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Secondary metabolites and antifertility potential of *Atriplex farinosa* Forssk

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Abstract

Atriplex farinosa Forssk was evaluated for its phytochemical contents. Moreover, its effect on the reproductive organs of male rats was studied. Five compounds were isolated from this plant (Three flavonoids and two coumarins). No alterations were observed in the fertility parameters of the male rats after the exposure to *A. farinosa* extract in a dose of 100 mg/kg (group B) for 6 weeks. Significant reduction was observed in the relative weight of reproductive organs of the male rats of groups C (200 mg/kg) and D (400 mg/kg). Administration of the ethanol extract of *A. farinosa* to groups C and D significantly reduced the serum levels of testosterone. The test extract significantly increased the serum level of prolactin in groups C and D. Significant reduction was observed in sperm count, sperm motility and sperm viability of the male rats of groups C and D. The observed sperm abnormalities included detached head and coiling of end tail. In conclusion, daily administration of *A. farinosa* extract to male Albino rats may lead to reduction of their fertility.

Keywords: *Atriplex farinosa*, flavonoids, male fertility

Introduction

The genus *Atriplex* comprises about 200 species and belongs to subfamily Chenopodiaceae. *Atriplex farinosa* is a tall shrub of yellow white appearance with large, naked panicles, but leaf base cordate with long, obtuse auricles, fruit bracts entire, longer than broad, acute (Tackholm, 1974). Some reports suggested the presence of naringin, naringenin 7-*O*-glucoside, isorhamnetin-3-*O*-rhamnosyl (1-6) glucopyranoside and isorhamnetin-7-*O*-glucopyranoside in *A. farinosa* (Al-Jaber et al, 1991).

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In traditional medicine, a cocktail of minerals in *A. halimus* is used to benefit glycaemic control in diabetic patients (Day, 1990). Like other halophytes, it used in veterinary medicine to combat internal parasites (Bayoumi & El-Shaer, 1990). *A. halimus* produces the polyphenols and other bioactive substances which potentially useful for medicinal properties and as natural food preservation (Benhammou et al, 2009). *A. confertifolia* has significant bioactivity against human breast cancer cell lines; the bioactivity of *A. confertifolia* extract on these cells was compared to a FDA-approved cancer drug; Onxol and an industry-standard leukocyte control cell line. Active portions of the extract were found primarily in the polar fractions of the plant. A dose-response curve of the extracts displayed significant cell death similar to Onxol (Capua, 2010).

One of the fundamental areas of human life is fertility and conception. Therefore, attention is being given to plants with antifertility properties. It is possible that a steady decline in the sperm count over the years in the future might lead to oligospermia and infertility. The aphrodisiac, antifertility, and fertility-enhancing properties of some plant extracts have been reported in previous studies. The present study was designed to investigate the phytochemical contents of *A. farinosa* plant and to evaluate the effects of its ethanol extract on the reproductive organs of adult male Albino rats.

Materials and Methods

Plant Material

Atriplex farinosa Forssk was collected from the South Eastern corner of Egypt, during autumn season 2007. The collected plant was identified by Prof. Dr. Ahmed Morsy Ahmed, Plant Ecophysiology, Desert Research Center (DRC), Cairo, Egypt. A specimen from this plant has been deposited in the Herbarium of the DRC, Cairo, Egypt (No. 11073 HDRC). The plant was dried under shade and then grinded to fine powder. The dried powder (one kg) was extracted with 70% aqueous ethanol by percolation in the solvent with occasional shaking for 48 h and the process was repeated three times. The ethanol extract was combined and concentrated under vacuum to obtain a dry crude extract (75 g). The dry crude extract yield was 7.5% (7.5 g extract/100 g raw material).

Acid Hydrolysis

From each of the recrystallized compounds, 2 mg were dissolved in 2 mL of methanol: water (1:1, v: v), mixed with 1 mL of 2N HCl, and refluxed at 60 ^{o}C for 3 h. The reaction mixture was subsequently extracted with ethyl acetate to give a glycone moiety and the neutralized aqueous part afforded sugars moiety (Stahl, 1969).

Apparatus

UV spectra of the isolated compounds were measured on Shimadzu 1201 spectrophotometer. Mass spectral data were done using Esquire-LC-00142 mass spectrometer. ^{1}H and ^{13}C NMR spectra, using external electronic referencing through the deuterium resonance frequency of the solvent, were determined at 400 and 100 MHz respectively with Avance NMR spectrometer fitted with an auto-tune 5mm X/H probe. ^{1}H-^{13}C correlations

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were established by using HMQC and HMBC pulse sequences respectively. ^{1}H-^{1}H correlate-ons were determined by double quantum filtered COSY.

Acute toxicity and median lethal dose (LD_{50}) test

The acute oral toxicity and median lethal dose (LD_{50}) of the ethanol extract of *A. farinosa* were estimated in male adult Albino rats (Finney, 1964). In a pilot experiment, three groups each of five rats received the tested extract suspended in a vehicle at doses of 10, 100, and 1000 mg/kg b.wt, respectively. Control animals were received the vehicle and kept under the same conditions. Animals were observed for 24 h for signs of toxicity and number of deaths.

According to the results of the preliminary test, doses of 2000, 3000 and 4000 mg/kg b.wt. of *A. farinosa* extract were administered to new animal groups, each of 5 rats. Signs of acute toxicity and number of deaths per dose within 24 h were recorded and the LD_{50} was calculated. The value of LD_{50} was used as a reference for the choice of the experimental doses of this work. Accordingly, doses of 100, 200 and 400 mg/kg that equal to $^{1}/_{40}$, $^{1}/_{20}$ and $^{1}/_{10}$ of the maximum possible dose of the extract that didn't cause mortalities in rats were selected to be given orally.

Effect on male fertility

Twenty four adult male Albino rats weighing 180-200 g were divided into 4 groups of 6 animals each and fed a standard laboratory chow and water *ad libitum*. *A. farinosa* extract was suspended in the vehicle and administered orally to the rats by gavage every morning for 6 weeks. Group A: Control rats received 0.5 ml/day of the vehicle. Groups B, C and D: Rats were treated with the ethanol extract of *A. farinosa* at 100, 200 and 400 mg/kg/rat/day, respectively.

Sample collection

The animals were weighed, 24 h after the last dose of treatment. Blood samples were collected under light ether anesthesia by cardiac puncture into centrifuge tubes and sera were separated, stored frozen and used within 12 h of preparation for the estimation of testosterone (Chen, 1991), prolactin (Tietz, 1995), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Uotila et al, 1981). Moreover, liver functions were evaluated by measuring the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Reitman & Frankel, 1957).

Serum concentrations of urea (Wills & Savory, 1981) and creatinine (Kroll et al, 1987) were determined colorimetrically as measures of kidney functions. Rats were sacrifice-ed and weights of testes, seminal vesicle and ventral prostate were recorded as relative weights (organ weight/body weight×100). Sperm count and sperm motility were determined according to the methods of (Sönmez et al, 2007, Sönmez, et al, 2005), respectively. Sperm viability test was done by the method described by WHO (WHO, 1999). In addition, total sperm abnormalities were assessed (Türk et al, 2007).

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Results

Phytochemical studies

The ethanol extract of *A. farinosa* (25 g) was separated into fractions on a silica gel (450 g, mesh 60-120) column (5 cm in diameter x 130 cm in length) and eluted with ethyl acetate (fractions 1-8), and ethyl acetate: methanol (95:5, v:v) (fractions 9- 21), and ethyl acetate:methanol (80:20, v:v) (fractions 22-37). Fractions (1-8) were applied to a silica gel (100 g of mesh 60-120) column (3 cm in diameter x 70 cm in length) eluted with chloroform, from which compounds 4 and 5 were isolated.

Fractions 22-37 (5 g) were applied to a second silica gel (150 g of mesh 60-120) column (3 cm in diameter x 90 cm in length) and eluted with ethyl acetate : methanol : water (70:5:4, v:v:v) and then with ethyl acetate:methanol : water (30:5:4, v:v:v), from this column, compounds 1, 2 and 3 were isolated.

Compound 1 (quercetin-4'-methoxy-7-glucorhamnoside)

The EI-mass spectrum of compound 1 revealed peaks at (M-1) 648 and other important peaks at M/e 331(100%). UV: λ max (MeOH): (nm) 254, 354, (NaOMe) 272, 401, 406, ($AlCl_3$) 269, 381, ($AlCl_3$/ HCl) 268, 379, (NaOAc) 273, 321,369, (NaOAc/H_3BO_3) 254,266,355. ^{1}H NMR (MeOD), δ: δ 7.62 integrated for one proton *J* = 8.5 Hz for 2', δ 7.47 (1H, dd, *J* = 8.5, *J* = 2.5 Hz, H-6'), δ 6.87 (1H, d, *J* = 8.5 Hz, H-5'), δ 6.22 (1H, d, *J* = 2.5 Hz, H-8), δ 6.07 (1H, d, *J* = 2.5 Hz ,H-6), δ 5.1 (1H, d, *J* = 2 Hz, H1'' rhamnose) , δ 4.52 (H , d, *J* = 2 Hz, H1''' glucose), δ 3.9 (3H , OCH_3), δ 3.20-3.7 (m, remaining sugar protons) and δ 1.17(3H, d, *J* = 6 Hz, CH_3 rhamnose). ^{13}C NMR (MeOD):177.4 (C-4), 164 (C-7), 162.69 (C-5), 157.01 (C-2), 156.90 (C-9), 149.93 (C-4'), 147.39 (C-3').133.49 (C-3),122.76 (C-6'), 121.54 (C-1'), 113.71 (C-2'), 115.76 (C-5') 104.27(C-10). 101.43 (C-1''), 101.77 (C-1''') 99.36 (C-6), 94.38 (C-8), the remaining sugar carbons appeared at 63.6-76.89, 56.29(-OCH_3), 18.24(C-6''').

Compound 2 (kaempferol-4'-methoxy-3-glucorhamnoside)

UV: λmax (MeOH): (nm) 271, 323, 336, (NaOMe) 275, 400, ($AlCl_3$) 272, 353, ($AlCl_3$/ HCl) 274, 360, (NaOAc) 272, 352 (NaOAc/H_3BO_3) 272,353. ^{1}HNMR (MeOD), δ 7.99 integrated for two protons *J* = 8.5 Hz for 2'and 6', δ 6.89 (2H, d, *J* = 8.5, H-3'-5'), δ 6.8 (1H, d, *J* = 2.5 Hz, H-8), δ 6.51 (1H, d, *J* = 2.5 Hz, H-6), δ 5.32 (1H, d, *J* = 2 Hz, H1'' rhamnose), δ 4.37 (1H , d, *J* = 2 Hz, H1''' glucose), δ 3.70 (3H , OCH_3), δ 3.0-3.75 (m, remaining sugar protons) and δ 0.97 (3H, d, *J* = 6 Hz, CH_3 rhamnose). ^{13}C NMR (MeOD): 178.03 (C-4), 160 (C-7), 58.52 (C-5), 157.34 (C-2), 152.30 (C-9), 149.93 (C-4'), 147.39 (C-3').133.37(C-3), 131.38 (C-6', C-2'), 121.54 (C-1'), 115.76 (C-5') 104.27 (C-10). 101.43 (C-1''), 101.72 (C-1''') 99.36 (C-6), 94.38 (C-8), the remaining sugar carbons appeared at 63.6-76.89, 56.29 (-OCH_3), 18.24 (C-6''').

Compound 3 (quercetin-6, 4'-dimethoxy-3-glucorhamnoside)

UV: λmax (MeOH): (nm) 255, 348, 350, (NaOMe) 272, 334, 385, ($AlCl_3$) 270, 380, ($AlCl_3$/ HCl) 269, 380, (NaOAc) 255, 361, (NaOAc/H_3BO_3) 258, 362. ^{1}HNMR (MeOD), δ 7.89 (1H, dd, *J* = 8.5 Hz, *J* =2.5 Hz, H- 2'), 7.62 (1H, dd, *J* = 8.5 Hz, J =2.5 Hz, H-6'), 6.91

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(1H, d, *J* = 8.5 Hz, H-5'), 6.47 (1H, s, H-8). 5.21 (1H, d, *J* = 2 Hz, H1'' rhamnose) , 4.52 (1H , d, *J* = 2, H1'''), 3.95, 3.87 sugar protons, 3.28-3.36 (m, remaining sugar protons) and 1.1 (3H, d, *J* = 6, CH_3 rhamnose. ^{13}C NMR (MeOD): 179.2 (C-4), 158.64 (C-7), 150.81 (C-5), 149.20 (C-2), 148.30 (C-9), 147.38 (C-4'), 135.06 (C-3'), 132.1 (C-3), 123.95 (C-6'), 123.00 (C-1'), 116.07 (C-2'), 114.46 (C-5'), 104.51(C-10), 102.48 (C-1''), 101.90 (C-1''') 98.8 (C-6), 93.7 (C-8), 60.88 and 56.37 (two $-OCH_3$ groups), The remaining sugar carbons appeared at 63.6-76.9, 18.24 C-6''. Mass spectrum (M-1) 678 and another important peak at M/e 331 (100%), also from DQF-COSY, HMQC and HMBC.

Compound 4 (Scopoletin)

White crystals, m.p. 203-204 ^oC. λmax (MeOH): (nm) 229, 252, 261, 294, 346, (NaOAc) 244, 277, 390. E-MS: m/z 191. ^1H NMR (DMSO-d6) δ 7.92 (1H, d, J = 9, H- 4), δ 7.2 (1H, s, H- 5), δ 6.75 (1H, s, H- 8), δ 6.2 (1H, d, J = 9, H- 3) and δ 3.8 (3H, s, OCH3). ^{13}C-NMR (DMSO-D6): δ 160.8 (C- 2), 111.4 (C- 3), 143.9 (C-4), 109.3 (C- 5), 145.2 (C- 6), 152.0 (C- 7), 102.3 (C- 8), 149.1 (C- 9), 110.5 (C- 10), 56.2 ($-OCH_3$).

Compound 5 (Scopolin)

White crystals, m.p. 126 -128 ^oC UV: λmax (MeOH): (nm) 276, 349, (NaOAc) 259, 390. ^1HNMR (DMSO-d_6) δ 7.93 (1H, d, *J* = 9.5, H-4), δ 7.3 (1H, d, *J* = 8.4, H-5), δ 7.1 (1H, dd, *J* = 8.4, 2.2, H-8), δ 6.3 (1H, d, *J* = 9.5, H-3), and 3.8 (3H, s, OCH_3). δ 5.1 (1H, d , *J* = 9, H-1' glucose) and δ 3-3.8 sugar protons. ^{13}C NMR (DMSO-d_6) 160.2 (C-2), 1121 (C-3), 145.8 (C-4), 109.5 (C-5), 113.2 (C-6), 149.8 (C-7), 102.8 (C-8), 153.6 (C-9), 112.3 (C-10), 100.6 (C-1'), 73.3 (C-2'), 76.7 (C-3'), 69.4 (C-4'), 77 (C-5'), 61 (C-6') and 55.9 ($-OCH_3$).

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Acute toxicity and median lethal dose (LD_{50}) test

The total ethanol extract of *A. farinosa* did not produce any symptom of acute toxicity in rats in doses up to 4000 mg/kg b.wt. and none of the rats died during 24 h of observation. Accordingly, it suggested that oral LD_{50} of the tested extract was higher than 4000 mg/kg. The non-toxic nature of the ethanol extract of *A. farinosa* in acute toxicity study is well supported by the normal levels of ALT, AST, urea and creatinine following 6-weeks treatment period in rats (data not shown).

Effect on male fertility

In the present investigation, the body weight of rats was not altered following treatment with *A. farinosa* extract in doses of 100, 200 and 400 mg/kg for 6 weeks (Table 1). In addition, the fertility of treated male rats reduced in the 200 and 400 mg/kg b.wt. groups as evidenced by the significant reduction in the relative weights of the testes (1.16±0.08 and 1.11±0.09 g/100 g b.wt *vs* 1.65±0.11 g/100 g b.wt), seminal vesicles (0.41±0.02 and 0.40±0.02 g/100 g b.wt *vs* 0.53±0.03 g/100 g b.wt) and ventral prostate (0.40±0.02 and 0.37±0.03 g/100 g b.wt *vs* 0.48±0.01 g/100 g b.wt).

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Table 1. Effect of oral administration of ethanol extract of *A. farinosa* for 6 weeks on the relative weights of reproductive organs of male rats, (n=6).

Group	Treatments and doses	Initial b.wt (g)	Final b.wt (g)	Relative weight of reproductive organs (g/100 g b.wt)		
				Testes (Pair)	Seminal vesicles	Ventral prostate
A	Control (0 mg/kg)	225.8±6.83	238.2±6.57	1.65±0.11	0.53±0.03	0.48±0.01
B	*A. farinosa* (100 mg/kg)	228.0±6.52	241.7±6.83	1.38±0.12	0.48±0.02	0.45±0.02
C	*A. farinosa* (200 mg/kg)	224.2±6.28	235.3±6.49	1.16±0.08**	0.41±0.02**	0.40±0.02**
D	*A. farinosa* (400 mg/kg)	221.6±6.44	230.5±6.60	1.11±0.09**	0.40±0.02**	0.37±0.03**

Significant at ** $P \leq 0.01$

A highly significant decline in serum testosterone level (Table 2) was observed in rats of groups C and D (2.91±0.24 and 2.70±0.21 ng/mL, respectively) when compared with group A (4.42±0.35 ng/mL). On the contrary, serum level of prolactin was significantly increased in rats of groups C and D. Serum levels of LH and FSH remained within the control levels by all the doses.

The epididymal sperm count of control animals was 62.78±3.55 × 10^6/mL, motility was 92.70±3.68% and viability was 93.52±3.88% (Table 3). Treatment with *A. farinosa* extract in doses of 200 and 400 mg/kg for 6 weeks showed a dose-dependent decrease in sperm count to 52.65±2.61 and 50.25±3.70×10^6/mL, respectively, sperm motility to 77.43±3.72% and 75.92±3.98%, respectively and viability to 81.15±3.26% and 78.40± 3.95%, respectively. Semen analysis of male rats of groups C and D showed a dose-dependent increase in sperm abnormalities: 5.24±0.31 and 5.52±0.28% respectively compared to 3.11±0.26% in group A. The morphological abnormalities of sperms were detached head and coiling of end tail.

Discussion

Compound 1: yellow crystals (10 mg), (m.p. 230 ^{o}C) soluble in methanol. It gave positive result with Molisch's test. Compound 2: yellow crystals (20 mg), (m.p.228-33°C) soluble in methanol. It gave positive result with Molisch's test. Compound 3: yellow crystals (35 mg), (m.p 232 ^{o}C) soluble in methanol. It gave positive result with Molisch's test.

Acid hydrolysis of compounds 1-3 yielded glucose and rhamnose as sugar moiety and Quercetin, Kaempferol, and Quercetin as aglycone, respectively. Compounds 1-3 were identified as quercetin-4'-methoxy-7-glucorhamnoside, kaempferol-4'-methoxy-3- glucorha-

Table 2. Effect of oral administration of ethanol extract of *A. farinosa* for 6 weeks on serum levels of reproductive hormones of male rats, (n = 6).

Groups	Treatments and doses	Testosterone (ng/mL)	Prolactin (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
A	Control (0 mg/kg)	4.42±0.35	0.68±0.05	7.38±0.25	0.59±0.02
B	*A. farinosa* (100 mg/kg)	3.51±0.33	0.85±0.06	7.00±0.37	0.55±0.03
C	*A. farinosa* (200 mg/kg)	2.91±0.24**	1.15±0.11**	7.07±0.29	0.62±0.04
D	*A. farinosa* (400 mg/kg)	2.70±0.21**	1.22±0.12**	7.12±0.41	0.57±0.04

Significant at ** $P \leq 0.01$

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Table 3. Effect of oral administration of ethanol extract of *A. farinosa* for 6 weeks on semen characteristics of male rats, (n = 6).

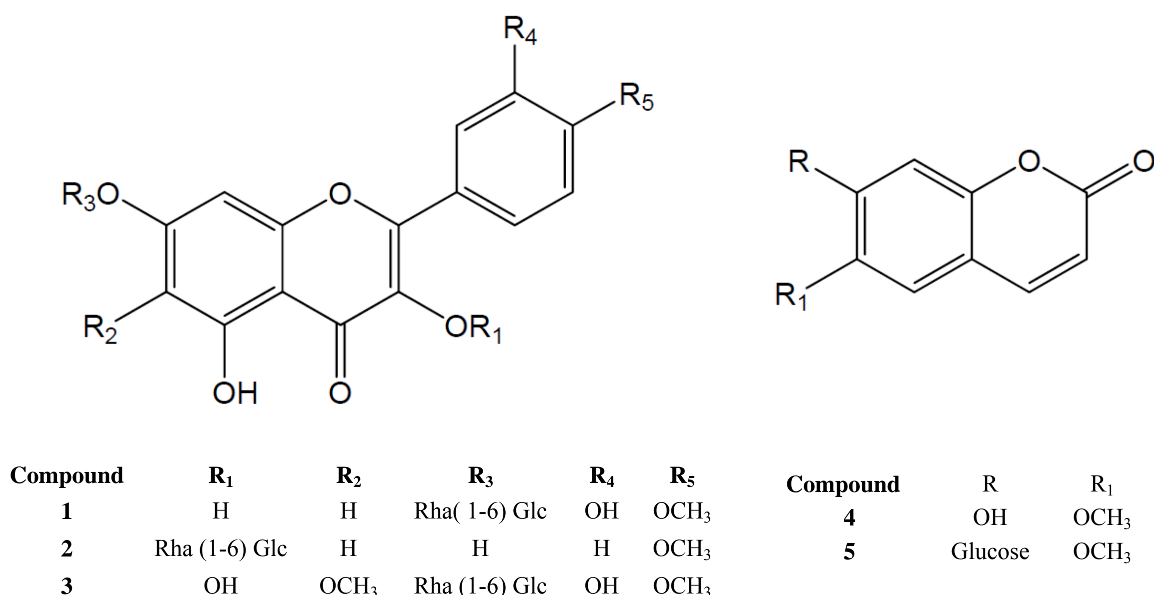
Groups	Treatments and doses	Sperm count (X 10^6/mL)	Sperm motility (%)	Viable sperms (%)	Total sperm abnormality (%)
A	Control (0 mg/kg)	62.78±3.55	92.70±3.68	92.52±3.88	3.11±0.26
B	*A. farinosa* (100 mg/kg)	58.16±3.84	90.13±4.11	89.36±4.15	3.68±0.29
C	*A. farinosa* (200 mg/kg)	52.65±2.61*	86.43±3.72	81.15±3.26*	4.24±0.31*
D	*A. farinosa* (400 mg/kg)	50.25±3.70*	85.52±3.93	78.40±3.95*	4.52±0.28**

Significant at *P ≤ 0.05, ** P ≤ 0.01

mnoside, quercetin-6,4'-dimethoxy-3-glucorhamnoside, respectively by using the different spectral techniques (UV, Mass spectra, ^{1}H NMR, ^{13}C NMR, DQF-COSY, HMQC and HMBC. Compounds 4-5 were identified as scopoletin and scopolin, respectively by using different spectral data and comparing with the published data on these compounds (Khamis et al, 1999). The first three compounds were isolated for the first time from this plant.

It suggested that the LD_{50} of *A. farinosa* extract was higher than 4000 mg/kg. The dose of 4000 mg/kg corresponds to an incredible intake of 280 g of the extract or 3.68 kg of the dried powder of *A. farinosa* per day for a 70 kg adult human. *A. farinosa* extract is therefore, can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg/kg are non-toxic (Buck et al, 1976). The non toxic nature of *A. farinosa* extract in acute toxicity study is well supported by the normal serum levels of biochemical data (ALT, AST, urea and creatinine) following 6 weeks treatment period in rats. By these indicators, ethanol extract of *A. farinosa* is therefore, not hepatotoxic and not nephrotoxic in rats.

Infertility is one of the major health problems in life, and approximately 30 % of this problem is due to male factors (Isidori, 2006). Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. The current study was carried out



Compound	R_1	R_2	R_3	R_4	R_5
1	H	H	Rha(1-6) Glc	OH	OCH_3
2	Rha (1-6) Glc	H	H	H	OCH_3
3	OH	OCH_3	Rha (1-6) Glc	OH	OCH_3

Compound	R	R_1
4	OH	OCH_3
5	Glucose	OCH_3

Figure 1. Isolated compounds from *Atriplex farinosa*.

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In the present investigation, the body weights of rats were not significantly different from the control group following oral administration of *A. farinosa* extract (100, 200 and 400 mg/kg for 6 weeks), indicating that the general metabolic conditions of the animals were within the normal range. In this connection, monitoring body weight provides information on the general health level of animals, which can be important to the interpretation of reproductive effects (USEPA, 1996).

Moreover, the weight of testes, seminal vesicles and ventral prostate are sensitive end points that can be used to assess the direct effect of a compound on testicular cells and accessory organs (Creasy, 2003). The present data show that the administration of *A. farinosa* extract for 6 weeks induced a significant dose-dependent loss in testes and accessory sex organ weights, which are known to be mostly related to the number of spermatids and spermatozoa in the tissue. The decreasing weight of the reproductive organs of male rats clearly indicated that *A. farinosa* (200 and 400 mg/kg) caused structural and functional alteration in the male reproductive organs (Banerji et al, 2000).

It is well known that circulating levels of testosterone are required for the maintenance of accessory sex organ functions. The reduced weights of seminal vesicle and ventral prostate support the suppressed concentration of testosterone (Lohiya & Ansari, 1999). In addition, the elevated level of prolactin is known to suppress testosterone synthesis and male fertility through prolactin induced hypersecretion of adrenal corticoids or by inhibiting the secretion of GnRH through prolactin receptors on hypothalamic dopaminergic neurons (Albertson et al, 1987). Accordingly, the fact that serum LH and FSH levels were unaltered in the treated animals suggested that *A. farinosa* extract was acting directly on the testes (Murono et al, 2006).

It is known that the structure and function of the epididymis are dependent on androgens (Cooper, 1992). Treatments with *A. farinosa* extract (200 and 400 mg/kg) for 6 weeks showed a dose-dependent decrease in sperm count, motility and viability. Reduction of sperm count and motility suggested an undersupply of testosterone to the epididymis, thereby possibly causing impaired epididymal function. In the present study, suppressed testosterone level was further confirmed by the decreased number of sperms which is completely testosterone dependent (Dym et al, 1979). The morphological abnormalities in spermatozoa of treated rats (detached head and coiling of end tail) could possibly be due to either a direct effect of *A. farinosa* on maturing germ cells or interference with sperm maturation process in epididymis.

In the present study, suppressed testosterone level was further confirmed by the decreased number of sperms which is completely testosterone dependent (Dym et al., 1979). The morphological abnormalities in spermatozoa of treated rats (detached head and coiling of end tail) could possibly be due to either a direct effect of *A. farinosa* on maturing germ cells or interference with sperm maturation process in epididymis.

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