Original Article

Safety Assessment of Hydro-ethanolic Extract of *Falcaria vulgaris* in Wistar Rats: Acute and Subchronic Toxicities

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Abstract

Background: *Falcaria vulgaris* is a medicinal plant with culinary uses and widespread therapeutic applications. Despite already proven as a very promising dietary supplement, its safety and possible effects on the human body are yet to define. This study was designed to investigate the acute and subchronic toxic effects of hydroethanolic *F. vulgaris* in male and female Wistar rats. **Experimental:** To evaluate the safety of a hydroethanolic extract of *F. vulgaris*, acute and subchronic toxicity in Wistar rats treated with extract was investigated. For investigation of acute toxicity of *F. vulgaris*, both genders of rats were treated for 45 days with a single dose of the extract (4000 mg/kg) via gavage. Also for sub-chronic testing, the extract was administrated orally at the doses of 150, 300, and 450 mg/kg for 45 days. At the end of the study, the animals were sacrificed and the hematological, biochemical, and histopathological parameters were assayed. **Results:** After a single oral administration of *F. vulgaris* (4000 mg/kg), no mortality was observed in both control and groups in either sex. Also, histopathological inspection of vital organs and tissues revealed no obvious alteration in these organs. The obtained results showed a significant reduction in the weight of heart and liver in male rats that received the highest dose of the extract. The level of red blood cell distribution width (dose of 450 mg/kg) from the hematological parameters and the level of serum creatinine (dose of 150 and 450 mg/kg) from the biochemical parameters increased significantly in male rats. On the contrary, during treatment the concentration of all examined minerals remained unchanged. Histopathological inspection indicated that liver, kidney, and testis were found to be affected by subchronic exposure to *F. vulgaris* extract. **Conclusion:** The results of the acute study revealed that *F. vulgaris* may be nontoxic even at doses less than 4000 mg/kg body weight. However, the result of subchronic study confirmed the liver dysfunctions in Wistar rats and also suggested the significant effect of *F. vulgaris* on testicular tissue, which may cause serious male infertility. The ability to impair male fertility by such a medicinal plant has not been reported yet. It can be concluded that the no observed adverse effect level (NOAEL) of *F. vulgaris* are 150 and 450 mg/kg for male and female rats, respectively.

Keywords: *Acute toxicity, Falcaria vulgaris, male infertility, medicinal plant, subchronic toxicity*

Introduction

Nowadays, there is an increase in the usage of medicinal plants as dietary supplements and alternatives in daily health care as well as a treatment against chronic and infectious diseases.[1] Proven efficacy, general perception of safety, and low price of medicinal herbs are major reasons for the increasing attention and support of them in both developing and developed countries.[2,3] However, it has been reported that many of these medicinal plants and their products are potentially toxic, mutagenic, and carcinogenic.[3,4]

The sickleweed (*Falcaria vulgaris* Bernh, Umbelliferae), domestically known as "Ghaz-e-yaghi," is grown naturally in the western parts of Iran[5] and traditional has been potently used in the treatment of digestive problems.[6] It has scientifically been proved that the *F. vulgaris* cooked leaves or its infusion possesses the ability in decreasing blood pressure.[7,8] Other herbal health benefits include acting as a carminative, febrifuge, vulnerary, stomachic, and hemostatic.[7] In addition to the beneficial therapeutic effects, its culinary uses, ranging from mixing with plain yogurt to baked stew as a nutrient source, are popular in Iran.[2]

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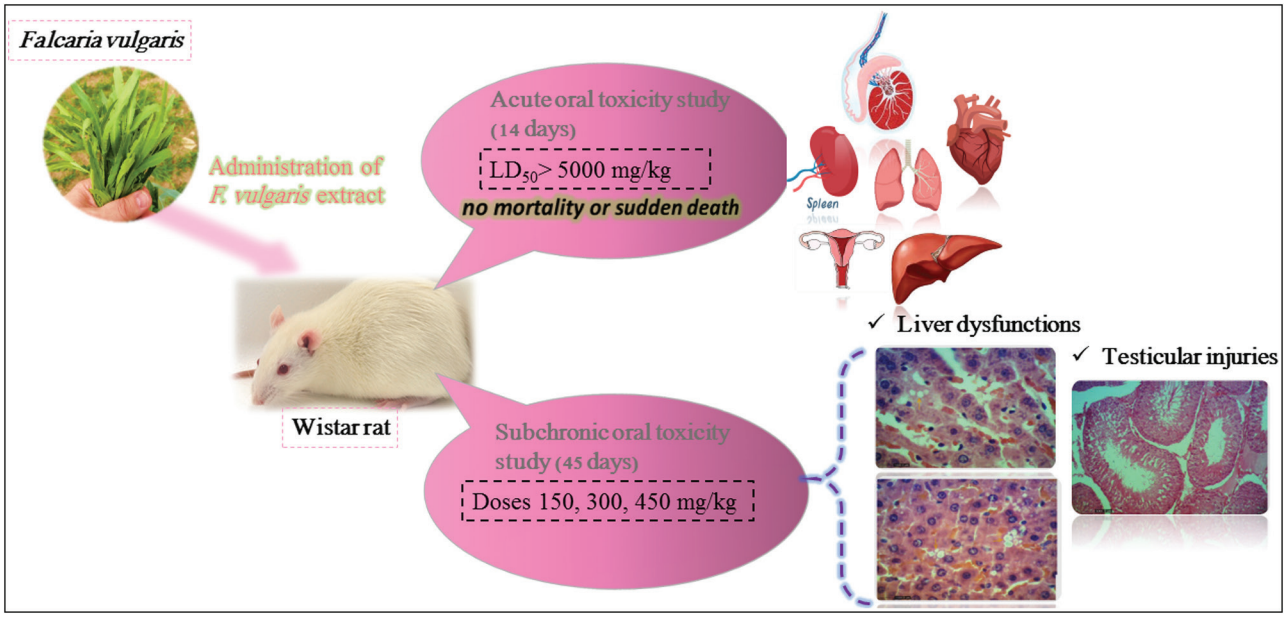

The nutritional and medicinal value of *F. vulgaris* can be attributed to the many active phytochemical compounds such as spathulenol, carvacrol, α-pinene, and limonene.[6] Furthermore, the presence of nonpolar phenolics and flavonoid components in this plant result in an antibacterial effect against both gram-positive and gram-negative of bacteria.[9] Considering all these outstanding features, *F. vulgaris* can be applied as a promising dietary supplement. However, safety evaluations of the medicinal herb before administration to determine the possible toxicological actions and especially the consequence of prolonged usage are critical for therapeutic application.

Although there are lots of studies to evaluate the pharmacological effects and prove the medicinal potential of *F. vulgaris*, the toxicological effects and long-term feeding of this extract have not been investigated.

The present work is provided the *F. vulgaris* safety concentrations focusing on acute toxicity and 45-day subchronic toxicity in male and female Wistar rats. Therefore, the effect of oral administration of the hydroethanolic extract of *F. vulgaris* on physiological and biochemical parameters in Wistar rats was assessed. Moreover, histopathological examinations of liver, kidney, lung, heart, spleen, ovary (in females), and testis (in males) were conducted at the end of the treatment period.

Materials and Methods

Ethical considerations

This study protocol was approved by the Research Ethics Committees of Kermanshah University of Medical Sciences with the ethical code: IR.KUMS.REC.1397.556 on 2018-10-10. https://ethics.research.ac.ir/ProposalCertificateEn.php?id=26888&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true.

The protocol of the study was in accordance with Helsinki's (1994) declaration.

Preparation, authentication, and extraction of *Falcaria vulgaris*

The aerial parts of *F. vulgaris* were collected from Sar Firuzabad rural district, Kermanshah province, Iran, in May 2018. The authentication of the plant was done by Dr. S. M. Masoumi (Razi University Herbarium, Kermanshah, Iran). The voucher sample has also been entrusted to the herbarium (identification number: 1167 (RUH)). Dried, powdered aerial parts of *F. vulgaris* (212 g) were extracted three times with ethanol: water (7:3) by maceration. After filtration of the extracts, the filtrate was concentrated using a rotary evaporator (Heidolph, Germany), and then a freeze-drier (Christ, Alpha 2–4 LD plus, Germany) to obtain a dried powder. The dried extract was stored at –20°C for further investigation. The extraction yield was 21.6%.

Experimental animals

Wistar rats of both genders (weighing 170 ± 8 g 8–10 weeks in the acute study, 130 ± 12 4–6 weeks in subchronic study) were purchased from KUMS breeding house, Kermanshah University of Medical Sciences (Kermanshah, Iran) and used for evaluating acute toxicity of *F. vulgaris*. Before the experiments, the rats were kept in the laboratory in special cages for 2 weeks, whereas the temperature (23 ± 2°C) and humidity were kept constant. Animals were kept under 12 h light-dark cycles. Rats were fed with specific food and tap water without any limitation, following an overnight fasting

before experimentations. All the procedures in this study were strictly conducted in compliance with internationally accepted principles for laboratory animal use and care as found in the US guidelines[10] and were approved by the Animal Ethics Committee of Kermanshah University of Medical Sciences.

Experimental design for single oral dose toxicity study of *Falcaria vulgaris*

Twenty 8–10 weeks Wistar rats of both genders weighing 170 ± 8g were used for acute toxicity of *F. vulgaris*. There were two groups of rats (treatment and control groups), each containing five males and five females. Treatment rats were administered with a single dose of extract, 5000 mg/kg via gavage. Control rats only received an equal volume of distilled water. The behaviors of all the rats were followed for 8 h to observe the survival time and clinical signs. Then, at specific daily time intervals rats were monitored for observing any possible toxicity symptom and recording the duration of these symptoms. Furthermore, the alteration in the behaviors, weight, and physical appearance of the animals were monitored for 2 weeks. Finally, animals were euthanized and the relative organ weight was calculated for all the rats and the organs were examined either macroscopically and microscopically.[11]

Experimental design for repeated oral dose toxicity study of *Falcaria vulgaris*

To study the subchronic toxicity, rats of either sex (4–6 weeks) with an initial weight of 130 ± 12 g were divided into four groups in either sex. The animals of the control group received normal saline and daily doses of *F. vulgaris* extract (150, 300, and 450 mg/kg BW) were orally administered to the treatment groups for 45 days. Just before the commencement of dosing, different doses of test substance were prepared freshly and administrated *via* gavage, then observations were made to record any possible physical and behavioral abnormality. The body weight of each animal was recorded once a week. Finally, the body weight of each rat was recorded and rats were euthanized by intraperitoneal administration of ketamine hydrochloride (40 mg/kg) and xylazine (5 mg/kg), sacrificed blood was collected, whereas the liver, kidney, lung, heart, spleen, ovary (in females), and testis (in males) were excised to further investigate histopathological parameters. The relative organ weight was calculated for all the rats and the organs were also macroscopically examined.[11]

Hematological, biochemical, and blood electrolytes analyses

The blood sample of each rat was individually collected in heparin-containing tubes, to examine the effect of *F. vulgaris* extract treatment on different hematological parameters. For examining the biochemical parameters, using a serum separation reagent, blood samples were centrifuged at 2016 *g* for 15 min.[12]

Serum biochemical parameters included: blood sugar, creatinine, globulin, cholesterol, triglycerides, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin total (Bit), and bilirubin direct (Bid). The hematological parameters and serum biochemistry were determined using Sysmex K1000 fully automated hematology analyzer and COBAS Mira S chemistry analyzer (Roche Diagnostic Systems, West Sussex, England).

Histopathological analysis

At the end of the trial and after sacrificing control and treatment groups, in order to investigate the tissues microscopically, the fragments of different organs including heart, liver, kidney, spleen, and lung, ovary and testis of rats were kept in 10% neutral buffered formalin. Then they were embedded in paraffin blocks sectioned and stained with hematoxylin and eosin.[12]

Statistical analysis

In this study, all the measured parameters are reported as the mean value ± standard error of the mean. The difference between treatment and control groups was separately determined for males and females using the one-way analysis of variance (ANOVA) followed by Tukey's test. A value of $P < 0.05$ was considered statistically significant.

Results

Acute toxicity of *Falcaria vulgaris*

After a single oral administration of *F. vulgaris*, the behaviors and mortality of Wistar rats were monitored for 14 days. Throughout this period, not only no visible spontaneous changes and abnormal behavior were observed in animals but also their food and water consumption remained without significant change. Furthermore, no mortality or sudden death was observed in both control and treatment groups in either sex. The alteration in body weights of experimental and control groups was negligible [Figure 1]. The organ coefficient of vital organs was also within the normal range [Table 1]. Histopathological inspection of vital organs and tissues after acute administration of hydroethanolic extract of *F. vulgaris* revealed no obvious alteration in these organs (data not shown).

Subchronic oral toxicity

Observational study, body weight and organ coefficients

Throughout 45 days of repeated administration of *F. vulgaris,* no treatment-related adverse effects were observed. During this period, all the rats were weighed at specific time intervals and the results are illustrated in Figure 2. It could be observed that none of the experimental rats underwent a significant change in the total body weight. The extract did not cause any abnormality and significant alteration in the body weight of the treated animals in comparison with controls. The calculated organ coefficients

of vital organs after fulfillment of subchronic study are also summarized in Table 2.

The organ coefficients of ovary, testis, spleen, and lung were statistically similar in administered and control animals of either sex. However, the weight of heart and liver of male rats treated with concentration of 450 mg/kg of *F. vulgaris* diminished significantly compared to control animals. The organ coefficient of the kidney also decreased in the female group receiving 150 mg/kg of the extract that this alteration was not dose-dependent.

Biochemical and hematological parameters

At the termination of 45 days of oral administration of *F. vulgaris* extract, the biochemical and hematological parameters in blood of all the treated and control rats were determined [Tables 3 and 4]. At a glance, it could be obvious that for most of the hematological parameters, there was no significant treatment-related alteration. The level of red blood cell distribution width (Rdw-cv) increased significantly ($P < 0.05$) in male rats treated with 450 mg/kg of the extract. All the blood parameters of female rats did not alter significantly and were all in the normal range.

Among all the biochemical parameters, the level of serum creatinine, which is an index for renal function, increased significantly in male rats receiving 150 and 450 mg/kg, after subchronic study period ($P < 0.05$). The level of triglyceride in male rats showed a statistically significant decrease after administration of *F. vulgaris* at the highest dose of extract (450 mg/kg). Furthermore, a considerable increase was observed in the level of SGOT, as one of the indices of

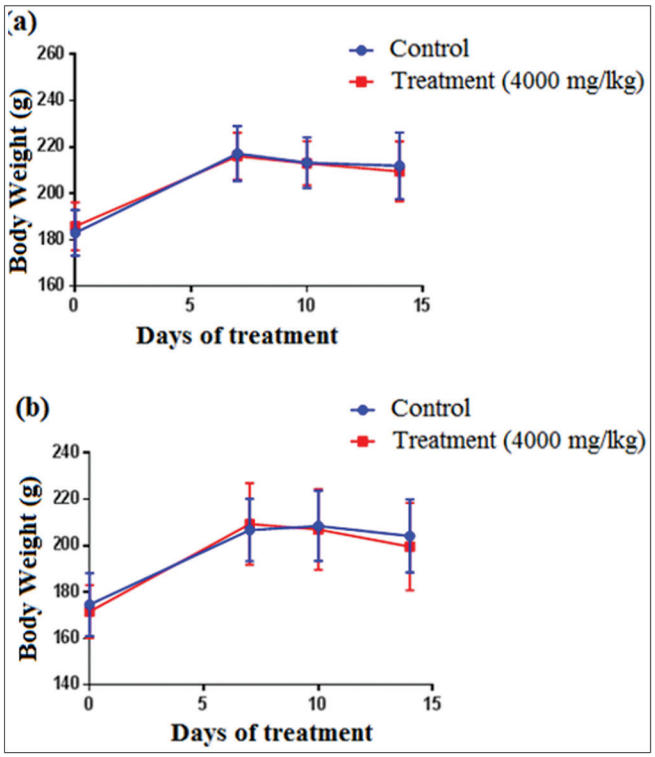

Figure 1: Relative body weight of (A) male and (B) female rats during acute treatment (*significantly different from control, $P < 0.05$)

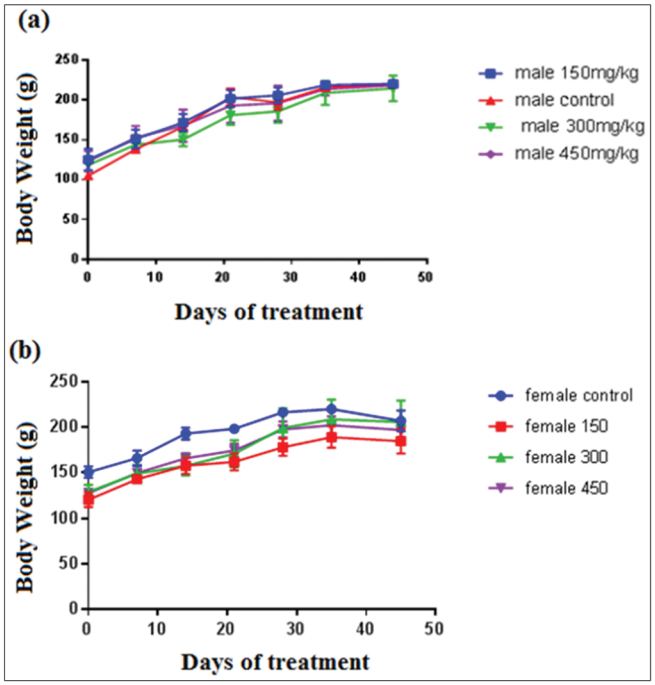

Figure 2: Relative body weight of (A) male and (B) female rats during subchronic treatment (*significantly different from control, $P < 0.05$)

Table 1: Relative weights of organs at termination of acute toxicity study (g % body weight)

Sex	Dose mg/kg	Liver%	Kidney%	Heart%	Lung%
Male	Control	3.974 ± 0.838	0.359 ± 0.001	0.374 ± 0.067	0.704 ± 0.091
	4000	3.648 ± 0.111	0.409 ± 0.041	0.421 ± 0.053	0.783 ± 0.109
Female	Control	2.867 ± 0.434	0.310 ± 0.012	0.309 ± 0.045	0.670 ± 0.078
	4000	3.126 ± 0.580	0.349 ± 0.044	0.379 ± 0.043	0.743 ± 0.114
Sex	**Dose mg/kg**	**Ovary%**	**Testis%**	**Stomach%**	**Spleen%**
Male	Control		0.929 ± 0.144	0.674 ± 0.003	0.524 ± 0.181
	4000		1.081 ± 0.087	0.612 ± 0.052	0.513 ± 0.034
Female	Control	0.025 ± 0.004		0.639 ± 0.089	0.362 ± 0.059
	4000	0.038 ± 0.012		0.736 ± 0.075	0.407 ± 0.053

Data presented as mean ± SEM for $N = 5$

Table 2: Relative weights of organs at termination of subchronic treatment (g % body weight)

Sex	Dose mg/kg	Ovary%	Testis%	Spleen%		
Male	Control		0.696 ± 0.049	0.514 ± 0.145		
	150		0.788 ± 0.043	0.470 ± 0.050		
	300		0.832 ± 0.063	0.429 ± 0.089		
	450		0.800 ± 0.085	0.480 ± 0.015		
Female	Control	0.031 ± 0.018		0.532 ± 0.056		
	150	0.027 ± 0.002		0.620 ± 0.154		
	300	0.029 ± 0.009		0.712 ± 0.097		
	450	0.025 ± 0.001		0.669 ± 0.074		
Sex	**Dose mg/kg**	**liver%**	**kidney%**	**Heart%**	**Lung%**	
Male	Control	5.561 ± 0.351	0.408 ± 0.044	0.454 ± 0.001	0.681 ± 0.048	
	150	4.850 ± 0.589	0.452 ± 0.062	0.453 ± 0.031	0.679 ± 0.082	
	300	4.797 ± 0.412	0.411 ± 0.081	0.429 ± 0.046	0.732 ± 0.054	
	450	4.058 ± 0.319**	0.362 ± 0.032	0.380 ± 0.027*	0.670 ± 0.029	
Female	Control	4.513 ± 0.244	0.516 ± 0.073	0.503 ± 0.124	0.804 ± 0.011	
	150	3.901 ± 0.751	0.391 ± 0.053*	0.445 ± 0.055	0.698 ± 0.110	
	300	3.606 ± 0.493	0.466 ± 0.053	0.435 ± 0.032	0.707 ± 0.080	
	450	4.023 ± 0.316	0.446 ± 0.040	0.457 ± 0.042	0.816 ± 0.110	

Data presented as mean ± SEM for $N = 5$
*Significantly different from control at $P < 0.05$
**Significantly different from control at $P < 0.01$

Table 3: Hematological parameters of blood samples of the Wistar rats after subchronic study

Sex	Dose mg/kg	WBC (1000/µL)	RBC (10^6/µL)	Hb (g/dL)	HCT (%)
Male	Control	4.066 ± 0.838	8.090 ± 0.072	15.430 ± 0.152	50.860 ± 0.709
	150	4.300 ± 0.880	8.218 ± 0.369	15.440 ± 0.427	52.040 ± 1.588
	300	3.500 ± 0.621	8.200 ± 0.429	15.500 ± 0.683	51.575 ± 2.098
	450	4.300 ± 0.458	8.226 ± 0.617	15.160 ± 0.723	51.066 ± 1.966
Female	Control	4.300 ± 1.473	7.713 ± 0.388	14.360 ± 0.723	44.560 ± 2.074
	150	4.680 ± 1.731	7.692 ± 0.286	14.660 ± 0.680	44.260 ± 2.609
	300	6.160 ± 1.936	7.720 ± 0.413	14.560 ± 0.971	43.980 ± 2.956
	450	4.000 ± 1.166	7.860 ± 0.537	14.540 ± 0.712	44.400 ± 1.823
Sex	**Dose mg/kg**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**	**Platelets (1000/µL)**
Male	Control	62.930 ± 1.242	19.030 ± 0.208	30.300 ± 0.360	887.00 ± 122.50
	150	63.440 ± 2.252	18.760 ± 0.658	29.620 ± 0.535	979.80 ± 82.41
	300	62.975 ± 0.741	18.875 ± 0.170	30.000 ± 0.282	963.50 ± 113.36
	450	62.233 ± 2.967	18.400 ± 0.624	29.660 ± 0.416	1086.66 ± 331.00
Female	Control	57.830 ± 0.763	18.566 ± 0.680	32.166 ± 0.680	827.66 ± 36.96
	150	57.560 ± 1.506	19.000 ± 0.367	33.120 ± 0.846	868.60 ± 229.56
	300	57.000 ± 1.263	18.800 ± 0.583	33.060 ± 0.461	844.60 ± 110.08
	450	56.660 ± 1.607	18.460 ± 0.493	32.680 ± 0.502	954.00 ± 138.45
Sex	**Dose mg/kg**	**Mpv fL**	**Pdw**	**Rdw-cv %**	**Rdw-sd fL**
Male	Control	7.600 ± 0.608	15.166 ± 0.115	16.566 ± 0.416	36.966 ± 1.332
	150	8.260 ± 1.043	15.160 ± 0.054	17.780 ± 0.683	38.360 ± 1.307
	300	1015.060 ± 289.640	15.100 ± 0.182	17.150 ± 0.776	36.625 ± 1.500
	450	7.833 ± 0.776	15.230 ± 0.057	18.260 ± 0.251*	38.466 ± 1.387
Female	Control	6.830 ± 0.251	15.260 ± 0.152	15.360 ± 0.493	33.130 ± 0.577
	150	6.740 ± 0.114	15.300 ± 0.291	15.240 ± 0.687	33.400 ± 1.821
	300	6.920 ± 0.178	15.580 ± 0.295	15.980 ± 0.753	33.360 ± 0.817
	450	6.460 ± 0.260	15.220 ± 0.295	15.000 ± 1.129	32.140 ± 1.910

Data presented as mean ± SEM for $N = 5$
*Significantly different from control at $P < 0.05$

Table 4: Serum biochemical parameters of the Wistar rats after subchronic study

Sex	Dose mg/kg	Fasting blood sugar (FBS) (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Male	Control	183.300 ± 51.665	0.586 ± 0.056	74.333 ± 3.215	113.000 ± 4.583
	150	150.320 ± 43.832	0.688 ± 0.035*	81.600 ± 2.510	126.800 ± 31.180
	300	219.140 ± 48.618	0.662 ± 0.037	81.200 ± 6.907	86.200 ± 14.377
	450	168.200 ± 51.423	0.678 ± 0.035*	84.600 ± 7.162	58.000 ± 18.908*
Female	Control	17.830 ± 56.271	0.626 ± 0.077	79.660 ± 5.033	43.660 ± 7.234
	150	73.840 ± 35.853	0.692 ± 0.081	83.600 ± 2.302	48.600 ± 13.428
	300	71.320 ± 11.843	0.670 ± 0.057	83.000 ± 14.646	36.800 ± 14.601
	450	146.780 ± 32.747	0.612 ± 0.051	79.600 ± 12.422	39.800 ± 5.805
Sex	**Dose mg/kg**	**Bit (mg/dL)**	**Bid (mg/dL)**	**CK (U/L)**	**LDH (U/L)**
Male	Control	0.076 ± 0.005	0.050 ± 0.000	505.23 ± 32.50	594.33 ± 53.46
	150	0.090 ± 0.012	0.054 ± 0.005	1343.30 ± 347.72	1742.60 ± 647.27
	300	0.086 ± 0.016	0.052 ± 0.004	1015.06 ± 289.64	1181.60 ± 443.80
	450	0.100 ± 0.018	0.058 ± 0.017	1527.24 ± 790.77	1636.80 ± 743.40
Female	Control	0.106 ± 0.011	0.060 ± 0.017	493.86 ± 237.37	675.33 ± 213.68
	150	0.114 ± 0.026	0.080 ± 0.012	574.48 ± 148.81	692.40 ± 435.17
	300	0.116 ± 0.037	0.080 ± 0.023	577.94 ± 311.15	598.80 ± 267.59
	450	0.100 ± 0.028	0.074 ± 0.019	384.02 ± 204.83	417.60 ± 254.43

Data presented as mean ± SEM for $N = 5$
*Significantly different from control at $^*P < 0.05$

Table 5: Concentration of minerals in blood samples of the Wistar rats after subchronic study

Sex	Dose mg/kg	Calcium (mg/dL)	Phosphorus (mg/dL)	Sodium (mEq/L)	Potassium (mEq/L)
Male	Control	10.83 ± 0.513	4.00 ± 0.300	139.00 ± 2.00	5.33 ± 0.378
	150	10.92 ± 0.567	3.80 ± 0.187	139.80 ± 3.564	5.70 ± 0.400
	300	11.52 ± 0.825	3.48 ± 0.356	140.40 ± 4.159	5.58 ± 0.376
	450	11.20 ± 1.239	3.66 ± 0.378	141.40 ± 3.362	5.42 ± 0.432
Female	Control	9.46 ± 1.097	3.76 ± 0.416	139.00 ± 8.021	4.23 ± 0.133
	150	10.22 ± 0.497	3.76 ± 0.336	140.20 ± 2.490	4.34 ± 0.238
	300	10.24 ± 1.571	3.86 ± 0.194	138.00 ± 2.120	4.26 ± 0.363
	450	9.74 ± 1.664	3.92 ± 0.228	139.40 ± 32.470	4.18 ± 0.622

Data presented as mean ± SEM for $N = 5$

liver function, in male rats fed with 450 mg/kg *F. vulgaris* hydroethanolic extract. ALP was another biochemical parameter that witnessed a significant decrement in male rats after administration of 450 mg/kg *F. vulgaris*.

The blood electrolytes

Apart from different hematological and biochemical parameters examined and after administration of the hydroethanolic extract of *F. vulgaris*, the possibility of any significant alteration in concentration of electrolytes in blood samples of animal, after treatment with the extract, was also examined. According to the results tabulated in Table 5, the concentration of all examined minerals remained unchanged, after treatments.

Histopathological results

Histopathological inspection indicated that among all the examined organs, liver, kidney, and testis were found to be affected by subchronic exposure to *F. vulgaris* extract [Figure 3]. The microscopic examination revealed normal architecture of other tissues and no histological alteration in other organs was shown. According to the results, a slight degeneration of hepatocytes and presence of several fat globules within liver of male rats receiving 150 mg/kg, slight-to-moderate degeneration of hepatocytes and several fat globules within hepatocytes of male rats receiving 300 mg/kg, and moderate fatty degeneration of hepatocytes in liver parenchyma and several fat globules within hepatocytes of male rats receiving 450 mg/kg *F. vulgaris* extract were the identified abnormalities in the liver of treated male rats in different groups. However, in female rats there were several fat globules in the liver of all the treated rats, and sinusoid congestion in liver of female rats receiving 150 mg/kg, slight fatty degeneration of liver parenchyma in female rats receiving 300 mg/kg, and congestion and slight to moderate degeneration of hepatocytes and congestion of central veins in rats receiving 450 mg/kg, were observed. The results of microscopic observation of kidney revealed that

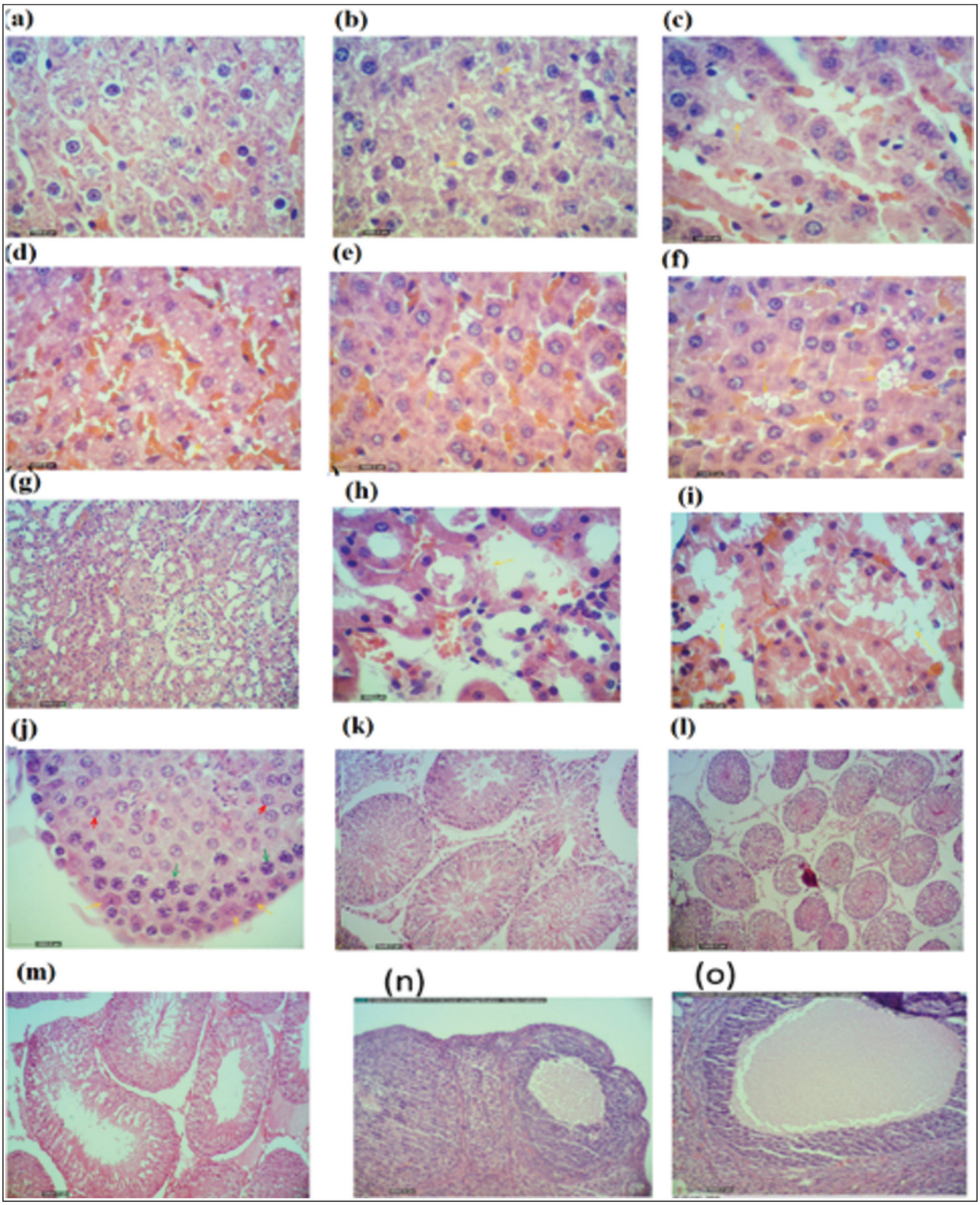

Figure 3: Selected histopathology photographs of (A–C) liver of male rates (150, 300, and 450 mg/kg) (D–F) liver of female rats (150, 300, and 450 mg/kg) (G) kidney of control rats (H, I) kidney of male and female rats (450 mg/kg), and (J–M) testis of male rats (control, 150, 300, and 450 mg/kg)

the prepared extract no toxic manifestation on kidney of rats exposed to the lowest and middle dosages of *F. vulgaris* (150 and 300 mg/kg) in either sex. In the kidney of female and male groups exposed to 450 mg/kg of *F. vulgaris* extract a slight focal cortical tubular epithelial degeneration was observed.

Histopathological results of testis in male rats exposed to the medium and highest dosages of *F. vulgaris* (300 and 450 mg/kg) showed a significant reduction in the number of spermatogonial, primary spermatocytes, spermatid, Sertoli and Leydig cells. The reduction was found to be more significant in rats that received 450 mg/kg of extract.

Discussion

Medicinal plants have long been consumed as modern alternatives to chemical drugs. *Falcaria vulgaris* is a dietary plant with widespread medicinal properties such as antibacterial, antioxidant,[9] antidiabetic,[13,14] anti-inflammatory, and antifungal.[15] Despite its wide application and frequent consumption, there is no prior scientific study in short-term and long-term periods to determine the safety of *F. vulgaris*. As per the fact that *F. vulgaris* has found its way to the food chain of lots of Iranian people, it would be critical to investigate the acute and subchronic toxicological effects of this plant to ensure its safety for the human body. To investigate the possible toxic effects of *F. vulgaris*, the acute and subchronic toxicity studies, for the first time, were carried out on the male and female Wistar rats. No comprehensive phytochemical study has been done on the aerial parts of *F. vulgaris.* However, the previous reports identified existing components in the essential oil of the known species of *F. vulgaris*.[16,17] These reports could somewhat help predicting the probable bioactivities of *F. vulgaris.* The main compound of germacrene-D as a sesquiterpenoid compound was identified from the essential oil of the aerial parts of *F. vulgaris* growing in Germany.[17] This compound shows a low order of oral toxicity, according to the common classification of relative toxicity of the substances.[18] On the contrary, the essential oil of *F. vulgaris* from Iran contains of a major component named Carvacrol,[16] which has been introduced as an inhibitor of the mitochondrial electron-transferring chain in mammalian cells.[19] The median lethal dose of carvacrol in rats has been 810 mg/kg of body weight when administered by oral gavage. Qualitative summing-up of the results obtained with the cyto/genotoxicity tests for carvacrol suggested the marginal toxicity of the compound and the feasibility of its operation at DNA level according to the observed nuclear fragmentation.[20]

In this study, *F. vulgaris* extract was evaluated for possible toxicological effects. The acute study revealed that LD_{50} of the extract exceeded 4000 mg/kg, which expresses that the hydroethanolic extract of *F. vulgaris* in doses up to 4000 mg/kg is nontoxic and safe. The long-term subchronic examination revealed no significant alteration in the body weight, clinical signs, and coefficient of vital organs. Only there was a reduction in the weight of the heart and the liver of male rats receiving 450 mg/kg of the extract. Liver is the primary organ involved in drug metabolism. Levels of biochemical parameters of liver functions, such as AST and ALT, usually determine the degree of liver damage or any toxicity effects.[21]

An increase in the hepatoenzymes activity is indicating a liver disease.

Moreover, an abnormally elevated SGOT level in the blood indicates cholestasis diseases such as gallstone or tumor blocking the bile duct.[22] After studying the biochemical parameters, the significant change in level of Aspartate Aminotransferase (SGOT) suggested that this extract at doses up to 450 mg/kg could probably affect liver. In line with these results, the histopathological inspection of organs revealed liver toxicity of *F. vulgaris* extract at all three doses. Creatinine levels can be used as a criterion for evaluating renal function.[17] Increased levels of creatinine can also be caused by chronic and acute kidney disease. In the current study, the level of creatinine increased in the male rat which was significant in the lowest and highest dose group. However, in microscopic inspection, no obvious abnormality was observed in kidney of male rats to support our premise about renal toxicity. Other studies, however, proved the nephroprotective effect of *F. vulgaris.* Zangeneh *et al.*[13] proved *F. vulgaris* aqueous extract at dose 1800 µg/ml can potentially reverse the dysfunctions induced by streptozotocin (STZ) in kidney of diabetic mice. In another study, after induction of renal injury by ethanol in rats, oral administration of hydroethanolic extract of *F. vulgaris* (50, 100, and 150 mg/kg) attenuated the injury in a dose-dependent manner.[23]

The results of microscopic observation of testis in male rats showed serious toxic manifestation and reduction in quantity of spermatogonial cells, primary spermatocytes, spermatid cells, sertoli cells, and leydig cells. This is the first study corroborating the testis toxicity of *F. vulgaris.* Jalili *et al.*[24] found that *F. vulgaris* (50, 100, and 150 mg/kg) can attenuate the abnormalities of testicular tissue and sperm parameters (count, morphology, viability, and motility) in diabetic mice and can be taken into account as a potent agent in the treatment of infertility in diabetic men. However, our study showed that this plant at doses up to or below 300 mg/kg probably causes infertility problems in male consumers. Regarding that these alterations were dose-dependent, it would be important to notice this side effect of *F. vulgaris*, which should be investigated in complementary works.

Conclusion

The major goal of this study was to assess whether the hydroethanolic extract of *F. vulgaris* induces any toxicity after acute and subchronic administration in Wistar rats or not. The results obtained by acute toxicity study revealed that the *F. vulgaris* may be non-toxic at doses less than 4000 mg/kg body weight, thus its safety in use. In addition, subchronic oral toxicity was assessed and liver dysfunctions in Wistar rats were confirmed.

The last of our findings was the significant toxic effect of *F. vulgaris* on testicular tissue, which may cause serious male infertility. Since *F. vulgaris extract* presents itself as a promising dietary supplement especially in Iran, the achievements of this study will pave a new way for many researches in the future. However, more studies are suggested to identify the exact mechanism of performance involved in the testicular toxicity properties of *F. vulgaris* in male infertility. The ultimate goal in safety assessment studies is the determination of no observed adverse effect level, NOAEL. Based on the obtained results, it can be concluded that the NOAEL of *F. vulgaris* are 150 and 450 mg/kg for male and female rats, respectively.

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Conflicts of interest

There are no conflicts of interest.

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