the plant was combined and concentrated under vacuum to give the total extract of (159.63 g).

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

In vitro anti-oxidant activity

Antioxidant activity using DPPH radical scavenging assay: Various concentrations of the *F. duranii* extract were prepared. The assay mixtures contained in total volume of 1 mL composed of 500 µL of the extract, 125 µL prepared DPPH and 375 µL solvent. Ascorbic acid was 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 (Brand-Williams *et al*., 1995). The radical scavenging activity was calculated as:

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

Ferric-reducing antioxidant power assay: The assay was done according to Rosalind *et al.* (2013) using potassium ferricyanide-ferric chloride system. One milliliter of *F. duranii* solution (0.2, 0.5, 0.8 and 1.0 mg mL^{-1}) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was heated at 50°C for 20 min then cooled. Trichloroacetic acid (2.5 mL, 10%) was added and the content was centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) 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. Increasing absorbance of the reaction mixture indicates increasing reducing power.

In vivo acute toxicity and antidiabetic activity

Animals used: Adult male albino mice (27-30 g b.wt) were used in the acute toxicity test. Adult male Wistar rats (180-200 g b.wt) were used in the antidiabetic study. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA. Animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and given fresh purified potable water *ad libitum*.

Acute toxicity test: Five groups of adult male albino mice (n = 10) were fasted for 12 h prior to the experiment. Mice were given *F. duranii* extract orally in graded doses from 1000-4000 mg kg^{-1} b.wt. Mice were closely observed for 48 h for changes in behavior, symptoms of toxicity and death. The oral LD_{50} of the tested extract was calculated mathematically.

Justification for dose selection: The ethanolic extract of *F. duranii* was safe at 4.0 g kg^{-1} so, 1/20th (200 mg kg^{-1}) and 1/10th (400 mg kg^{-1}) of this dose were selected as experimental doses.

Induction of diabetes: After overnight fasting, DM was induced by IP administration of STZ (45 mg kg^{-1}) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5 (Aslan *et al*., 2007). Normal control rats received buffer alone. The FBG was estimated following 72 h of STZ administration and animals with FBG higher than 250 mg dL^{-1} were included in our experiment.

Experimental design: Thirty male Wistar rats (180-200 g b.wt.) were separated into five groups (n = 6). Groups 1 (Normal control) and 2 (Diabetic control) animals received the vehicle in a dose of 5 mL kg^{-1}. Diabetic rats of groups 3 and 4 received *F. duranii* extract at doses of 200 and 400 mg kg^{-1}, respectively. Group 5 (reference group) was diabetic rats given glibenclamide at a dose of 0.6 mg kg^{-1}. Vehicle, extract and glibenclamide were given orally by gavage as single daily treatments, for 28 days.

Biochemical evaluation: Blood samples were withdrawn by retro orbital puncture 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. 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 collected 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. The Hb A1c and the lipid parameters viz. TG, TC and HDL-C were evaluated according the method of Nayak and Pattabiraman (1981), Foster and Dunn (1973), Zlatkis *et al*. (1953) and Burstein *et al*. (1970), respectively. Level of plasma LDL-C was estimated according to Friedewald *et al*. (1972). 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 superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities and reduced glutathione (GSH) level in hepatic and pancreatic tissues were estimated (Sun and Zigman, 1978; Mohandas *et al*., 1984; Chance and Maehly, 1955; Beutler *et al*., 1963, respectively). Lipid peroxidation products were estimated by determining malondialdehyde (MDA) content in the hepatic and pancreatic tissue according to the procedure of Jain *et al*. (1990).

Histopathological study: Samples of the liver and pancreas were obtained and kept in 10% formalin for 48 h, then embedded within paraffin. Sections of 4 µm thickness were prepared using a rotary microtome. Tissue sections were stained with haematoxylin and eosin (H and E) and then observed by light microscopy for histopathological examination.

Statistical analysis: Results are expressed as Means±SE and statistical analysis was performed following 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. duranii* (Table 1) indicated the presence of alkaloids, carbohydrates, glycosides, saponins, steroids, triterpenoids, phenolic, flavonoids, proteins and amino acids.

Effect of *F. duranii* extract on *in vitro* anti-oxidant activity
Antioxidant activity using DPPH radical scavenging assay: The DPPH radical scavenging activity was presented as % inhibition (Fig. 1a). *F. duranii* showed considerable antioxidant potential in a concentration-dependent manner. Moreover, ascorbic acid revealed higher % inhibition indicating better antioxidant potential. *Ferula duranii* at concentrations of 10, 50, 100, 500 and 1000 µg mL^{-1} showed scavenging activities of 5.6, 6.0, 10.8, 23.0 and 42.0%, respectively while those of ascorbic acid were 58.5, 77.3, 87.6, 94.0 and 97.2%, respectively at same concentrations. The EC_{50} values for *F. duranii* and ascorbic acid were calculated to be 1751 and 6.038 µg mL^{-1}, respectively.

Table 1: Preliminary phytochemical screening of *Ferula duranii* methanolic extract

Phyto constituents	Tests	*Ferula duranii* methanolic extract
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	+

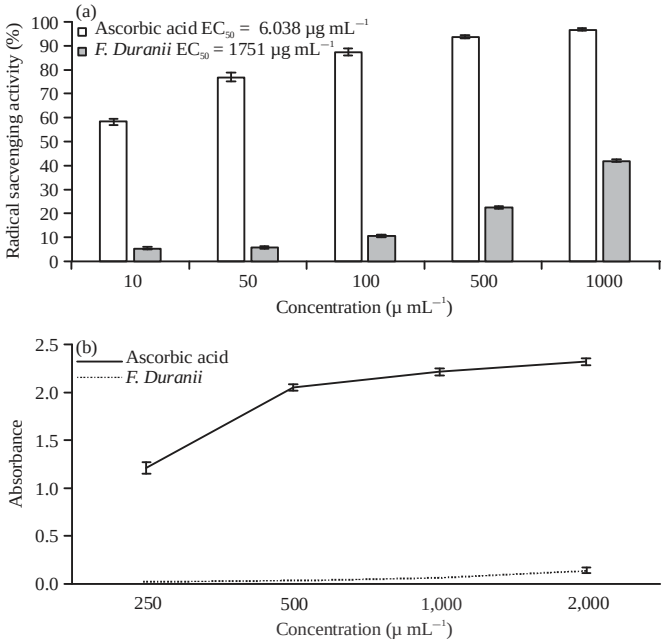

Fig. 1(a-b): Effect of *Ferula duranii* extract on *in vitro* anti-oxidant activity, (a) Antioxidant activity using DPPH radical scavenging assay and (b) Ferric-reducing antioxidant power assay

Ferric-reducing antioxidant power assay: As observed in Fig. 1b, *F. duranii* has minimum reducing power of 0.140% at concentration of 2000 µg mL^{-1}. As compared to ascorbic acid, that showed higher absorbance indicating more reducing power (2.337%) at concentration of 2000 µg mL^{-1}.

Acute toxicity test: Oral administration of the extract did not cause death in the highest dose of 4 g kg^{-1} b.wt. No visible signs of toxicity were reported in the mice exposed to different doses of *F. duranii* indicating it to be safe. Accordingly, the oral LD_{50} of *F. duranii* extract was determined to be higher than 4 g kg^{-1} b.wt which is the highest tested dose.

Biochemical evaluation: Diabetic animals exhibited a significant increase in FBG at days 14 and 28 of treatment (327.6 and 315.3 mg dL^{-1}, respectively) as compared to normal control rats (102.4 and 106.7 mg dL^{-1}, respectively). The elevated FBG of diabetic rats showed a tendency toward normal levels after dosing of *F. duranii* 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 (6.5 and 6.9 U L^{-1}, respectively) compared with normal controls (15.8 and 15.2 U L^{-1}, respectively). Medication of diabetic animals with 200 and 400 mg kg^{-1} b.wt of *F. duranii* extract increased the plasma insulin levels at days 14 (11.7 and 12.8 U L^{-1}, respectively) and 28 (11.9 and 12.9 U L^{-1}, respectively) of treatment compared with diabetic control rats. In addition, glibenclamide administration results in the restoration of plasma insulin to control levels.

Effect on total hemoglobin and HbA1c: The contents of total hemoglobin and HbA1c in control and experimental groups are shown in Table 3. Control diabetic animals showed a considerable reduction in the level of total hemoglobin (10.7 mg dL^{-1}) and a significant elevation in HbA1c content (11.5% Hb) in comparison with normal controls (14.4 mg dL^{-1} and 5.8% Hb, respectively). Administration of *F. duranii* extract (200 and 400 mg kg^{-1}) to diabetic rats revert back the levels of total hemoglobin and HbA1c to normal conditions (Table 3). Similarly, the levels of total hemoglobin and HbA1c 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 experimental rats are illustrated in Table 4. Diabetic rats had increased levels of plasma TG, TC and LDL-C and decreased level of HDL-C compared to normal control group. Oral administration of *F. duranii* (200 and 400 mg kg^{-1}) 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. duranii* 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

Table 2: Levels of glucose and insulin in plasma of control and experimental rats after 14 and 28 days of treatment with *Ferula duranii* extract

	FBG (mg dL^{-1})		Fasting insulin (U L^{-1})	
Groups	14 days	28 days	14 days	28 days
Normal control	102.4±2.53#	106.7±3.63#	15.8±0.14#	15.2±0.15#
Diabetic control	327.6±3.63*	315.3±5.83*	6.5±0.13*	6.90±0.12*
DC+*F. duranii* (200 mg kg^{-1})	218.3±2.20*#	205.4±4.54*#	11.7±0.10*#	11.9±0.10*#
DC+*F. duranii* (400 mg kg^{-1})	195.7±2.31*#	186.5±4.15*#	12.8±0.11*#	12.9±0.13*#
DC+glibenclamide	144.2±2.37*#	136.6±2.74*#	14.2±0.14*#	14.5±0.12*#

Values represent the 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, FGB: Fasting blood glucose

Table 3: Levels of total hemoglobin and HbA1c of control and experimental rats after 28 days of treatment with *Ferula duranii* extract

Groups	Total hemoglobin (mg dL^{-1})	HbA1C (Hb%)
Normal control	14.4±0.58#	5.80±0.13#
Diabetic control	10.7±0.30*	11.5±0.17*
DC+*F. duranii* (200 mg kg^{-1})	12.1±0.31*#	8.30±0.11*#
DC+*F. duranii* (400 mg kg^{-1})	12.4±0.27*#	8.70±0.10*#
DC+glibenclamide	13.9±0.40#	7.50±0.18*#

Values represent the 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

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 duranii* extract

Groups	TG (mg dL^{-1})	TC (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})
Normal control	42.5±1.64#	56.6±1.93#	29.3±0.93#	17.67±0.54#
Diabetic control	89.4±2.70*	79.1±2.87*#	20.9±0.89*	35.30±0.96*
DC+*F. duranii* (200 mg kg^{-1})	58.0±1.91*#	64.3±2.71*#	24.7±0.87*#	26.40±0.84*#
DC+*F. duranii* (400 mg kg^{-1})	52.3±1.62*#	65.0±2.70*#	25.3±0.95*#	28.10±0.98*#
DC+ glibenclamide	85.2±3.85*	60.0±2.88#	27.3±0.96#	21.00±0.88*#

Values represent the 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, TG: Triglycerides, TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, ALP: Alkaline phosphatase

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 duranii* extract

Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Bilirubin (mg dL^{-1})	Total protein (g dL^{-1})
Normal control	42.3±1.90#	86.4±4.58#	64.2±3.56#	0.57±0.04#	8.50±0.15#
Diabetic control	110.5±2.88*	195.2±10.40*	143.4±3.07*	1.620±0.06*	3.40±0.09*
DC+*F. duranii* (200 mg kg^{-1})	61.7±2.16*#	116.4±2.32*#	79.6±2.13*#	0.810±0.04*#	6.70±0.18*#
DC+*F. duranii* (400 mg kg^{-1})	66.7±1.59*#	105.2±5.65*#	73.3±2.97*#	0.74±0.05*#	7.0±0.03*#
DC+glibenclamide	58.0±1.97*#	97.8±3.41*#	82.7±4.61*#	0.680±0.04*#	7.70±0.60*#

Values represent the 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 duranii* extract

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	49.2±1.35#	3.8±0.11#	14.7±0.25#	11.3±0.15#	44.5±1.32#
Diabetic control	31.8±1.26*	1.7±0.10*	8.5±0.21*	6.2±0.12*	72.4±2.13*
DC+*F. duranii* (200 mg kg^{-1})	37.5±1.26*#	2.8±0.11*#	10.7±0.14*#	8.3±0.17*#	60.2±2.73*#
DC+*F. duranii* (400 mg kg^{-1})	39.7±1.84*#	3.0±0.12*#	11.3±0.17*#	8.5±0.13*#	59.4±2.17*#
DC+glibenclamide	44.5±1.85*#	3.3±0.13*#	12.9±0.19*#	9.9±0.15*#	53.3±1.59*#

Values represent the 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 activities, GSH: Reduces glutathione, MDA: Malondialdehyde

plasma protein in normal control and diabetic rats were shown in Table 5. In diabetic rats, there was a meaningful reduction in the protein level when compared with normal group. When *F. duranii* extract was dosed to diabetic animals for 28 days, the total protein level reverted to near normal.

Effect on oxidative stress markers in hepatic and pancreatic tissues: Table 6 and 7 clearly illustrate the effect of *F. duranii* extract 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 200 and 400 mg kg^{-1} of *F. duranii* extract 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. duranii* medication.

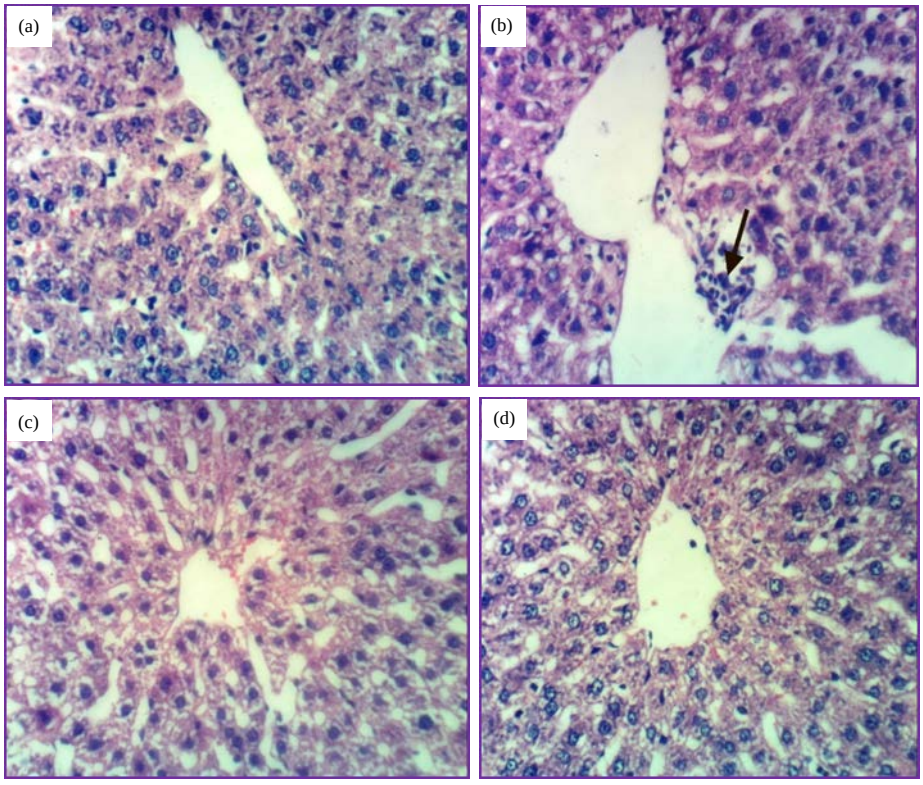

Fig. 2(a-d): Liver of rat from (a) Normal control group showing the normal histological structure of hepatic lobule (H and E× 400), (b) Diabetic control group showing focal hepatic necrosis associated with inflammatory cells infiltration (H and E×400), (c and d) *Ferula duranii* 200 and 400 mg kg^{-1}, respectively showing no histopathological changes (H and E×400)

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 *Ferula duranii* extract

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	34.5±1.56$^{\#}$	3.2±0.17$^{\#}$	17.1±0.44$^{\#}$	9.5±0.27$^{\#}$	32.7±1.55$^{\#}$
Diabetic control	22.7±1.85*	1.6±0.14*	10.4±0.38*	5.4±0.22*	58.2±2.73*
DC+*F. duranii* (200 mg kg^{-1})	28.0±1.66*$^{\#}$	2.5±0.13*$^{\#}$	14.3±0.47*$^{\#}$	8.1±0.25*$^{\#}$	48.5±2.85*$^{\#}$
DC+*F. duranii* (400 mg kg^{-1})	29.2±1.52*$^{\#}$	2.7±0.18*$^{\#}$	15.0±0.41*$^{\#}$	8.4±0.28*$^{\#}$	42.6±2.33*$^{\#}$
DC+glibenclamide	32.5±1.96$^{\#}$	3.0±0.15$^{\#}$	16.3±0.53$^{\#}$	9.0±0.27$^{\#}$	37.5±1.72$^{\#}$

Values represent the 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, CAT: Catalase activities, GSH: Reduces glutathione, MDA: Malondialdehyde

Histopathological findings: The liver of non-diabetic rats showed normal hepatocytes with central vein and portal triad (Fig. 2a). The injury to the hepatocytes including focal hepatic necrosis associated with inflammatory cells infiltration can be observed in the liver of diabetic control animals (Fig. 2b). The harm to the hepatocytes was reversed in *F. duranii* treated groups (Fig. 2c and d). Non-diabetic rats showed normal histological architecture of pancreas (Fig. 3a). Pancreatic sections of the diabetic rats revealed necrosis of cells of islets of Langerhans (Fig. 3b) Oral treatments of diabetic rats with *F. duranii* extract (200 mg kg^{-1}) showed slight vacuolation of pancreatic acinar epithelium (Fig. 3c) while 400 mg kg^{-1} repaired the morphological alterations in the pancreas toward normal (Fig. 3d).

DISCUSSION

More than 70 Ferula species have been exposed to phytochemical evaluation and the results have led to the identification of this genus as a good source of bioactive compounds including terpenoid derivatives (Iranshahi *et al*., 2007, 2009). To our knowledge, data regarding phytochemical analysis of *F. duranii* have been lacking. In our study, the methanolic extract of *F. duranii* was found to contain alkaloids, glycosides, saponins, steroids, triterpenoids, phenols, tannins and flavonoids. Some flavonoids have antidiabetic properties as they ameliorate altered glucose and oxidative metabolisms of the diabetic conditions (Song *et al*., 2005). They also exert a stimulatory effect on insulin secretion by modifying Ca^{++} concentration (Hii and Howell, 1985).

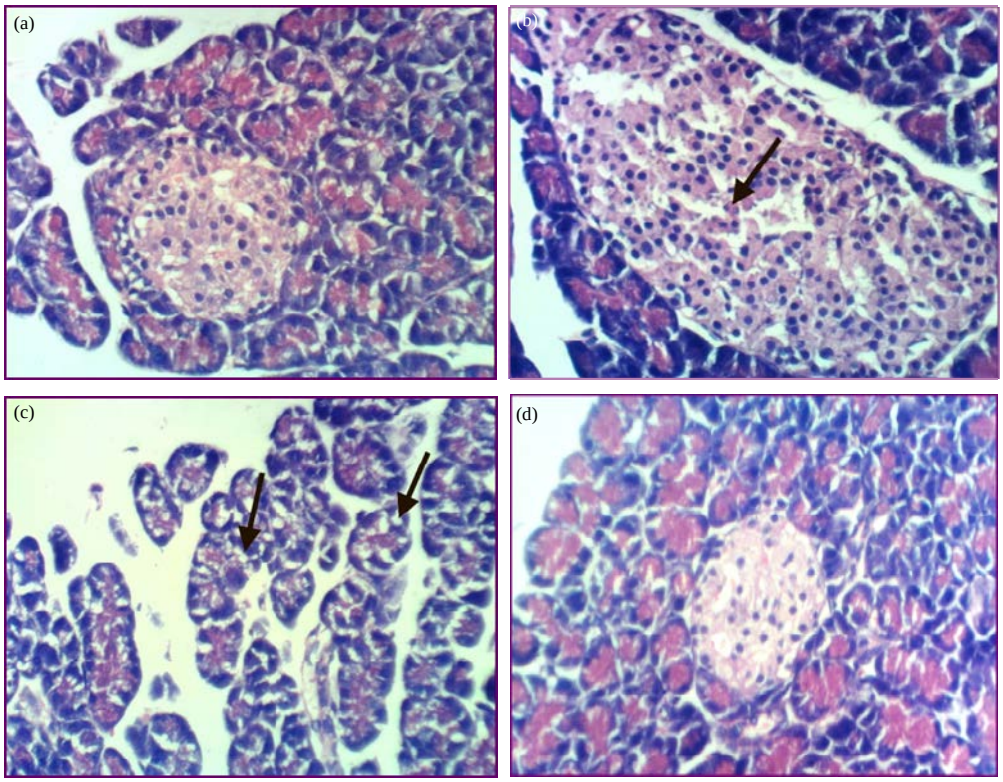

Fig. 3(a-d): Pancreas of rat from (a) Normal control group showing no histopathological changes (H and E×400), (b) Diabetic control group showing necrosis of cells of islets of Langerhans (H and E×400), (c) *Ferula duranii* 200 mg kg^{-1} showing vacuolation of pancreatic acinar epithelium (H and E×400) and (d) *Ferula duranii* 400 mg kg^{-1} showing no histopathological changes (H and E×400)

We have shown that the *F. duranii* extract displays *in vitro* antioxidant activity from the dose-dependent inhibition on DPPH. Additionally, *F. duranii* extract also possesses ferric-reducing antioxidant activity. The antioxidant activity of *F. duranii* extract could be due to the presence of various bioactive compounds such as alkaloids, glycosides, saponins, steroids, triterpenoids, phenols, tannins and flavonoids which were reported to possess antioxidant capabilities (Yu *et al*., 2005).

Evaluation of the potential toxicity of natural products is usually an initial step in screening for their pharmacological activities. Acute toxicity test disclose that oral administration of *F. duranii* at doses up to 4 g kg^{-1} did not induce verifiable acute toxic impact or mortality in all groups of mice during 7 days of observation. None of the animals display depression, seizures, excitement, fever and respiratory distress. The oral LD_{50} value for the tested extract was indeterminable being in excess of 4 g kg^{-1} b.wt. In general, the higher the LD_{50} value, the lower toxic the compound. Therefore, *F. duranii* can be categorized as relatively non-toxic extract since substances possessing LD_{50} more than 4 g kg^{-1} by oral route are regarded as being safe or practically nontoxic (Kennedy *et al*., 1986).

Non-insulin dependent DM is the much more widespread form of diabetes accounting for more than 90% of all diabetes cases and causes serious socioeconomic problems especially in developing countries (Cheng, 2005). Antihyperglycemic effect of medicinal plants is achieved by different mechanisms including decreasing glucose absorption from intestine, promoting insulin secretion from β-cells, stimulating glucose uptake by tissues, blocking glucose production in liver and stimulating pancreatic tissue regeneration and/or presence of insulin-like agents in plants. This investigation aimed at studying the antidiabetic and antihyperlipidemic activities of *F. duranii* extract in diabetic animals.

In this study STZ was injected IP to induce DM in rats. It causes alkylation of pancreatic deoxyribonucleic acid inducing the generation of superoxide, hydrogen peroxide, nitric oxide and hydroxyl radicals which are responsible for β-cells damage and necrosis resulting in diabetes (Szkudelski, 2001). This effect was represented in the current study through the elevation of FBG and reduction of insulin levels in diabetic control rats. In diabetic rats, FBG and insulin were returned toward their normal levels following the administration of *F. duranii* extract (200 and 400 mg kg^{-1}). The action of *F. duranii* seems to be lower to that of glibenclamide. The antidiabetic activity of *F. duranii* may be due to stimulating insulin secretion from the existing pancreatic β-cells. The antidiabetic effect of the extract was correlated with an elevation in the level of insulin in plasma suggesting that *F. duranii* favors insulin secretion from the remnant β-cells or from regenerated β-cells. Increment of non-enzymatic

glycosylation is one of the probable mechanism joining increased blood glucose level and vascular complications of DM. In case of diabetes, the increased glucose level in the blood reacts with hemoglobin to form HbA1c (Kondeti *et al*., 2010). Hence, the estimation of HbA1c is a well-established marker useful in the management and prognosis of the disease (Chang and Noble, 1979). HbA1c was found to elevate in diabetic patients and the level of increase is directly proportional to the FBG level (Alyassin and Ibrahim, 1981). In our investigation, the diabetic animals had shown higher concentration of HbA1c in comparison with those in normal animals, indicating their poor glycemic control. The concentrations of HbA1c were well regulated near to normal levels in *F. duranii* treated diabetic groups which might be due to progress in insulin secretion upon *F. duranii* treatment. Further, since the non-enzymatic glycosylation of hemoglobin is an oxidative reaction (Yadav *et al*., 2000); the antioxidant effect of *F. duranii* is expected to inhibit the reaction.

Diabetes is often associated with dyslipidemia, a main risk factor for coronary heart diseases (Pari *et al*., 2014). The elevation of serum lipid levels due to insulin insufficiency is correlated with hypercholesterolemia which may attribute to metabolic aberration (Murali *et al*., 2002). The cluster of lipid abnormalities defined by high triglycerides and low HDL-C levels and also increased LDL-C levels and hypertriglyceridemia can actively contribute to β-cell failure and thus to manifestation of diabetes mellitus (Rutti *et al*., 2009). In the present study, plasma of diabetic animals showed a marked increase of TG, TC and LDL-C while HDL-C was declined. A diversity of alterations in metabolic and regulatory mechanisms, due to insulin insufficiency is responsible for the observed accumulation of lipids (Rajalingam *et al*., 1993). Treatment with *F. duranii* significantly decreased the TG, TC, and LDL-C concentrations in a dose dependent manner while elevating the beneficial HDL level to a great extent. It is well known that injection of insulin to diabetics not only increases the activity of lipoprotein lipase enzyme but also reduces the plasma TG concentrations and the production of LDL-C particle (Langhi and Cariou, 2010). Accordingly, the decline in plasma lipid profiles in *F. duranii* administered diabetic rats may relate to the insulinotropic activity or insulin secretagogue effect of the plant. In addition, it is supposed that *F. duranii* may induce its hypocholesterolemic effect either due to reduced intestinal absorption or impaired cholesterol biosynthesis. One of the most common side effects of some antihyperlipedemic drugs, that decrease TC, is the reduction of the cardioprotective HDL-C (Wilson, 1990). Interestingly, *F. duranii* not only lowered TC and LDL levels but also increased HDL-C level.

In case of DM, the metabolic disorders of protein, lipid and carbohydrate together with oxidative stress are possibly to influence liver functions. Accordingly we tested the protective effect of *F. duranii* against liver injury induced by DM. The activities of ALT, AST and ALP enzymes were used in the evaluation of hepatic injury. In the present study, the increased activity of these enzymes in plasma reflects liver injury. Increased activities of ALT and AST under insulin deficiency have been correlated with elevated gluconeogenesis and ketogenesis during diabetes (Felig *et al*., 1970). Oral dosing of *F. duranii* to diabetic rats resulted in decrease in the activities of these enzymes in plasma compared to the control diabetic rats. Kondeti *et al.* (2010) have reported that STZ induced diabetic rats account for the observed decrease in the total protein content.

Oxidative stress has been considered to be a pathogenic factor of diabetic complications. In the current investigation we estimated the levels of antioxidant enzymes and lipid peroxidation in STZ-diabetic rats. The study revealed a marked reduction in antioxidant enzymes including SOD, GPx and CAT in diabetic animals with decreased GSH and increased MDA levels indicating oxidative stress. A significant improvement in these indicators of oxidative stress in the liver and pancreas of *F. duranii* treated diabetic groups. This confirms that *F. duranii* extract is eligible of providing protection against hyperglycemia-induced oxidative stress in the hepatic and pancreatic tissues of rats. This effect of *F. duranii* on antioxidants was found to be better than those of glibenclamide treated diabetic rats. Glutathione protects different tissues against free radical induced oxidative damage (Bandyopadhyay and Chattopadhyay, 2006). Protective effect of *F. duranii* extract on one of the most powerful endogenous anti-oxidants, GSH, was evaluated in the hepatic and pancreatic tissues of diabetic animals. Exposure to STZ caused significant reduction in GSH level compared with normal control. Treatment with *F. duranii* (200 and 400 mg kg^{-1}) was noticed to protect dose-dependently against the changes in the content of GSH in both tissues. Lipid peroxidation is the main biomarker of oxidative stress (Buege and Aust, 1978). The increase in the level of lipid peroxidation, due to the modified intracellular ratio between free radicals and antioxidant systems, has been recently related to diabetes (Altomare *et al*., 1992). In this study, STZ significantly elevated hepatic and pancreatic MDA level, as a marker of lipid peroxidation, when compared to normal rats. Oral dosing of *F. duranii* (200 and 400 mg kg^{-1}) to diabetic rats was capable of reducing the elevated MDA level significantly in liver and pancreas. The increased SOD, GPx and CAT activities and GSH content in addition to decreased MDA level in response to *F. duranii* extract indicates the role of the extract as antioxidant. Accordingly, we assumed that *F. duranii* extract is able to improve liver and pancreas injury induced by hyperglycemia in rats by reducing oxidative stress. In this connection, *F. duranii* is proved in the present study to contain several biologically active constituents. The antioxidant effect of the extract has been connected to its main components, phenolics and total flavonoids which can scavenge free radicals and decrease levels of reactive oxygen species (Ibrahim *et al*., 2015). These results are in accordance with many studies carried out by other researchers who attributed the antioxidant activities to the presence of phenolic and polyphenolic compounds in many of the medicinal plants (Vinson *et al*., 1995). The phenolic substances of natural origin show their antioxidant effects by different mechanisms including their ability to scavenge free radicals or stimulate various antioxidant enzymes and prevent oxidases (Kulkarni *et al*., 2004).

Results obtained from histopathological investigations of liver and pancreas of rats are in accordance with the obtained biochemical investigations. In STZ-diabetic animals, administration of *F. duranii* resulted in normalizing the hepatic and pancreatic histoarchitecture quite remarkably. The increase in the number of β-cells in the pancreas showed that they were regenerated. In addition, the increased secretory granules in the cells indicate that the cells were stimulated for insulin synthesis.

Our study had a few limitations. We did not measure the active components in the methanol extract of *F. duranii*. In conclusion the *F. duranii* extract has both an antidiabetic effect and an antidyslipidemic activity. This is the first research record for the hypoglycemic and hypolipidemic effects of *F. duranii*. The probable mechanism of the antihyperglycemic activity may be through a stimulation of insulin release from the remnant pancreatic β-cells. Both antidiabetic and antidyslipidemic effects may in part be due to its antioxidant activity. Future studies are needed to isolate the antidiabetic compounds and to elucidate their real mechanisms of action.

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