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Antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes

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Abstract: *Fumaria officinalis* belongs to family papaveraceae and is traditionally used to treat hypertension, hepatitis and diabetes. The current study was conducted to evaluate in vitro and in vivo antidiabetic activity of *Fumaria officinalis*. Aerial parts of the plant were sequentially extracted with n-hexane, chloroform, methanol and water. Phytochemical analysis was carried out on all extracts. Antioxidant activity was determined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) inhibition method. *In vitro* alpha-amylase inhibitory activity was performed on all extracts by using dinitrosalicylic acid. Effect of aqueous and methanolic extracts of *F. officinalis* on blood glucose was evaluated in normo-glycaemic rats and alloxan induced diabetic rats. Glimepiride 0.2 mg/kg was used as standard therapy in diabetic rats. Results showed that methanolic extract exhibited the maximum percentage inhibition of DPPH (86.30%) and alpha-amylase inhibition (94.01%) at 500 µg/ml and 16 mg/ml concentration respectively. Administration in normo-glycaemic rats did not show any significant decrease in blood glucose level at 500 and 750 mg/kg dosage. Aqueous and methanolic extracts exhibited a significant hypoglycaemic effect ($p<0.05$) at all doses. A significant increase in the body weight and an improvement in liver and kidney function tests of diabetic rats were observed. These extracts also reduced the damage to the cells of glomeruli, interstitial inflammation, necrosis of tubular cells and thrombosis in the kidney, the enlargement of sinusoids and steatosis in the liver of diabetic rats. This study concludes that *F. officinalis* may have antidiabetic potential possibly due to its antioxidant and alpha-amylase inhibitory activities.

Key words: Diabetes mellitus; Antioxidant; Ethnomedicine; Nutraceutical; Histopathology; Alpha-amylase inhibitor; Antioxidant.

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder in which chronic hyperglycemia occurs due to insufficient release or any defect in the action of insulin. Long-term damage to several organs especially kidneys, heart, blood vessels, eyes and nerves may occur due to improper control over DM (1). Common symptoms of DM include polydipsia, polyuria, weight loss and blurred vision. Long-term complications such as irregularities in lipoprotein metabolism, retinopathy, nephropathy and autonomic neuropathy are associated with DM. Cardiovascular, gastrointestinal, sexual and genitourinary disturbances may be evident as a result of DM induced neuropathy (2).

Two major types of DM affect humans. Type I DM mainly depends upon insulin whereas type 2 is non-insulin dependent. DM affects approximately 20 million individuals from all age groups worldwide (3). The annual rise in the prevalence of DM is about 5.49% in teenagers of less than 15 years. The pathogenesis of type 2 DM is complex. Obesity, genetic and environmental factors are the primary contributors to type 2 DM and cause insulin resistance in liver and muscles. These factors lead to the destruction of β cells and disrupt the regular secretion of insulin (4). Meanwhile, type 2 DM is responsible for 90% cases of DM. Its prevalence is higher in developing countries (69%) than the developed (20%). Geriatrics are less affected than the younger ones (5). The rate of type 2 DM in developed countries is rising sharply due to obesity, fat rich diet and decreased physical activity. Insulin secretion and insulin sensitivity are the fundamental tools for the regulation of blood glucose level. Recent studies have shown that the pre-diabetes and diabetes do not occur until the peripheral resistance of insulin is not compensated by pancreatic beta cells (6).

Any disruption in the balance of pro- and anti-oxidants due to drugs, xenobiotics, aging, addiction and inflammation is known as oxidative stress. The excessive production and inadequate deduction of the reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) lead to oxidative stress (7). Free radicals activate extracellular-signal-regulated kinase (ERK) and Mitogen activated protein kinase (MAPK) pathways. These pathways play a vital role in cell death. ROS are involved in gene transcription, signal transduction and regulation of other events

in the cell cycle. Oxidative stress is the foremost cause of the progression of type 2 DM. The elevated levels of ROS in DM produce oxidative stress that culminates in vascular complications (8). The decreased production or inhibition in synthesis of endogenous antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase elevates the level of ROS in the body (9). Epidemiological studies have shown that the death rate in DM patients is higher due to vascular complications than hyperglycemia (8). Use of exogenous antioxidants may therefore decrease the vascular complications and delay the progression of DM.

Treatment strategies for DM include use of hormone replacement therapy with insulin and oral hypoglycemic agents. Oral hypoglycemic agents act through a variety of mechanisms including increased production of insulin, increased utility and decreased production of glucose (10). A decrease in the formation of glucose may occur through inhibition of alpha-glucosidase and alpha-amylase. However, about 80% population in developing countries depends on traditional therapies for the prevention, management and cure of ailments. Several biologically active substances are present in medicinal plants. Local people in rural and remote areas utilize plants for cure because of their efficacy, cost effectiveness and lack of modern health facilities (11).

The plant, *F. officinalis* belongs to family Papaveraceae. It is widely found in Pakistan, Turkey and India (12). It is used traditionally as a potent hepatoprotective, hypotensive, blood purifier, diuretic, antidiabetic and antioxidant plant (13). It is also helpful in rheumatism, abdominal cramps, fever, conjunctivitis and diarrhoea (14). Recent studies carried out on *F. officinalis* demonstrated its pharmacological use in the treatment of hyperglycemia, hyperthermia, helminthic infections and as antimicrobial agent against Methicillin resistant *staphylococcus aureus* (MRSA) (12). *F. officinalis* comprises of tannins, potassium salts and microsomal enzyme inducer alkaloids such as protopine and allocryptopine (15).

The present study was designed to investigate the *in vitro* antioxidant and alpha-amylase inhibitory activities and *in vivo* anti-diabetic potential of the different extracts of *F. officinalis* to validate its folklore use as an antidiabetic agent.

Materials and Methods

Plant collection, identification and extraction

Aerial parts of *F. officinalis* were collected from the northern areas of Pakistan. The voucher number confirming identification "LHA# 032017A" of *F. officinalis* was obtained from the Department of Botany, University of the Punjab, and the plant specimen was deposited in a herbarium.

The aerial part of the plant was washed, shade dried and grounded to coarse particles. Four different extracts of *F. officinalis* (n-Hexane, chloroform, methanol and aqueous) were prepared sequentially by hot extraction technique from low to high polarity. The filtrates were concentrated by using a rotary evaporator at low temperature (40-45°C) under reduced pressure and dried extracts were kept in airtight containers in a refrigerator until further studies.

Preliminary phytochemical analysis

All the plant extracts were subjected to different phytochemical tests for the detection of alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, steroids, fixed oil/fats, glycosides, proteins, amino acids, gums and mucilages according to the methods described previously (16).

Evaluation of antioxidant activity

The antioxidant activity of aqueous, methanolic, chloroform and n-Hexane extracts of *F. officinalis* was evaluated by 2,2-diphenyl-1-picryl hydrazyl (DPPH) inhibition method at 500, 250, 125, 62.50 and 31.25 µg/ml concentration according to the previously described method with slight modifications (17). DPPH solution was prepared by adding 0.004 mg DPPH in 100 ml methanol. Methanol (1 ml) and DPPH (2 ml) was used as control solutions. Ascorbic acid served as standard antioxidant drug. 1 ml of each test solution was added to test tube along with freshly prepared solution of DPPH (2 ml) and methanol (1 ml). The mixture was shaken briskly and allowed to stand in dark for 30 min at 25ºC. The absorbance was measured at 517 nm wavelength. The experiment was repeated in triplicate. The percentage inhibition of DPPH radical was calculated by the following equation (18).

Percentage inhibition (%) = (absorbance of control reaction − absorbance of test or standard drug) × 100 / absorbance of control reaction

Alpha-amylase inhibitory activity

The in-vitro alpha amylase inhibitory activity was performed on aqueous, methanolic, chloroform and n-hexane extracts of *F. officinalis* at 2, 4, 8, 16 and 32 mg/ml concentration according to the previous method with slight modifications (19). 1 ml extract and 1ml alpha-amylase solution (prepared in 1 mg/ml of sodium phosphate buffer pH 6.9) were mixed and incubated for 30 min at 25ºC. 1 ml starch (1% w/v solution in pH 6.9 buffer) was added to each test tube. Test tubes containing reaction mixture were incubated for 10 min at 37ºC. Dinitrosalicyclic acid (DNS) reagent (1 ml) was added to each tube and reaction mixture was heated on a water bath for 5 min at 90ºC and cooled to room temperature. Distilled water was added to make the final volume up to 10 ml. The absorbance was measured at 540 nm using UV-visible spectrophotometer. The absorbance of control (containing buffer instead of the test sample) and blank (containing buffer instead of alpha-amylase solution) were also measured. Acarbose was used as a standard drug. The percentage inhibition of alpha-amylase was calculated by using the above formula (10).

Experimental animals

Wistar rats of either sex weighing 150–200 g were purchased from The University of Lahore. All the experimental animals were housed at 25 ± 2°C in a 12 h light and dark cycle. All the animals received rodent standard pellet diet and water *ad libitum*. All animals were kept in plastic cages at room temperature until end of the experiment. The study was approved from and performed according to the guidelines of the Animal Ethics Committee of the University of Lahore.

Oral glucose tolerance test (OGTT) in normoglycemic rats

Wistar rats were divided into five groups with 5 rats in each group. 500 and 750 mg/Kg aqueous and methanolic extracts were administered orally in separate groups for 7 days. Animals were fasted overnight on the specified days. The blood glucose level of all the experimental animals was measured before glucose administration. Aqueous and methanolic extracts at doses of 500 and 750 mg/kg were orally administered to normal rats (non- diabetic), 2 g/kg glucose solution was administered orally after 10 min of dosing. The blood glucose level was determined 3 h post glucose administration (20).

Anti-diabetic activity against Alloxan induced diabetes

Freshly prepared alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) was administered at a single dose of 150 mg/Kg through i.p. route in the rats (21). Fasting blood glucose level in Wistar rats was measured with glucometer 72 h post- alloxan administration. Rats exhibiting fasting blood glucose level of more than 200 mg/dl were selected for antidiabetic activity (22). Normal control rats (non-diabetic) and negative control (untreated diabetic rats) rats received normal saline orally. Glimepiride (0.2 mg/kg) p.o. served as standard therapy. Aqueous and methanolic extracts were evaluated for their antidiabetic potential in diabetic rats at 250, 500 and 750 mg/Kg dose.

The fasting blood glucose level in rats was determined daily. The body weight of all rats was recorded. After 14 days of therapy, rats were anesthetized and killed by cervical dislocation. Blood was collected by heart puncture for haematological evaluation, whereas liver and kidney function tests were carried out to determine the effect of plant extract on secondary changes in DM. The liver and kidney were removed and stored in 10% formalin. Tissues were embedded in paraffin wax and 5 µm sections were prepared using a semi-automated rotary microtome. The tissue sections were stained with hematoxylin and eosin (23). Tissue sections were observed under a microscope and pathological lesions were scored on a scale of 5.

Statistical analysis

Antioxidant and alpha-amylase inhibitory activities were evaluated by one way ANOVA followed by post hoc test. OGTT was evaluated by one way ANOVA. Body weight, ALT, ALP, AST and blood urea level were statistically analysed by one way ANOVA followed by a Tukey's multiple comparison test. Data of alloxan induced diabetes were analysed by two way repeated measure ANOVA followed by Tukey's post hoc test. The statistical analysis was performed by GraphPad Prism (version 6) software.

Results

Aerial parts of *F. officinalis* were extracted sequentially with n-hexane, chloroform, methanol and distilled water. The % age yields of n-hexane, chloroform, methanol and aqueous extracts of *F. officinalis* were found to be 0.6, 1.3, 4.8 and 9.9% w/w respectively.

Preliminary phytochemical analysis

Phytochemical analysis of *F. officinalis* extracts showed that the alkaloids, saponins and flavonoids were present in aqueous and methanolic extracts. Methanolic extracts also contained phenols, tannins and steroids. The chloroform and n-hexane extracts confirmed the presence of fats and fixed oils. Chloroform extract also confirmed the presence of proteins. Qualitative phytochemical analysis of different extracts of *F. officinalis* is shown in Table 1.

Antioxidant activity

The antioxidant potentials of all the extracts were significantly lower than the standard drug (Ascorbic acid) at the corresponding concentrations. Aqueous extract showed the highest antioxidant activity among all the extracts at respective concentrations. The results showed that percentage inhibition of DPPH shown by ascorbic acid was significantly higher than the corresponding concentration of plant extracts. However, statistically analysed data showed that the percentage inhibition of DPPH exhibited by methanolic and aqueous extracts was statistically comparable to that of ascorbic acid at 500 µg/ml concentration. The antioxidant activity of aqueous extract was the most pronounced among all plant extracts. Antioxidant activity of the different extracts of *F. officinalis* is shown in Table 2.

Alpha-amylase inhibitory activity

All the plant extracts showed variable values of alpha-amylase inhibition in terms of percentage. Howe-

Table 1. Qualitative phytochemical analysis of *Fumaria officinalis* extracts.

Metabolites	Name of Tests	Aqueous	Methanol	Chloroform	n-Hexane
Alkaloids	Mayer's Wagner's & Hager's test	+	+	_	_
Carbohydrates	Fehling's & Benedict's test	_	_	_	_
Saponins	Foam test	+	+	-	-
Phenols	Ferric chloride & Gelatine test	-	+	-	-
Tannins	Lead acetate test	-	+	_	_
Flavonoids	Alkaline reagent test	+	+	_	_
Steroids		_	+	_	_
Fixed oil/fats	Spot test	_	_	+	+
Glycosides	Borntrage's & Legal's test	_	_	_	_
Proteins and amino acids	Millon's, Biuret & Ninhydrin test	_	-	+	_
Gums and mucillages	_	_	_	_	_

Table 2. Antioxidant activity of *Fumaria officinalis* extracts expressed as Percentage inhibition of DPPH.

Concentrations (µg/ml)	Percentage inhibition of DPPH				
	Ascorbic acid	Methanol	Aqueous	Chloroform	n-Hexane
500	94.05±1.07	86.3±2.91$^{n.s.}$	84.0±2.43$^{n.s.}$	73.4 ± 0.75*	68.3±2.27*
250	90.81±2.41	64.3±1.76*	81.66±2.93$^{n.s.}$	30.03 ± 2.25*	40.36±5.19*
125	87.94±4.51	43.7±1.0*	64.66±4.24*	25.25 ± 2.50*	25.30±3.32*
62.5	85.01±3.04	35.5±2.71*	48.66±1.05*	20.47 ± 3.91*	32.5±1.07*
31.25	80.96±2.79	28.2±1.37*	35.66±2.99*	21.50 ± 4.0*	31.32±2.81*

Where * shows the statistically significant difference when the % inhibition of extract was compared to ascorbic acid at the corresponding concentrations.

Table 3. Percentage inhibition of alpha-amylase activity with different extracts of *Fumaria officinalis.*

Concentrations (mg/ml)	Percentage inhibition of Alpha-amylase				
	Acarbose	Methanolic	Aqueous	Chloroform	n-Hexane
16	97.23±1.29	94.85±1.27$^{n.s.}$	84.29±1.07*	74.70±2.99*	80.85±1.46*
8	93.74±2.43	81.10±2.94$^{n.s.}$	83.42±2.50$^{n.s.}$	70.77±1.0*	79.42±2.24*
4	91.64±2.18	49.85±3.11*	81.00±2.07$^{n.s.}$	48.70±0.27*	61.56±1.90*
2	90.89±1.62	23.57±3.07*	80.42±1.12$^{n.s.}$	42.8±3.91*	49.57±4.51*

Where * shows the statistically significant difference when the % inhibition of extract was compared to ascorbic acid at the corresponding concentrations.

Table 4. Oral glucose tolerance test in non-diabetic rats treated with *Fumaria officinalis* extracts.

Treatment Days	Control		Methanolic extract of *Fumaria officinalis*				Aqueous extract of *Fumaria officinalis*			
	Normal saline		500 mg/Kg		750 mg/kg		500 mg/Kg		750 mg/kg	
	Before	After	Before Dosing	After Dosing	Before Dosing	After Dosing	Before Dosing	After Dosing	Before Dosing	After Dosing
Day 7	106± 6.41	114± 6.1	97± 5.20	82± 11.86*	112± 10.0	105± 6.0	113± 5.20	100 ± 4.10	136 ± 8.96	111± 9.58
Day 14	99± 10.1	110± 3.01	115± 2.44	110± 5.94	119± 7.16	107± 7.65	101± 9.24	95 ± 10.8	130 ± 6.01	114 ± 5.27

Where * shows statistically significant difference when the extract treated group was compared to that of corresponding control group ($p<0.05$).

ver, aqueous extract had shown the maximum inhibition of alpha-amylase activity at 8 mg/kg or lower concentrations among all extracts. Alpha-amylase inhibitory activities of all the extracts of *F. officinalis* are shown in Table 3.

OGGT in normo-glycaemic rats

The treatment with methanolic and aqueous extracts at 500 and 750 mg/kg doses for 14 days resulted in a reduction of OGGT in non-diabetic rats. It showed reduction in blood glucose level compared to rats treated with normal saline. However, no risk of mortality was observed at the highest dose of plant i.e. 750 mg/kg as shown in Table 4.

Anti-diabetic activity against Alloxan induced diabetes

Diabetic rats were treated with 250, 500 and 750 mg/kg dose of *F. officinalis* aqueous or methanolic extracts for 14 days. The hypoglycemic effect of *F. officinalis* and glimepride treatment was more pronounced during the second week of therapy. All the treatments exhibited a significant reduction in the blood glucose level compared to untreated diabetic rats on day 3 and later as shown in Table 5.

Effect on body weight

Untreated diabetic rats showed a significant decrease in body weight. Administration of *F. officinalis* in diabetic rats decreased the weight loss in most cases. However, weight gain was not evidenced while treating with *F. officinalis* aqueous (250 mg/kg) and methanolic (750 mg/kg) extracts. Effect of *F. officinalis* extracts on body weight of diabetic rats is shown in Figure 1.

Effect on liver and kidney function tests

Elevated levels of ALT, AST and ALP in diabetic rats compared to normal rats indicated the alloxan induced liver injury. Levels of ALT and bilirubin (direct and total) decreased in response to treatment with aqueous and methanolic extracts. Rise of AST and ALP, gamma GT, albumin, creatinine and urea were evidenced while treating with plant extracts. The effect of treatment with *F. officinalis* extracts on the liver and kidney function tests is shown in Table 6.

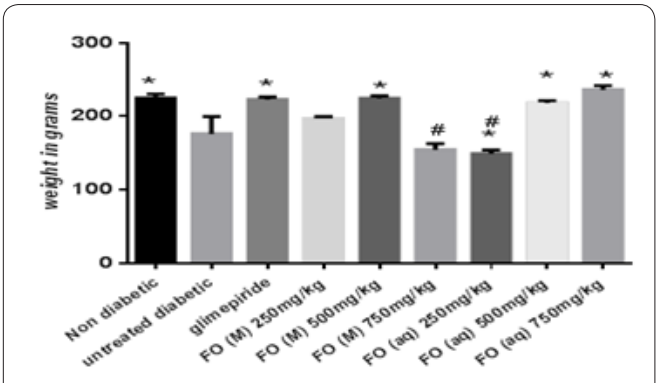

Figure 1. Effect of *Fumaria officinalis* extracts on body weight of diabetic rats.

Table 5. Effect of *Fumaria officinalis* on fasting blood glucose level (mg/dl) in alloxan-induced diabetic rats.

Treatments/ days	Non-diabetic rats	Disease control	Glimepiride	Methanolic extract of *F. officinalis*			Aqueous extract of *F. officinalis*		
				250 mg/Kg	500 mg/Kg	750 mg/Kg	250 mg/Kg	500 mg/Kg	750 mg/Kg
Day 1	105± 2.1	228± 17.9	248± 3.5	208± 9.7	227± 8.5	230± 7.1	210± 10.7	239± 15.4	239± 17.0
Day 2	108± 4.2	256± 12.9	158± 2.9*	170± 5.4*	200± 5.6*	213± 4.8	200± 7.5*	257± 7.4	201± 9.7*
Day 3	115± 3.9	301± 15.3	156± 4.4*	200± 3.4*	201± 7.1*	179± 3.5*	191± 9.1*	201± 4.9*	168± 5.0*
Day 4	100± 8.1	296± 16.0	134± 9.1*	168± 8.7*	184± 4.9*	174± 5.7*	169± 3.5*	204± 8.4*	121± 3.6*
Day 5	95± 10.3	299± 12.3	119± 3.1*	134± 2.2*	119± 4.6*	141± 3.2*	132± 3.5*	208± 10.7*	118± 3.8*
Day 6	88± 9.2	285± 13.0	125± 2.5*	121 ± 3.4*	115 ± 6.6*	127± 2.1*	128± 4.9*	181± 5.9*	111± 5.7*
Day7	110± 5.5	244± 14.6	117± 5.8*	105 ± 9.0*	119 ± 2.7*	111± 2.0*	101± 9.1*	174± 9.7*	100± 9.9*
Day8	108± 3.8	253± 14.9	100± 2.3*	115 ± 6.7*	117 ± 2.9*	119± 1.9*	107± 7.0*	152± 4.6*	105± 4.5*
Day9	106± 3.2	269± 13.5	114± 4.6*	122 ± 4.5*	116 ± 4.7*	127± 4.8*	100± 5.8*	162± 5.8*	109± 9.7*
Day10	96± 8.5	254± 15.9	116± 5.0*	102 ± 9.0*	110 ± 7.3*	120± 2.7*	104± 5.3*	175± 3.9*	107± 4.6*
Day11	111± 4.0	288± 16.0	100± 10.1*	123 ± 2.9*	119 ± 5.0*	122± 3.4*	112± 3.1*	116± 4.0*	101± 8.9*
Day12	93± 7.3	226± 17.9	105 ± 8.0*	111 ± 6.4*	105 ± 3.9*	110± 5.8*	121±3.4*	156±12.0*	94± 6.7*
Day13	99±6.9	221± 15.8	100 ± 5.8*	123 ± 2.5*	108 ± 2.5*	104± 9.4*	116±1.0*	88± 8.2*	101± 8.9*
Day 14	100±2.0	227 ± 13.0	96 ± 10.6*	116 ± 5.6*	105 ± 4.7*	98± 2.6*	109±2.1*	121±5.3*	100± 4.5*

Values are mean ± SD (n=05). Where * show the treated group was statistically different from the untreated diabetic control group ($p<0.05$).

Table 6. Effect of *Fumaria officinalis* on Liver and renal function markers of diabetic rats.

Markers	Normal Rats	Disease Control	Glimepiride	Aqueous extract of *F. officinalis*			Methanolic extract of *F. officinalis*		
				250 mg/kg	500 mg/kg	750 mg/kg	250 mg/kg	500 mg/kg	750 mg/kg
ALT (u/l)	26± 10.2	67±2.1	57± 8.50	62± 2.3*	57± 1.01*	51± 3.21*	62± 3.5	44± 2.3*#	62± 1.50*#
AST (u/l)	116± 11.0	113± 4.3	105± 5.7	155± 8.6#	133± 2.47*#	147± 1.50*#	154± 8.3#	145± 7.50*#	171± 3.56*#
ALP (u/l)	242± 2.04	165± 5.8	268± 7.80*	351± 19.7*#	296± 13.07*	204±16.07*#	276± 11.9*	252± 12.87*	226±12.94*#
Bilirubin Total (mg/dl)	0.1± 0.06	10.8±0.32	0.1± 0.04*	0.1± 0.03*	0.2± 0.07*	0.08± 0.02*	0.1± 0.05*	0.15± 0.07*	0.07± 0.05*
Bilirubin Direct (mg/dl)	0.01±0.005	3.88±0.6	0.03± 0.009	0.01±0.04*	0.02± 0.01*	0.01± 0.003*	0.1± 0.04*	0.03± 0.01*	0.02± 0.01*
Total Protein (g/dl)	6.7± 1.79	14.08±1.43	6.1± 1.13	5.6± 1.1*	5.8± 1.91*	6.2± 2.1*	5.9± 1.3*	6.5± 1.65*	5.8± 1.21*
Albumin (g/dl)	4.5± 7.04	0.1±0.03	4.6± 4.0	3.7± 1.01*	4.1± 1.14*	4.3± 1.23*	4.1± 1.02*	4.8± 1.05*	4.4± 1.43*
Gama GT (u/l)	3± 2.01	0.04±0.03	7.0± 4.02	6.3±1.29*	8± 1.01*	8.8± 1.47*	4.2±1.12*	5.8± 2.4*	5.2± 1.94*
Urea (mg/dl)	58.4± 4.51	0.01±0.006	36± 5.04	69.3±3.5*#	68.1±3.34*#	63.7± 1.37*#	31.8±2.7*	33.6± 2.80*	47.2± 1.04*#
Creatinine (mg/dl)	0.4± 0.9	0.03±0.001	0.3± 0.05	0.26±0.064	0.3± 0.037*	0.3± 0.04*	0.27±0.11*	0.3± 0.07*	0.2± 0.04*

* showed statistically significant difference ($p<0.05$) when *F. officinalis* treated groups were compared to diabetic group. # showed statistically significant difference ($p<0.05$) *F. officinalis* treated groups were compared to glimepiride treated group.

Histopathological evaluation

Kidney sections of untreated diabetic rats showed slight damage to the cells of glomeruli. Treatment with methanolic and aqueous extracts of *F. officinalis* decreased the damage to the cells of glomeruli. Neither Alloxan administration nor the extracts of *F. offininalis* were associated with glomerular sclerosis. Diabetic rats showed severe necrosis of tubular cells. Both extracts

of *F. officinalis* decreased the alloxan induced inflammation in diabetic rats. Administration of either extract of *F. officinalis* also ameliorated alloxan induced thrombosis in kidney blood vessels. Severe interstitial inflammation was noticed among diabetic rats. Administration of *F. officinalis* extracts decreased diabetes induced interstitial inflammation. The effect of both plant extracts (aqueous and methanolic) was most prominent at the 750 mg/Kg dose. Effects of F. officinalis on diabetic kidney are shown in Figure 2.

Liver sections of diabetic rats showed the enlargement of sinusoids, steatosis and steato-hepatitis. Treatment with aqueous and methanolic extracts of *F. officinalis* corrected these alloxan induced histopathological changes in Wistar rats (Figure 2).

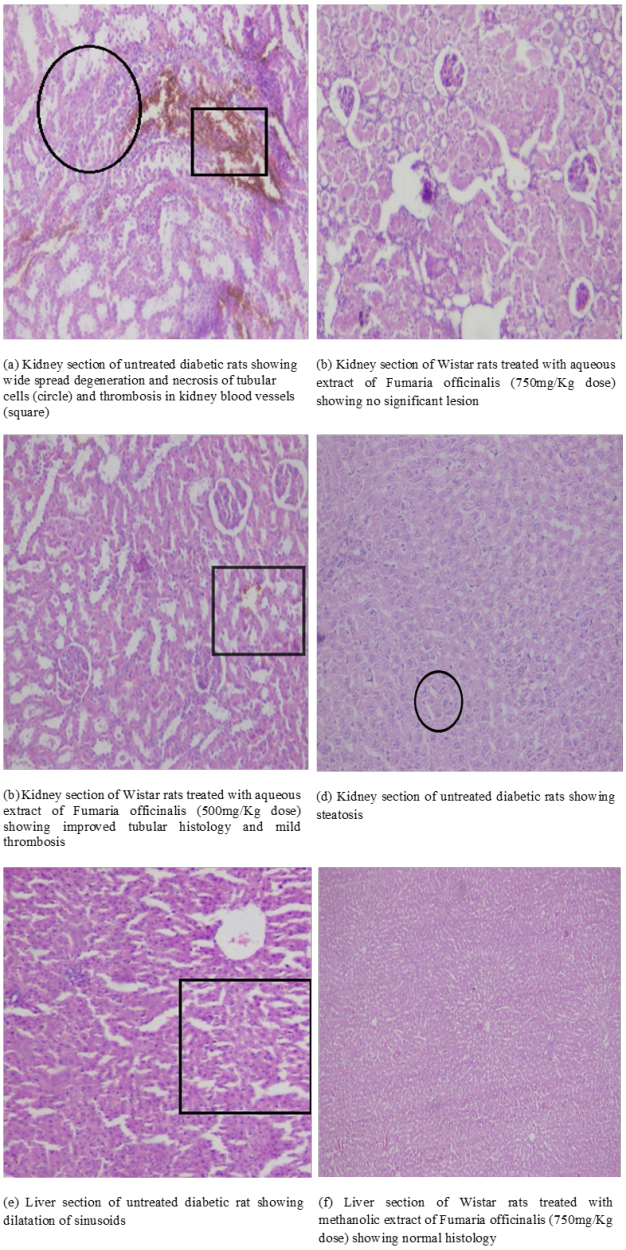

Figure 2. Effect of Fumaria officinalis on the kidney and liver of diabetic rats.

Discussion

F.officinalis is traditionally used to manage diabetic patients in many areas of Pakistan. The current study was conducted to determine the antidiabetic potential and in-vitro antioxidant activity of plant *F.officinalis* to validate its folkloric use in the management of diabetes. This study first time reports the antidiabetic potential of *F. officinalis* extracts. The present study also reveals that the aqueous and methanolic extracts *F.officinalis* were not lethal or showed no toxicity at designated doses in Wistar rats.

This study showed a significant antioxidant potential of all extracts of *F. officinalis*. Antioxidant activity of *F. officinalis* may be attributed to the presence of tannins, polyphenols and flavonoids which were detected in plant extract during preliminary phytochemical screening. This study also supports the previous finding which concluded the significant antioxidant potential of *Fumaria* species (14).

The evaluation in normo-glycaemic rats revealed that the extracts of *F. officinalis* did not show any hypoglycaemic effects. This finding is in accordance with the Previous studies which showed that *F. parviflora*, another plant of Fumaria genus, did not exhibit hypoglycaemic effect in non-diabetic rats (24).

It is well established that the decline in body weight is evident in diabetic individuals. In this study, there was a significant weight gain in diabetic rats treated with methanolic (500 mg/Kg) and aqueous extracts of F. officinalis. Weight gain in diabetic rats was more pronounced with aqueous extract than its methanolic counterpart. Therefore, it can be speculated that the extracts of *F. officinalis* altered the metabolic system of diabetic rats resulting in weight gain (25).

Alloxan is taken up rapidly and accumulates in pancreatic beta cell, liver and kidney. Uptake of alloxan by beta cells causes the formation of ROS and produces similar toxic effects in the liver and kidney of exposed animals. Because of its destructive effect on pancreatic beta cells, alloxan is extensively used to induce hyperglycaemia in experimental animals (26). Methanolic and aqueous extracts of *F.officinalis* showed a significant reduction ($P < 0.05$) in blood glucose level as compared to untreated diabetic rats. The hypoglycaemic effect of methanolic extract at all doses and aqueous extract at 250 and 750 mg/kg was statistically insignificant to rats treated with glimepiride. The current study revealed that the aqueous and methanolic extracts of *F officinalis* had shown significant hypoglycaemic effect in diabetic rats. It can be speculated that the antioxidant and alpha amylase inhibitory nature of *F. officinalis* is responsible for the antidiabetic activity in rats (27).

Liver and kidney function markers were used to evaluate the effect of liver and kidney disorders respectively. The increased level of these parameters indicates the damage to liver and kidney (28). Elevated creatinine and urea level in serum are substantial markers for renal dysfunction in diabetic hyperglycaemia (29). Both aqueous and methanolic extracts of *F. officinalis* significantly reduced the damage to kidney and liver. It is also evident from the histological studies of rats which showed that both extracts ameliorated the kidney and liver damage in alloxan treated rats. Both extracts reduced the sinusoidal enlargement, steatosis and steato-hepatitis in diabetic rats. Administration of these extracts reduced the damage to cells of glomeruli, interstitial inflammation, necrosis of tubular cells and thrombosis in the kidney of diabetic rats. Previous stu-

dies have shown that natural products might improve the kidney function owing to their antioxidant action (30). It has also been demonstrated that the antidiabetic herbal drugs may ameliorate the oxidative stress related parameters in liver and kidney to prevent the complications of DM (31). Based on these findings, it can be suggested that the extracts of *F. officinalis* may prevent the damage to kidney and liver in diabetic rats due to reduction of ROS.

The current study performed on *F. officinalis* concluded that the plant exhibited a potent hypoglycaemic effect in diabetic rats. The hypoglycaemic activity of *F. officinalis* in diabetes may be attributed to alpha amylase inhibition and antioxidant activity. Aqueous and methanolic extracts of the plant may also reduce diabetes induced secondary changes in the liver and kidney. However, more detailed phytochemical evaluation and activity based isolation of phytochemicals are needed to identify active principles in *F. officinalis* aerial parts.

Ethical disclosures

The authors declare that experiments on animals were approved by and carried out according to guidelines of the University of Lahore, Pakistan.

Conflict of interests

Authors declare that they have no conflict of interests.

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