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Antihyperglycemic and Antihyperlipidemic Effects of *Ferula assa-foetida* and *Ferula tenuissima* Extracts in Diabetic Rats

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ABSTRACT

The present study was carried to explore the potential antihyperglycemic and antihyperlipidemic activity of *Ferula assa-foetida* L. and *Ferula tenuissima* Hub-Mor & Pesmen extracts in streptozotocin (STZ) induced diabetic rats. Furthermore, phytochemical screening, *in vitro* antioxidant activity and acute toxicity study of both plants were performed. Both extracts showed considerable antioxidant potential *in vitro*. In diabetic rats, *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) showed significant elevation in plasma insulin level, total hemoglobin (Hb) and decrease in Fasting Blood Glucose (FBG) and glycosylated hemoglobin (HbA1c) levels. Significant elevations in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and level of reduced glutathione (GSH) in liver and pancreas homogenates were observed in diabetic animals following *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) treatments. The antihyperlipidemic effect of *F. assa-foetida* extract was demonstrated by a significant reduction in plasma triglycerides (TG), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and the increase of High Density Lipoprotein Cholesterol (HDL-C). Plasma activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and levels of Total Protein (TP) and bilirubin (BIL) in diabetic rats were recovered significantly after *F. assa-foetida* and *F. tenuissima* treatment in comparison with diabetic controls. The present data suggest that *F. assa-foetida* have both antihyperglycemic and antihyperlipidemic effects with enhancement of insulin-secreting activity.

Key words: Apiaceae, *Ferula*, antihyperglycemic, antihyperlipidemic, streptozotocin

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disturbance that threatens the life of world populations leading to hyperglycemia which is the main reason of diabetic complications. Hyperlipidemia is considered a major cardiovascular hazard factor in DM. The cardiovascular complications related to atherosclerosis are responsible for 70-80% of all reasons of mortality in patients with DM (Laakso, 2010). The essential aim of diabetes therapy is the protection against diabetic complications thereby, diabetics require a good glycemic control in addition to treatment for dyslipidemia (LeRoith, 2008). The pathogenesis of DM is controlled by insulin and oral antidiabetic agents. Regrettably, apart from having some adverse effects, none of the synthetic antidiabetic drugs have been effective in maintaining blood glucose level and reducing cardiovascular complications. Therefore, the search for new antidiabetic agents with more effectiveness and lesser side effects has continued. Plants have evermore been big source of medications and most of the currently used drugs have been derived from them.

The genus *Ferula*, belonging to the family Apiaceae, comprise around 170 species distributed in central Asia, the Mediterranean region and northern Africa (Maggi *et al*., 2009). Plants belong to this genus are documented in the traditional medicine of the Middle East area. Among the reported traditional medicinal uses of *Ferula* species are for treating convulsion, neurological disorders, diabetes, rheumatism, pain and inflammation (Asili *et al*., 2009). A number of medicinal properties including antibacterial, antioxidant, anti-inflammatory and hypotensive activities have been reported for *Ferula* species (Sahebkar and Iranshahi, 2011). *Ferula* has been documented as a useful origin of biologically active substances as coumarin derivatives, sesquiterpenes and sulphur containing compounds (Iranshahy and Iranshahi, 2011). 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; hence, this study was committed to investigate the potential antihyperglycemic and antihyperlipidemic activities of *F. assa-foetida* and *F. tenuissima* in STZ-diabetic rats.

MATERIALS AND METHODS

Plant material: *Ferula assa-foetida* oleo-gum-resin was purchased from the local market in Riyadh, Saudi market. Fresh roots of *F. tenuissima* was collected at summer in 2013 from Osmaniye, 2 km before Yarpuz, 940 m, Turkey. Taxonomic identification was determined by Assistant Professor Dr. Şüra Baykan Erel and a voucher specimen from *F. tenuissima* was deposited at the Herbarium of Faculty of Pharmacy (IZEF No.: 6046), Ege University, Izmir, Turkey.

Preparation of the extracts: The collected plant *F. tenuissima* was shade dried and then grinded to fine powders. About 490 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 91.12 g. A weight of 250 g from the oleo-gum-resin of *F. assa-foetida* 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 177.73 g.

Phytochemical screening: Preliminary phytochemical tests were carried out to identify the chemical constituents of the methanol extracts of *F. assa-foetida* and *F. tenuissima* 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 *F. assa-foetida* and *F. tenuissima* extracts were prepared. The assay mixtures was contained in a total volume of 1 mL composed of 500 µL of the extract, 125 µL prepared DPPH and 375 µL solvent (Brand-Williams *et al*., 1995). Ascorbic acid was used as the positive control.

Ferric-reducing antioxidant power assay: The assay was done according to Rosalind *et al.* (2013) using potassium ferricyanide-ferric chloride system.

Animals: Adult male Albino rats weighing 165-180 g were used in this study. Rats were obtained from Lab Animal Care Unit, Pharmacy College, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA. They were received standard rodent diet and given fresh purified potable water *ad libitum* in controlled conditions of temperature with a 12 h light and 12 h dark cycle.

Acute toxicity study: Acute toxicity study for *F. assa-foetida* and *F. tenuissima* extracts was carried in adult male Albino rats according to OECD-423 guidelines (OECD., 2001). Rats were kept fasting providing only water, after which *F. assa-foetida* and *F. tenuissima* were administered orally by gastric tube in different gradual doses (1000-4000 mg kg^{-1}), then observed for any toxic symptoms and mortality for 72 h.

Induction of diabetes: Diabetes was induced by intraperitoneal administration of STZ at 45 mg kg^{-1} (freshly prepared in 0.1 mol L^{-1} citrate buffer, pH 4.5) after fasting for 16 h. After 3 days of STZ injection, rats with FBG higher than 200 mg dL^{-1} were considered as being diabetic.

Experimental procedure: Forty two male Albino rats were randomly divided into seven equal groups.

Group 1 : Untreated normal control rats received the vehicle at 5 mL kg^{-1}

Group 2 : Untreated diabetic control rats received the vehicle at 5mL kg^{-1}

Groups 3-4 : Diabetic rats received *F. assa-foetida* extract (200 and 400 mg kg^{-1}, respectively)

Groups 5-6 : Diabetic rats received *F. tenuissima* extract (200 and 400 mg kg^{-1}, respectively)

Group 7 : Diabetic rats received glibenclamide (0.6 mg kg^{-1})

The vehicle, extracts and glibenclamide were administered orally via an orogastric cannula for 28 days.

Estimation of biochemical parameters: 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 was estimated using the glucose oxidase peroxidase method (Trinder, 1969) and insulin levels were determined through a radioimmunoassay procedure, using insulin kits according to the manufacturer's instructions. At the end of the experiment, 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) and HbA1c (Nayak and Pattabiraman, 1981). The second sample was centrifuged at 3500 rpm for 15 min to separate plasma. The TG, TC and HDL-C were evaluated using commercial kits. LDL-C was calculated by using Friedewald formula (Friedewald *et al*., 1972). The activities of ALT, AST and ALP and levels of BIL and TP in plasma were estimated according to the instructor manual of commercially available kits. Antioxidant activity was estimated by measuring the activity of SOD, GPx and CAT and levels of GSH and lipid peroxidation (LPO) product; MDA in the hepatic and pancreatic tissue homogenates using the specified kits from Biodiagnostic Chemical Company (Egypt) according to the instructions of the supplier.

Histopathological examination: Liver and pancreas samples were fixed in 10% formalin, processed routinely and embedded in paraffin. Five meter thick sections were prepared and stained with hematoxylin and eosin (H and E) for microscopic investigation. The stained sections were examined and photographed under a light microscope.

Statistical analysis: Results are expressed as Mean±Standard Error (SE) of mean. Statistical analysis was performed, using 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 version 17.0.

RESULTS AND DISCUSSION

Phytochemical screening: The preliminary phytochemical screening of *F. assa-foetida* and *F. tenuissima* extracts disclose the existence of alkaloids, steroids, triterpenoids, phenols, tannins and flavonoids (Table 1). In an earlier study, Dehpour *et al*. (2009) mentioned that plants of the *Ferula* genus are rich sources of gum-resin, total phenol and flavonoids. In addition, *F. assa-foetida* of the present study contains glycosides.

In vitro anti-oxidant activity

DPPH radical scavenging assay: The DPPH radical scavenging activities of *F. assa-foetida* and *F. tenuissima* extracts were recorded in terms of percentage inhibition as shown in Fig. 1a. Both extracts showed marked antioxidant potential. Ascorbic acid revealed higher percentage inhibition indicating better antioxidant potential. *Ferula assa-foetida* at concentrations of 10, 50, 100, 500 and 1000 μg mL^{-1} showed scavenging activities of 8.15, 35.20, 54.29, 77.45 and 87.43%,

Table 1: Preliminary phytochemical screening of *Ferula assa-foetida* and *Ferula tenuissima* extracts

Phyto-constituents	Test	*Ferula assa-foetida*	*Ferula tenuissima*
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	+	+

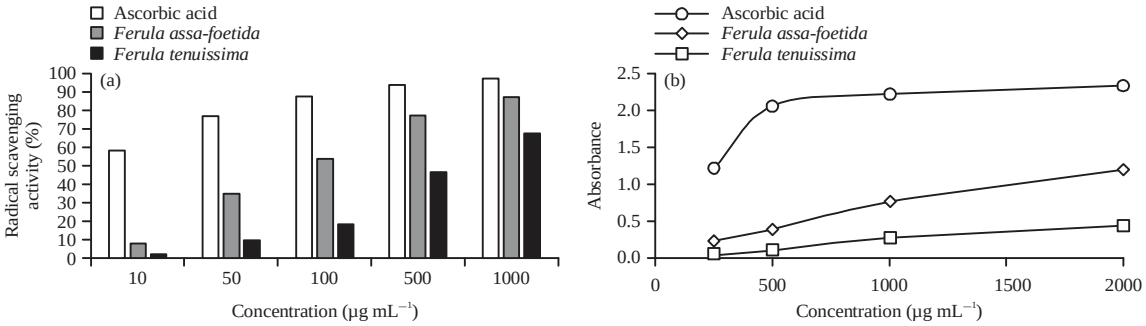

Fig. 1(a-b): Effect of *Ferula assa-foetida* and *Ferula tenuissima* extracts on anti-oxidant activity *in vitro* (a) DPPH radical scavenging assay and (b) Ferric-reducing antioxidant power assay

Table 2: Levels of FBG and insulin in plasma of the experimental rats after treatment with *Ferula assa-foetida* and *Ferula tenuissima* extracts (n = 6)

	FBG (mg dL^{-1})		Fasting insulin (U L^{-1})	
Groups	14 days	28 days	14 days	28 days
Normal control	97.3±3.00‡	102.1±2.27‡	17.5±0.36‡	17.9±0.44‡
Diabetic control	303.8±9.45*	317.3±9.52*	9.4±0.28*	9.0±0.32*
DC+*F. assa-foetida* (200 mg kg^{-1})	175.6±5.74*‡	173.3±6.28*	13.2±0.75*‡	13.7±0.51*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	166.7±5.71*‡	160.5±5.74*‡	15.6±0.67*‡	15.4±0.56*‡
DC+*F. tenuissima* (200 mg kg^{-1})	296.0±7.16*	299.8±8.15*	10.3±0.55*	10.3±0.53*
DC+*F. tenuissima* (400 mg kg^{-1})	204.4±6.33*‡	200.3±7.62*‡	12.2±0.74*‡	12.3±0.51*‡
DC+Glibenclamide	137.2±4.40*‡	131.3±5.13*‡	17.1±0.72*‡	16.8±0.46*‡

FBG: Fasting blood sugar, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

respectively while those of *F. tenuissima* showed 2.21, 9.64, 18.05, 46.86 and 67.89%, respectively. The EC_{50} values were calculated to be 99.05 and 511.0 µg mL^{-1} for *F. assa-foetida* and *F. tenuissima* extracts, respectively.

Ferric-reducing antioxidant power assay: As observed in Fig. 1b, *F. assa-foetida* extract has high reducing power of 1.20% at concentration of 2000 µg mL^{-1}. While, *F. tenuissima* extract reveals a lower reducing power (0.44%) at the same concentration. Moreover, ascorbic acid shows the highest absorbance indicating the most reducing power (2.34%) at the same concentration, too.

Acute toxicity study: Acute toxicity results revealed the non-toxic nature of *F. assa-foetida* and *F. tenuissima* extracts. The rats treated with different doses of *F. assa-foetida* and *F. tenuissima* did not exhibit any physical symptoms of toxicity throughout the experimental period and no mortalities were detected.

Antidiabetic study: In this study, we have explored the antidiabetic activity of *F. assa-foetida* and *F. tenuissima* extracts in STZ induced diabetic rats as well as antihyperlipidemic activity. It was found that methanolic extract of *F. assa-foetida* was more effective than *F. tenuissima* extract after 28 days of treatment. However, no statistical difference in any parameters between diabetic *F. tenuissima*-treated rats (200 mg kg^{-1}) and diabetic controls was observed.

Effect on levels of insulin, FBG and HbA1c: In the present study, STZ was used to induce DM in rats. It is excessively used animal model and is often associated with hepatotoxicity, oxidative stress and hypercholesterolemia. In this study, administration of *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) extracts to diabetic rats induced a significant elevation in the levels of insulin at the 14th and 28th days of treatment compared to the values of diabetic control rats (Table 2). The marked increase of plasma insulin may be due to the stimulation of insulin release from the existing β-cells of the pancreas. Alternatively, *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) treatment showed significant reduction in FBG levels at the 14th and 28th days of treatment, compared to the control values of diabetic rats. The reduction in FBG level of diabetic animals medicated with both extracts might be due to the increase in releasing of insulin, which in turn, stimulates the utilization of glucose by the tissues. As expected, treatment with glibenclamide improved the FBG and insulin levels in plasma of diabetic rats.

In hyperglycemic animals, Hb contents were found to be reduced when compared to normal rats, as Hb synthesis might also be depressed. *Ferula assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) treated rats showed increased levels of Hb because of their glucose lowering effect. In presence of hyperglycemia, there was an increase glycosylation of a number of proteins including Hb. The excess blood glucose reacts with Hb to form HbA1c (Koenig *et al*., 1976). Hence, the level of HbA1c is observed

Table 3: Levels of total hemoglobin and HbA1c of the experimental rats after 28 days of treatment with *Ferula assa-foetida* and *Ferula tenuissima* extracts (n = 6)

Groups	Total hemoglobin (mg dL^{-1})	HbA1c (% Hb)
Normal control	14.4±0.67‡	4.8±0.18‡
Diabetic control	10.1±0.53*	13.3±0.37*
DC+*F. assa-foetida* (200 mg kg^{-1})	12.6±0.61*‡	7.3±0.24*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	12.9±0.51*‡	7.1±0.28*‡
DC+*F. tenuissima* (200 mg kg^{-1})	10.5±0.67*	11.8±0.35*
DC+*F. tenuissima* (400 mg kg^{-1})	12.8±0.69*‡	8.7±0.21*‡
DC+Glibenclamide	13.9±0.58*‡	5.2±0.24*‡

DC: Diabetic control, HbA1c: glycosylated hemoglobin, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

Table 4: Levels of TG, TC, HDL-C and LDL-C in plasma of the experimental rats after 28 days of treatment with *Ferula assa-foetida* and *Ferula tenuissima* extracts (n = 6)

Groups	TG (mg dL^{-1})	TC (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})
Normal control	41.6±2.63‡	49.4±2.85‡	27.8±0.85‡	15.3±0.27‡
Diabetic control	74.2±2.39*	70.2±3.22*	19.5±0.33*	39.3±0.36*
DC+*F. assa-foetida* (200 mg kg^{-1})	62.8±2.63*‡	61.7±2.35*‡	22.3±0.46*‡	28.5±0.52*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	58.3±3.85*‡	56.5±2.71*‡	23.6±0.25*‡	25.3±0.44*‡
DC+*F. tenuissima* (200 mg kg^{-1})	73.7±3.88*	69.6±3.35*	20.3±0.49*	37.7±0.83*
DC+*F. tenuissima* (400 mg kg^{-1})	75.2±2.84*	68.4±3.79*	21.4±0.45*	37.4±0.88*
DC+glibenclamide	72.6±3.72*	59.3±3.26*‡	24.7±0.44*‡	19.6±0.36*‡

TG: Triglyceride, TC: Total cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, DC: Diabetic control, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

as a dependable indicator of diabetes control. In the present study, the diabetic rats have showed increased levels of HbA1c compared to normal controls (Table 3). Diabetic rats treated with *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) showed a marked reduction in HbA1c levels that might be due to the hypoglycaemic activity of both extracts. The improvement in HbA1c levels was predictable as an outcome of decreased glycemia upon *F. assa-foetida* and *F. tenuissima* treatments.

The probable antidiabetic mechanism of both extracts action could be linked to protection of β-cells of the islets of Langerhans against destructive effect of STZ. Second, they may trigger the restoration of partially degenerated β-cells, as pancreatic endocrine cells have the chance to proliferate after induction of STZ diabetes (Risbud and Bhonde, 2002). In addition, the antidiabetic effect of *F. assa-foetida* and *F. tenuissima* may be related to the antioxidant property of some of their active constituents. Earlier phytochemical investigation of *Ferula* plants led to the characterization of several flavonoids (Dehpour *et al*., 2009). It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas β-cells regeneration ability (Chattopadhyay, 1999). They also exert a stimulatory effect on insulin secretion by changing Ca^{++} concentration (Hii and Howell, 1985).

Effect on plasma lipid profile: The abnormal elevated level of plasma lipids in DM is mostly due to the uninhibited effect of lipolytic hormones on the fat stores mainly due to the effect of insulin. In normal condition, insulin stimulates lipoprotein lipase enzyme, which break down TG. However, in diabetic case lipoprotein lipase is not stimulated due to insulin insufficiency resulting in hypertriglyceridaemia (Pari and Satheesh, 2004). In addition, insulin insufficiency is linked with hypercholesterolaemia, because it has depressant action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of LDL-C (Saklani *et al*., 2012).

In the present study, there was marked increase in TG, TC and LDL-C levels and decrease in the plasma levels of HDL-C of diabetic rats. *Ferula assa-foetida* (200 and 400 mg kg^{-1}) significantly reduced the plasma levels of TG, TC and LDL-C; however, the plasma level of HDL-C was significantly elevated when compared to the diabetic control rats (Table 4). This implies that *F. assa-foetida* can reduce the complications of hyperlipidemia seen in some diabetics in whom hyperglycaemia and hypercholesterolaemia coexist quite often. The decline in plasma lipid profiles may be related to amelioration in insulin levels upon *F. assa-foetida* treatment. It is known that dosing of insulin to diabetic patients not only elevates lipoprotein lipase activity, but also reduces the plasma TG concentrations (Langhi and Cariou, 2010). These results are similar to earlier reports of hypocholesterolemic effect of *F. assa-foetida* wherein it was shown to decrease cholesterol absorption in rats (Nadkarni, 1976). In this study, *F. tenuissima* did not decrease plasma TG and TC levels suggesting no antihypertriglyceridaemic or antihypercholesterolaemic effects. Treatment with glibenclamide significantly decreased the blood levels of TC and LDL-C. However, the level of HDL-C significantly increased. Glibenclamide did not significantly change the blood level of TG, when compared to diabetic control rats.

Table 5: Activities of SOD, GPx and CAT and levels of GSH and MDA in liver homogenate of the experimental rats after 28 days of treatment with *F. assa-foetida* and *Ferula tenuissima* extracts (n = 6)

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	55.3±1.73‡	3.7±0.10‡	16.5±0.45‡	12.2±0.14‡	45.2±1.63‡
Diabetic control	32.7±1.24*	1.5±0.11*	9.3±0.21*	7.3±0.17*	77.8±2.73*
DC+*F. assa-foetida* (200 mg kg^{-1})	41.5±1.25*‡	2.2±0.11*‡	12.2±0.19*‡	9.2±0.15*‡	62.7±1.42*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	45.8±1.86*‡	2.5±0.12*‡	13.0±0.18*‡	9.5±0.12*‡	59.5±1.50*‡
DC+*F. tenuissima* (200 mg kg^{-1})	35.2±1.74*	1.6±0.11*	9.5±0.12*	7.6±0.15*	73.6±2.33*
DC+*F. tenuissima* (400 mg kg^{-1})	41.6±1.85*‡	2.4±0.17*‡	12.2±0.14*‡	8.5±0.11*‡	62.6±2.59*‡
DC+Glibenclamide	44.3±1.55*‡	3.0±0.13*‡	14.3±0.26*‡	10.2±0.15*‡	53.6±1.94*‡

SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Caralase, GSH: Glutathione, MDA: Melondialdihyde, DC: Diabetic control, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

Table 6: Activities of SOD, GPx and CAT and levels of GSH and MDA in pancreatic homogenate of the experimental rats after 28 days of treatment with *Ferula assa-foetida* and *Ferula tenuissima* extracts (n = 6)

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	43.8±1.75‡	3.5±0.15‡	19.4±0.36‡	9.3±0.29‡	39.2±1.16‡
Diabetic control	27.5±1.34*	1.7±0.11*	12.7±0.17*	5.3±0.25*	55.7±2.25*
DC+*F. assa-foetida* (200 mg kg^{-1})	33.8±1.63*‡	2.2±0.15*‡	14.5±0.27*‡	7.1±0.19*‡	48.3±1.55*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	34.2±1.34*‡	2.4±0.13*‡	15.4±0.23*‡	7.7±0.15*‡	45.7±1.74*‡
DC+*F. tenuissima* (200 mg kg^{-1})	28.8±1.55*	1.7±0.13*	13.1±0.09*	5.5±0.14*	52.2±2.73*
DC+*F. tenuissima* (400 mg kg^{-1})	34.7±1.34*‡	2.8±0.15*‡	15.5±0.17*‡	7.4±0.19*‡	47.3±2.50*‡
DC+Glibenclamide	35.8±1.38*‡	2.9±0.13*‡	16.3±0.29*‡	8.0±0.20*‡	44.7±1.42*‡

SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Caralase, GSH: Glutathione, MDA: Melondialdihyde, DC: Diabetic control, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

Table 7: Activities of ALT, AST and ALP and levels of bilirubin and total protein in plasma of the experimental rats after 28 days of treatment with *Ferula assa-foetida* and *Ferula tenuissima* extracts (n = 6)

Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Bilirubin (mg dL^{-1})	Total protein (g dL^{-1})
Normal control	49.4±1.48‡	70.2±2.73‡	92.2±3.28‡	0.51±0.03‡	8.3±0.18‡
Diabetic control	116.7±5.45*	153.5±6.50*	185.7±7.83*	1.26±0.08*	4.9±0.17*
DC+*F. assa-foetida* (200 mg kg^{-1})	96.4±4.54*‡	116.6±5.49*‡	147.2±5.33*‡	0.83±0.05*‡	6.4±0.11*‡
DC+*F. assa-foetida* (400 mg kg^{-1})	85.3±4.38*‡	95.8±4.74*‡	135.8±4.71*‡	0.77±0.04*‡	6.9±0.12*‡
DC+*F. tenuissima* (200 mg kg^{-1})	113.5±6.35*	144.5±5.84*	165.8±6.30*	1.14±0.09*	7.8±0.17*
DC+*F. tenuissima* (400 mg kg^{-1})	87.6±4.15*‡	99.4±4.88*‡	117.5±5.72*‡	1.17±0.08*‡	7.9±0.14*‡
DC+Glibenclamide	61.8±3.55*‡	82.7±3.38*‡	116.9±5.85*‡	0.60±0.03*‡	7.7±0.16*‡

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, *Significantly different from the values of normal control rats at $p<0.05$, ‡Significantly different from the values of DC rats at $p<0.05$

Effect on oxidative stress markers in hepatic and pancreatic tissues: Oxidative stress has been shown to play a role in the etiology of DM and thus, antioxidants may have a role in the reduction of diabetes. The STZ creates Reactive Oxygen Species (ROS) in the body, which induce pancreatic damage and could be responsible for hyperglycemia seen in animals. Low levels of insulin in DM also increase the activity of fatty acyl coenzyme-A-oxidase which initiates beta oxidation of fatty acids resulting in LPO and membrane damage. Estimation of MDA level provides an indicator of LPO which is one of the major mechanisms of cell injury. In the present study, STZ diabetes showed an increase in LPO in liver and pancreas homogenates compared with that of the normal control group indicating oxidative stress. *Ferula assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) extracts significantly reduced the MDA levels in the liver and the pancreas tissues of diabetic rats (Table 5-6) indicating their protective role during oxidative damage.

In addition to the changes in LPO, diabetic animals showed reduced activity of the key antioxidant enzymes viz. SOD, GPx and CAT and level of GSH in liver and pancreas homogenates. *Ferula assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) extracts showed a significant restoration in SOD, GPx and CAT activities and GSH level in liver and pancreas of diabetic rats (Table 5-6). The present results suggest that the possible mechanism of action by extracts could be related to their antioxidants activity. This may be due to the presence of phenols and flavonoids which have been shown to possess significant antioxidant activities (Van Acker *et al*., 1996).

Effect on markers of liver injury: Estimation of liver functions can be made by assessing plasma activities of liver marker enzymes (ALT, AST and ALP) and level of BIL. The elevation of liver biomarker enzymes has been observed in diabetic rats indicating hepatic damage (Kondeti *et al*., 2010). In addition, the elevated plasma level of BIL is the usual indicator of liver injury. In our study, STZ-diabetic rats developed marked hepatic injury as indicated by a significant increase in the activities of ALT, AST and ALP and level of BIL in plasma as compared with the normal control animals. The elevated serum activities of liver marker enzymes observed in diabetic control rats can be related to the injured structural integrity of the hepatocytes because these are cytoplasmic in nature and are liberated into blood stream after cellular damage (Chaitanya *et al*., 2012). In addition, the

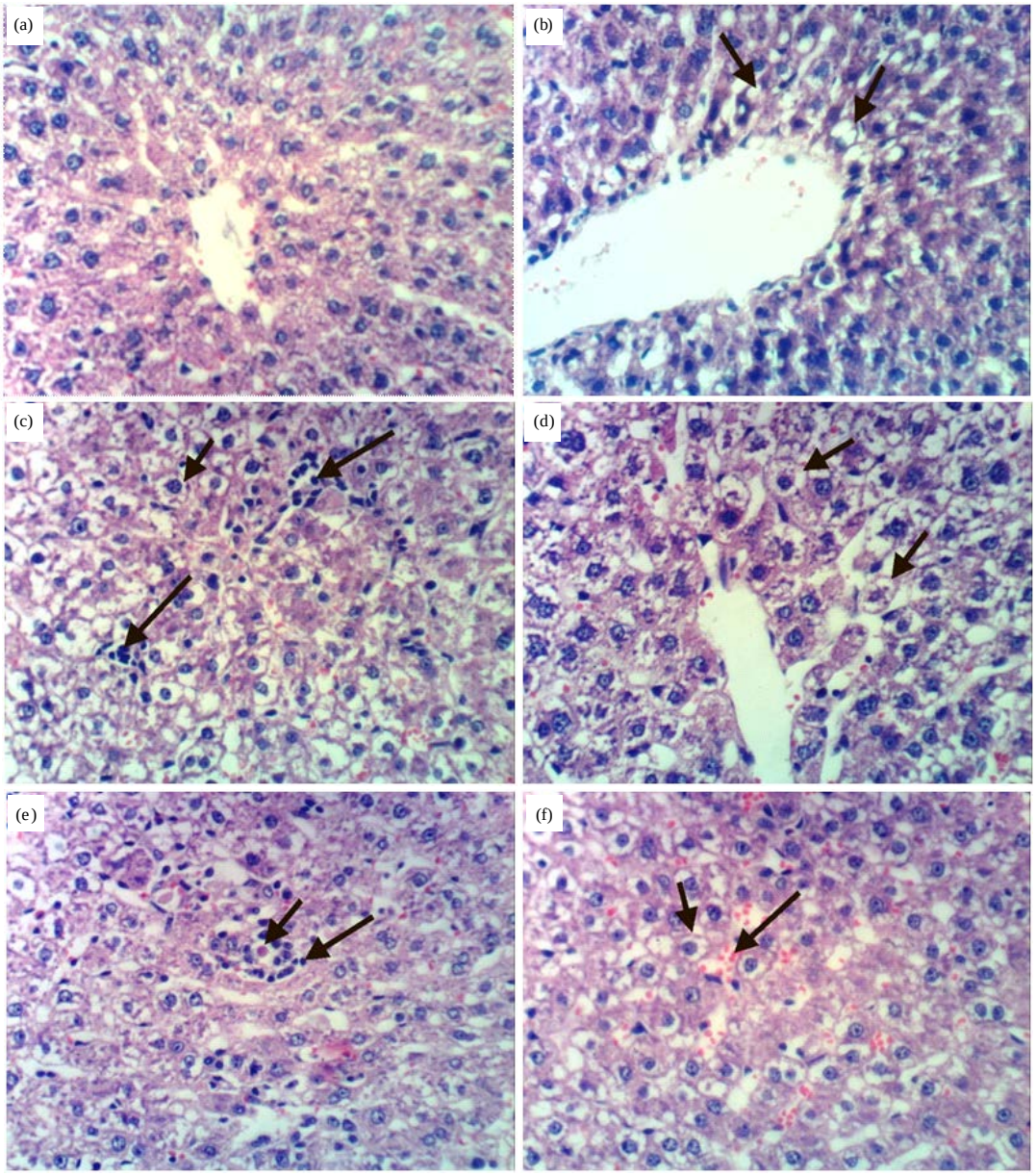

Fig. 2(a-f): Liver of rat from (a) Normal control group, (b) Diabetic control group, (c) Group treated with 200 mg kg^{-1} *Ferula assa-foetida*, (d) Group treated with 400 mg kg^{-1} *Ferula assa-foetida*, (E) Group treated with 200 mg kg^{-1} *Ferula tenuissima* and (f) Group treated with 400 mg kg^{-1} *F. tenuissima* (H and E X 400)

abnormal level of BIL in plasma of diabetic rats could be attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction (Blanckaert and Schmid, 1982). Administration of *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) to diabetic rats appear to be effective in reducing the increased activities of liver marker enzymes and level of BIL in plasma (Table 7).

Variation of plasma levels of total protein can reflect liver health status. In this study, it was noticed that STZ reduced the plasma level of total protein, compared with the normal controls. The reduction in plasma total protein may be due to increased rate of amino acids conversion to glucose and reduction of ribosomal protein synthesis (Rawi *et al*., 2011). Treatment with *F. assa-foetida* (200 and 400 mg kg^{-1}) and *F. tenuissima* (400 mg kg^{-1}) extracts showed a significant reversal of total protein levels toward the normal values and suggested the stabilization of endoplasmic reticulum that are responsible for protein synthesis.

Histopathological findings: Histologically, liver structure was normal in non-diabetic rats (Fig. 2a). Liver of diabetic control rats showed inflammatory cell infiltration and cytoplasmic

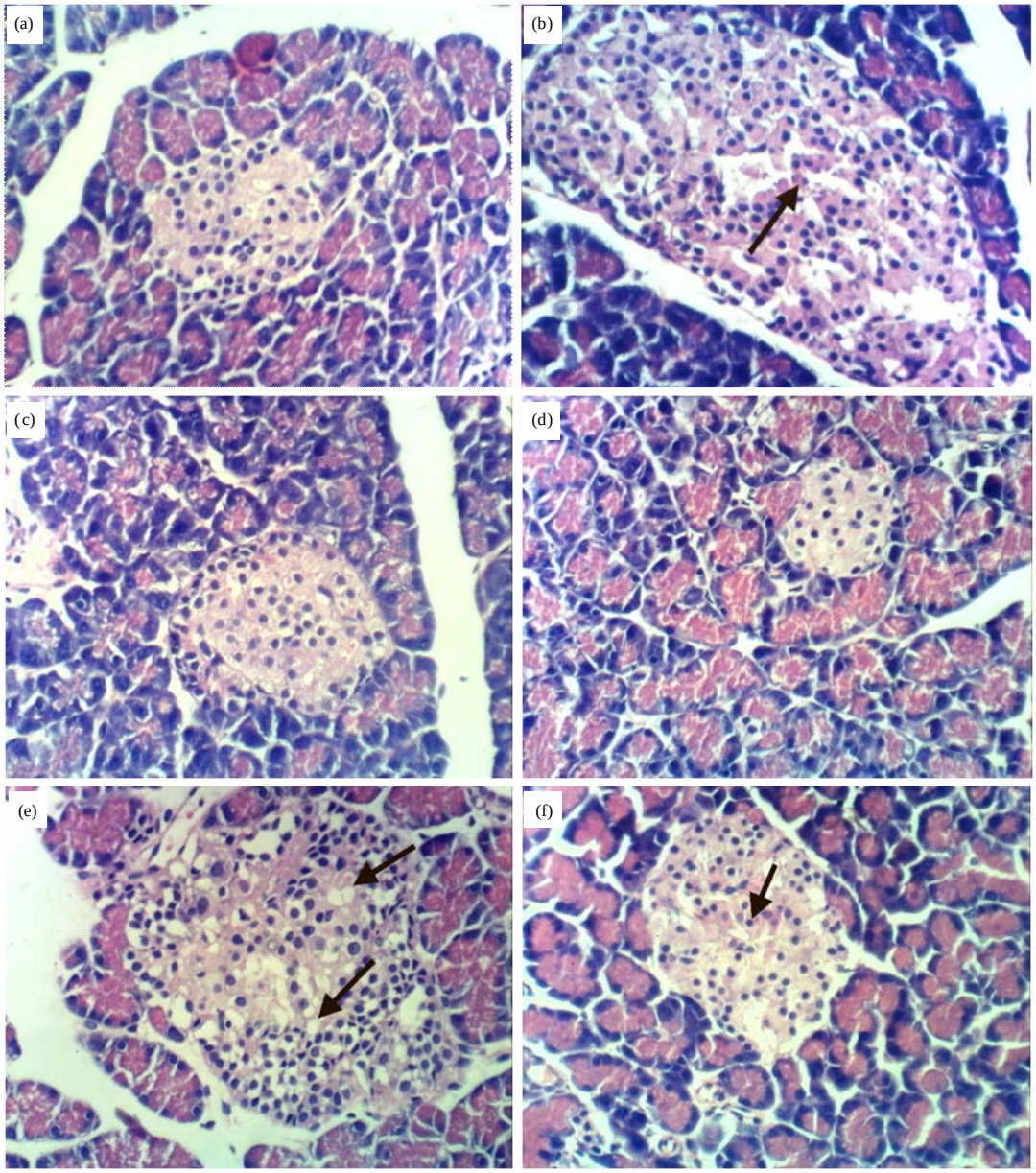

Fig. 3(a-f): Pancreas of rat from (a) Normal control group, (b) Diabetic control group, (c) Group treated with 200 mg kg^{-1} *Ferula assa-foetida*, (d) Group treated with 400 mg kg^{-1} *Ferula assa-foetida*, (e) Group treated with 200 mg kg^{-1} *Ferula tenuissima* and (f) Group treated with 400 mg kg^{-1} *F. tenuissima* (H and E X 400)

vacuolization of centrolobular hepatocytes (Fig. 2b). Oral treatments of diabetic rats with *F. assa-foetida* extract (200 mg kg^{-1}) showed hydropic degeneration of hepatocytes and sinusoidal leukocytosis (Fig. 2c), while 400 mg kg^{-1} repaired the morphological alterations in the liver toward normal (Fig. 2d). Livers of *F. tenuissima* treated rats (200 mg kg^{-1}) showed apoptosis of hepatocytes and sinusoidal leucocytosis (Fig. 2e), while those received 400 mg kg^{-1} showed slight hydropic degeneration of hepatocytes and congestion of hepatic sinusoids (Fig. 2f).

Pancreas of the normal control rats shows the normal lobular histological structure of pancreatic acini and langerhans islets cells as shown in Fig. 3a. Different records have shown that the islets appear to be preferentially affected in DM by damaging insulin-secreting β-cells (Kondeti *et al*., 2010). Figure 3b shows pancreatic tissues of diabetic control rats with necrosis and vacuolations of pancreatic acini and Langerhans islets cells. Treating diabetic rats with 200 and 400 mg kg^{-1} of *F. assa-foetida* extract nearly restored the pancreatic tissues to its normal state without histopathological

changes as shown in Fig. 3c-d. Oral treatments of diabetic rats with *F. tenuissima* extract (200 mg kg^{-1}) showed vacuolations of cells of islet's of langerhan's (Fig. 3e), while treatment with 400 mg kg^{-1} restored its normal structure without any pathological changes (Fig. 3f).

CONCLUSION

In conclusion, *F. assa-foetida* and *F. tenussima* extracts have an antidiabetic activity. *Ferula assa-foetida* has an antihyperlipidemic effect. The probable mechanism of antidiabetic activity of *F. assa-foetida* and *F. tenuissima* extracts may be through a stimulation of insulin secretion from the remnant pancreatic β-cells or may in part be due to their antioxidant activity. However, further studies are in progress in our laboratory to explain the exact mechanisms of action, as well as identify their active phytochemical constituents.

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