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Research Article

Acute and Sub-Acute Oral Toxicity Evaluation of *Astragalus hamosus* Seedpod Ethanolic Extract in Wistar Rats

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ABSTRACT

Background: Oral consumption of *Astragalus hamosus L.* (AH) seedpod has been widely prescribed in traditional medicine system. However, its toxicity evaluation has never been investigated. Hence, the current study was performed to evaluate the toxicological profile of AH seedpod in acute and subacute assessments based on the OECD-guidelines 425 and 407 in male and female Wistar rats.
Methods: In the acute study, ethanolic extract of AH at a single dose of 2000 mg/kg was orally administrated to six female rats. In the subacute assay, AH at the three different oral doses (75, 150 and 300 mg/kg) were administrated to both male and female rats for 28 consecutive days.
Results: No death or behavioural changes were observed in the treated animals. In subacute test, in both sexes, no changes in organ weights observed. Biochemically, compared to the control, AH at the dose of 300 mg/kg slightly increased ($p<0.05$) uric acid and creatinine and declined total cholesterol levels in both male and female rats. However, there is no statistically difference in other parameters such as albumin, triglyceride, blood urea, aspartate aminotransferase and alanine aminotransferase between AH treated groups and untreated controls. Hematologic parameters showed that AH at the maximum dose decreased red blood cells count only in male rats. Histopathological evaluation of liver and kidney exhibited no noticeable alterations in AH treated animals.
Conclusion: It could be concluded that high excessive and long term consumption of AH may lead to renal dysfunction and deficiency in hematopoietic system.

Introduction

Over the past two decades, there has been a tremendous increase in the use of herbal medicine worldwide. It has been estimated that three quarters of world population use herbal medicine. Despite this interest, there is still a significant lack of research data in this field.[1] There is a generally accepted belief that herbal products due to their natural origin are safer than synthetic drugs.[2] However, in contrast to popular view, there are some reports regarding serious adverse effects of herbal therapies, such as renal failure and liver injury caused by some plant species.[3,4] In this sense, experimental studies to determine the safety of medicinal plants are required.[5]

Astragalus hamosus L. is an annual herbaceous belongs to Fabaceae family. Its pod or seedpod is known as milk vetch, European milk vetch, Iklil-ul-Malik and Nakhonak.[6] The seedpod of *Astragalus hamosus L.* (AH) traditionally used for treatment of headache, vertigo, stroke, dementia, gastrointestinal upset, respiratory discomfort and urinary complications.[7] The result of a study on prescribed herbal medicine in traditional markets of Mashhad, Iran showed that this plant has been frequently recommended for kidney stone and arthrodynia as an anodyne, diuretic and carminative.[8] It has been approved that AH has anti-inflammator, analgesic, neuroprotective and cytoprotective effects.[9] Apart from the medicinal uses, the fresh seedpods of AH are also consume as a raw food.[10]

There are controversies regarding the scientific name of this plant. For example according to the reference book titled "Medicinal Plants" which wrote by Ali Zargari, the scientific name of Iklil-ul-Malik incorrectly was presented as *Melilotus officinalis*.[11] Subsequently, there are some studies in which claimed that Iklil-ul-Malik was used but its scientific name or presented picture was incorrect. Therefore, in those surveys we could not clearly understand the used plants were Iklil-ul-Malik (*Astragalus hamosus L.*) or yellow sweet clover (*Melilotus officinalis)*.[12-14] A group led by Dr. Zarshenas at Shiraz University of Medical Sciences recently published a study on authentication and phytochemical assessments of Iklil-ul-Malik (*Astragalus hamosus L.*).[7] Accordingly, the pods of AH consist of free amino acids, soluble sugars, polyphenols, triterpenes, glycosides and glycolipids and valuable percent of poly unsaturated fatty acids.

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In 2007, a new flavonol glycoside 7-O-methyl-kaempferol 4'-beta-D-galactopyranoside (rhamnocitrin 4'-beta-D-galactopyranoside) (RGP) was isolated from the leaves of AH.[15] Further research demonstrated that RGP exhibited anticancer potential against N-diethylnitrosamine-induced hepatic cancer in Wistar rats.[16] Moreover, antioxidant effects of RGP against 6-hydroxy (OH)-dopamine-induced oxidative stress in isolated rat brain synaptosomes and its cytoprotective potential in Bendamustine and/or Cyclophosphamide induced toxicity in isolated rat hepatocyte have been proven.[17] Evidence also shows that AH was inadvertently introduced into Australia from Iran, Turkey and Algeria. Todays, it widely grows in Australia lands and considered as a valuable and excellent sheep forage. To date, no problem has been reported due to livestock consumption of this plant; however, it is demonstrated that AH could synthesized nitro compounds in leaves.[18] Although the nitro contents of AH is so low but the results of a toxicological study have shown the leaves of AH could induce toxic effects in one-week-old chicken.[19] However, most of previous works have only focused on its leaves instead of seedpod as a common edible part. Moreover, its effects on function of vital organs like liver and kidney have not been dealt with in dept.

Hence, present investigation was conducted to identify toxicological profile of ethanol extract of AH seedpod after a single oral administration (acute toxicity) and 28 consecutive days treating (subacute toxicity) in rats.

Methods and Materials

Extract preparation

Astragalus hamosus fresh pods were purchased from traditional local market in Birjand, Iran. After identification of the plant by an expert botanist the voucher specimen (125) was kept in herbarium of Birjand University, Faculty of agriculture, Birjand, Iran. The seedpods (dried in shade and at the room temperature) were powdered using an electric miller. To prepare ethanolic extract, 100 grams of the powder was macerated in 80% ethanol (1000 ml) with constant stirring for 48 hours at room temperature. Afterwards, the mixture was passed through filter paper (Blue Ribbon, Grade 589, Germany), concentrated under vacuum evaporator, lyophilized using a freeze-dryer (Dena Vacuum Industry, model FD-5005-BT, Iran), and then stored at -20º C until use.

Animal and experimental design

Adult male and female Wistar rats weighting 250-300g (12 weeks old) were used in this study. The animals were housed in polypropylene cages, temperature-controlled room (22±2 °C) with a 12 h light/dark cycle and free access to commercial animal chow (Behparvar, Iran) and tap water during the study period. All animal procedures were conducted in accordance with the guide for the care and use of laboratory animals approved by the Ethics Committee of the Birjand University of Medical Sciences. (Ir.bums.REC.1396.17)

All efforts were made to minimize animal suffering and to reduce the number of animals used.

Acute toxicity

After 8-hour of fasting, a single oral dose of the ethanoic extract *of Astragalus hamosus* (AH) at the dose of 2000 mg/kg was administered to one female rat and at intervals of 48 h, the same dose was given to five other animals, totalling 6 female treated rats. Simultaneously, a control group (n=6) was treated with vehicle solution (saline) to establish a comparative negative control (NC) group.[20] For 14 consecutive days after the administration, the animals were monitored to evaluate behavioural parameters include; conscious state (general activity); motor system coordination and muscle toning (response to tail touch and grip, straightening, strength to grab); reflexes (corneal and headset); central nervous system activities (straub, convulsions, tremors, sedation, anaesthesia and ataxia) and activities on the autonomic nervous system (lacrimation, cyanosis, ptosis, salivation and piloerection).

Subacute toxicity

The animals were divided into four equal groups (n=10 per group, 5 female and 5 male) randomly. The extract at the doses of 75 mg/kg, 150 mg/kg and 300 mg/kg were orally administrated into three experimental groups (AH75, AH150 and AH300, respectively) for consecutive 28 days.[21] The control group (C) was treated with only vehicle solution (saline). At the 29th day and after an overnight fasting, all the animals were sacrificed under anaesthesia (ketamine-xylazine; 65:10 mg/kg IP)22 and their blood and organs include: heart, lung, liver, kidney, spleen, testis or ovaries were collected for further assessment.

Haematology and biochemical assessment

The following biochemical parameters: fasting blood glucose (FBG) total bilirubin (Bil.t), total cholesterol (TC), triglyceride (TG), electrolytes (sodium, potassium), liver function markers [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], renal function markers [blood urea, creatinine (Cr), uric acid, and albumin] were analysed using an automatic auto-analyzer (Roche Hitachi 912, Japan) and standard kits (Bionic, Iran). Also blood samples with anti-coagulant EDTA (K3) were immediately analysed for haematological parameters including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (HCT), and platelets (PLT) by using a haematology analyser (Sysmex KX-21N, Japan).

Histopathology

Immediately after blood collection and organ weighting, small pieces of liver and kidney were harvested and fixed in 10% buffered formalin. Tissue specimens of the liver and kidney were processed for paraffin-embedding and serial sections (5 um thickness) were prepared for staining with haematoxylin and eosin (H&E) or periodic acid-

Schiff (PAS). For each rat, three random sections were analysed under a light microscope (UPLAN FI, Japan) to study toxicity associated histological changes in the liver and kidney tissues. Pathological features including degeneration, congestion, infiltration and haemorrhage were assessed and scored for each microscopic field of kidney or liver sections according to a scoring checklist (0 = none, 1= mild, 2= moderate, 3= severe).[3,22,23]

Statistical analysis

Results are expressed as mean ± SD in all groups. Variance in data was checked for homogeneity by Kolmogorov–Smirnov test. Statistical differences between groups were detected by T-test, and one-way ANOVA test followed by Dunnett's test. Furthermore, histopathological grading scores were analysed between the groups using Kruskal-Wallis test. Statistical significance was inferred at $p<0.05$. The SPSS software, version 22 was used for all analysis.

Results

Acute toxicity evaluation

Likewise to control group, ethanolic extract of AH at the dose of 2000 mg/kg did not cause death in the animals. There was no difference in behavioural evaluated parameters between control and AH (2000mg/kg) treated group. Moreover, there was no significant difference in food consumption (p=0.79), water intake (p=0.69) and weight changes (p=0.908) between AH (2000 mg/kg) and control group (Table 1). It is very crucial to evaluate acute toxicity signs in attempt to establish a lethal dose causing of 50% of animals death (LD50).[20] According to these findings, it was assumed that AH LD50 dose is above 2000 mg/kg.

Subacute toxicity evaluation

Compared to the control, repetitive administration of AH at the all doses (75-300 mg/kg) showed no changes in food consumption, water intake and weight gain in both female and male rats (Table 2).

Table 1. Food consumption, water intake and weight changes after 14 days of single administration of ethanolic extract of *Astragalus hamosus* (AH) in acute toxicity study.

Parameters	Groups		T-test (p-value)
	Control	AH 2000 mg/kg	
Food consumption (g/day)	95.83±4.35	96.66±6.43	0.79
Water intake (ml/day)	175.83±12.36	178.33± 8.73	0.69
Weight changes (g)	28.16±2.22	28.00±2.60	0.908

Values are expressed as mean± standard deviation. n=6 animals per group.

Table 2. Food consumption, water intake and weight changes after 28 days repetitive oral administration of ethanolic extract of *Astragalus hamosus* (AH) in subacute toxicity study.

	Groups				ANOVA Test (p-value)
	Control	AH 75 mg/kg	AH 150 mg/kg	AH 300 mg/kg	
Female					
Food consumption (g/day)	95.87±3.27	102.62± 3.77	98.12±6.26	104.62±12.48	0.98
Water intake (ml/day)	165.25±5.87	168.62±11.08	165.37±12.87	168.62±10.55	0.84
Weight changes (g)	48.50±9.31	48.37±11.01	54.75±14.24	45.50±14.13	0.302
Male					
Food consumption (g/day)	106.10±5.37	102.23±4.32	99.41±5.11	101.14±3.23	0.98
Water intake (ml/day)	153.32±8.11	165.93±4.21	153.12±4.41	166.29±8.71	0.79
Weight changes (g)	41.13±7.49	51.79±13.21	49.72± 6.68	42.13±8.42	0.19

Values are expressed as mean± standard deviation. n=10 animals per group.

Table 3. Organ weights (g), after 28 days administration of ethanolic extract of *Astragalus hamosus* (AH) (75-300mg/kg) in subacute assay.

	Groups				ANOVA Test (p-value)
	Control	AH 75 mg/kg	AH 150 mg/kg	AH 300 mg/kg	
Female					
Heart	0.98±0.22	0.97±0.15	0.99±0.13	1.00±0.18	0.93
Lung	1.47±0.21	1.39±0.21	1.48±0.11	1.44±0.24	0.17
Liver	7.81±0.67	7.42±0.56	7.89±0.71	7.61±0.69	0.41
Kidney	0.89±0.03	0.91±0.04	0.86±0.07	0.89±0.05	0.33
Spleen	0.67±0.04	0.65±0.08	0.67±0.06	0.69±0.05	0.08
Ovary	0.09±0.01	0.09±0.00	0.88±0.03	0.09±0.02	0.19
Male					
Heart	1.01±0.07	1.11± 0.13	1.11±0.09	1.02±0.11	0.99
Lung	1.60±0.15	1.47±0.15	1.59±0.107	1.59±0.07	0.84
Liver	8.24±0.53	8.17±0.83	8.61±0.63	8.66±0.704	0.51
Kidney	1.01±0.09	0.96±0.07	1.00±0.04	1.02±0.09	0.23
Spleen	0.76±0.04	0.73±0.07	0.68±0.06	0.77±0.09	0.14
Testis	1.57±0.13	1.55±0.22	1.63±0.18	1.74±0.16	0.98

Values are expressed as mean± standard deviation, n=10 animals per group.

Table 4. Biochemical parameters in rats treated with ethanolic extract of *Astragalus hamosus* (AH) (75-300mg/kg) for 28 days.

	Groups			
	Control	**AH 75 mg/kg**	**AH 150 mg/kg**	**AH 300 mg/kg**
Female				
Glucose (mg/dl)	90.85±6.32	90.73±5.32	93.14±7.41	90.32± 8.19
Total bilirubin (mg/dl)	0.21±0.05	0.23±0.03	0.20±0.04	0.20±0.01
Total cholesterol (mg/dl)	98.16± 4.49	101.13±5.22	91.64±4.12	81.23±7.39*
Triglyceride (mg/dl)	51.18±4.32	57.28±6.11	50.23±7.48	49.61±8.13
Sodium (mmol/dl)	153.66±3.63	154.16±3.40	153.31±4.71	156.66±5.20
Potassium (mmol.dl)	3.85±0.23	3.95±0.06	3.91±0.04	3.96±0.07
Aspartate aminotransferase (U/L)	83.5± 8.45	75.40±9.11	86.42±6.34	76.11± 8.56
Alanine aminotransferase (U/L)	41.30± 7.14	39.27± 8.23	42.64± 6.18	40.33± 9.36
Blood urea (mg/dl)	60.83± 7.32	63.33± 8.42	61.52±6.38	58.11±9.79
Creatinine (mg/dl)	0.83± 0.03	0.79±0.06	0.78± 0.12	1.08±0.07*
Uric acid (mg/dl)	0.93±0.41	1.02± 0.33	1.05±0.44	1.53± 0.31*
Albumin (g/dl)	3.01±0.37	3.23± 0.28	3.06±0.38	3.00±0.42
Male				
Glucose (mg/dl)	94.25 ± 6.73	90.12 ± 5.02	89.87 ± 7.33	95.00 ± 10.19
Total bilirubin (mg/dl)	0.31 ± 0.09	0.26 ± 0.05	0.27 ± 0.04	0.25 ± 0.05
Total cholesterol (mg/dl)	98.87 ± 8.32	101.12 ± 9.89	100.00 ± 1.69	88.37 ± 6.20*
Triglyceride (mg/dl)	58.66 ± 13.48	57.66 ± 8.28	65.83 ± 15.65	45.16 ± 4.83
Sodium (mmol/dl)	153.00 ± 4.64	153.16 ± 5.37	152.16 ± 5.07	149.50 ±3.39
Potassium (mmol.dl)	3.56 ± 0.08	3.63 ± 0.12	3.56 ± 0.08	3.50 ± 0.18
Aspartate aminotransferase (U/L)	91.16 ± 17.30	105.83 ± 16.66	107.66 ± 4.41	94.00 ± 18.44
Alanine aminotransferase (U/L)	59.83 ± 13.89	63.83 ± 9.43	58.33 ± 10.01	51.00 ± 8.80
Blood urea (mg/dl)	48.83 ± 9.62	50.00 ± 7.32	45.00 ± 4.38	40.66 ± 5.95
Creatinine (mg/dl)	0.80 ± 0.06	0.88 ± 0.04	0.88 ± 0.04	1.01 ± 0.09*
Uric acid (mg/dl)	0.68 ± 0.32	1.13 ± 0.26	0.93 ± 0.33	1.25 ± 0.28*
Albumin (g/dl)	2.91 ± 0.29	3.30 ± 0.28	3.38 ± 0.33	3.21 ± 0.27

Values are expressed as mean± standard deviation. n=10 animals per group for subacute toxicity.* represents significant difference (p<0.05) in comparison with control group.

The results of organ weight of the animals are presented in Table 3. In both sexes, there was no significant difference in the weight of heart, lung, liver, kidney, spleen, testis/ovary between AH treated groups (75-300 mg/kg) and non-treated control group (p>0.05, each).

The results of biochemical parameters are presented in Table 4. On this ground, statistical differences were identified for TC, Cr and uric acid between groups. Compared to the control group, AH at doses of 300 mg/kg significantly decreased TC levels (p=0.034 in male, p=0.01 in female); However, it significantly increased Cr (p=0.001 in male, p=0.03 in female) and uric acid (p=0.021 in male and p=0.031 in female) in the animals.

The results of the haematological study are shown in Table 5. There was no significant difference in WBC, Hb, HCT and PLT values between the control and AH treated groups (p>0.05, each). However, compared to the control group, only in male rats, the RBC count significantly decreased in the AH 300 mg/kg treated group (p=0.01).

Histopathological evaluation of the liver and kidney of AH treated groups (75-300 mg/kg) did not show any alterations or signs of toxicity. The results of semi-quantitative histopathological evaluation are presented in Table 6. Kruskal-Wallis global comparison revealed no significant difference between the groups in both sexes (p>0.05, each).

There was no evident liver injury in the AH treated groups (75-300 mg/kg) and the liver sections showed normal architecture without any significant inflammations, sinusoidal haemorrhage and infiltration or hepatocyte degenerations. Moreover, kidney histopathology showed no degeneration in Bowman's capsule, glomeruli and proximal or distal tubules (Figure 1).

Table 5. Haematological parameters of rats treating ethanolic extract of *Astragalus hamosus* (AH) (75-300 mg/kg) for 28 days.

	Groups			
	Control	**AH(75 mg/kg)**	**AH(150 mg/kg)**	**AH(300 mg/kg)**
Female				
Erythrocytes ($x10^6$/ul)	7.92 ±0.14	7.84 ± 0.22	7.21±0.42	7.61±0.32
Leukocytes ($x10^3$/ul)	5.00 ± 1.08	4.45± 1.83	5.03± 1.22	5.55± 1.72
Hemoglobin (g/dl)	13.37± 0.94	14.62± 0.73	13.60±0.42	13.39±0.70
Hematocrit (%)	40.94± 1.39	41.49± 1.05	40.21± 1.42	40.85± 1.20
Platelets ($x10^3$/ul)	952.13± 93.17	998.00± 86.73	894.38± 62.86	994.60±72.88
Male				
Erythrocytes ($x10^6$/ul)	8.56 ± 0.09	8.17 ± 0.43	8.24 ± 0.408	7.98 ± 0.36*
Leukocytes ($x10^3$/ul)	7.54 ± 1.45	9.20 ± 3.37	8.08 ± 1.47	7.47 ± 1.009
Hemoglobin (g/dl)	14.91 ± 0.72	15.38 ± 0.62	14.75 ± 0.32	15.12 ± 0.61
Hematocrit (%)	43.91 ± 1.98	45.90 ± 1.39	43.47 ± 2.104	44.17 ± 2.21
Platelets ($x10^3$/ul)	835.42 ± 68.64	907.37 ± 39.10	850.12 ± 90.45	897.50± 72.11

Values are expressed as mean± standard deviation. n=10 animals per group for subacute toxicity.* represents significant difference (p<0.05) in comparison with control group.

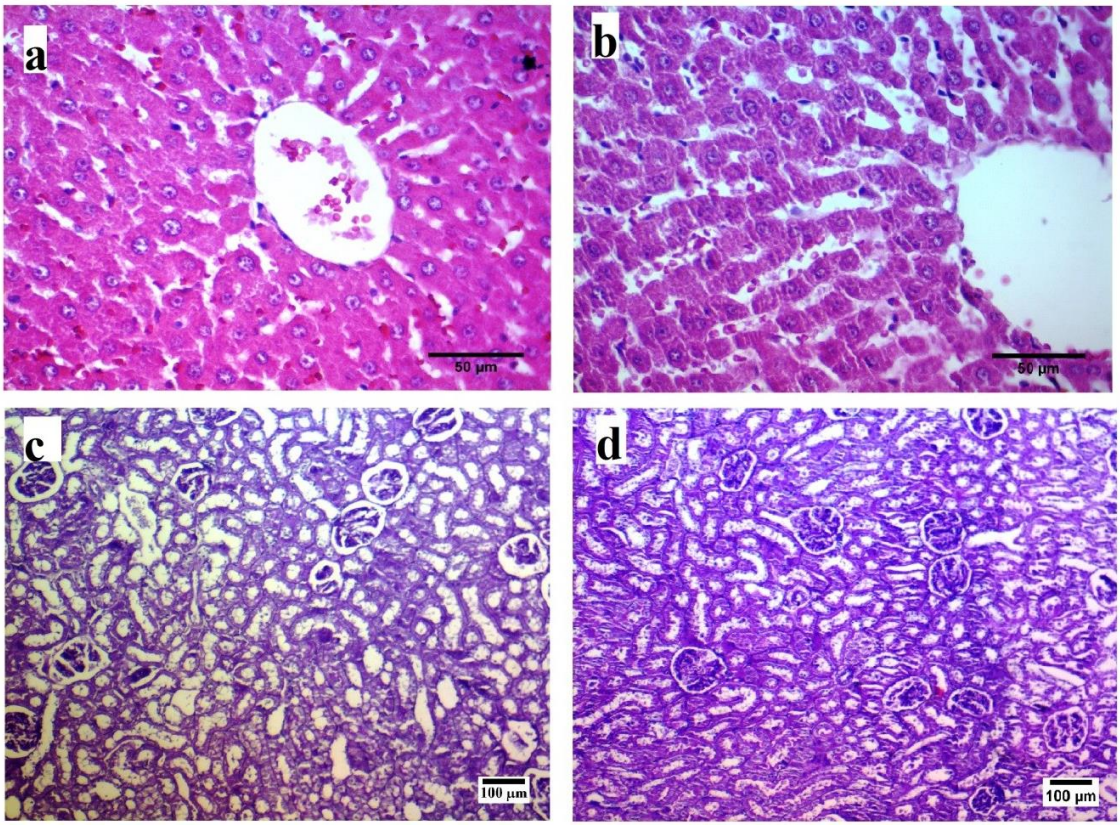

Figure 1. Histopathological assessment of the liver and kidney sections of rats in the control (a, c) and *Astragalus hamosus* ethanolic extract (AH 300 mg/kg) treated in the subacute toxicity test (b, d). The liver sections were stained with hematoxylin and eosin dyes (H&E, 400X) and the kidneys slides stained with periodic acid-Schiff technique (PAS, 100X). (a) Normal liver section belongs to untreated control group ;(b) normal appearance of liver tissue of AH treated rats with no evidence of pathological alteration; (c) kidney section belongs to untreated control group; (d) the kidney section showing adequate glomeruli and normal tubules, with no evidence of pathological damage in AH treated group.

Table 6. Histopathological evaluation of liver and kidney sections in rat treated with ethanolic extract of Astragalus hamosus (AH) (75-300mg/kg) for 28 days.

		Groups				Kruscal-Wallis Test (p value)
		Control	**AH 75mg/kg**	**AH 150 mg/kg**	**AH 300 mg/kg**	
Liver	**Congestion** ***(Mean rank)***	0.35 ± 0.12 *(12)*	0.33 ± 0.50 *(12)*	0.53 ± 0.50 *(18)*	0.53 ± 0.50 *(18)*	0.33
	Haemorrhage ***(Mean rank)***	0.46 ± 0.26 *(14.75)*	0.53 ± 0.50 *(18.50)*	0.51 ± 0.37 *(16.63)*	0.92 ± 0.50 *(16.13)*	0.81
	Degeneration ***(Mean rank)***	0.46 ± 0.25 *(16.38)*	0.35 ± 0.12 *(14.44)*	0.46 ± 0.25 *(16.38)*	0.75 ± 0.50 *(18.81)*	0.67
	Infiltration ***(Mean rank)***	0.51±0.37 *(18.13)*	0.46± 0.25 *(16.25)*	0.35± 0.12 *(14.38)*	0.92 ± 0.50 *(17.25)*	0.74
Kidney	**Congestion** ***(Mean rank)***	0.51 ± 0.37 *(13.13)*	0.53 ± 0.18 *(15)*	0.75 ± 0.70 *(18)*	0.87 ± 0.64 *(19.88)*	0.37
	Haemorrhage ***(Mean rank)***	0.35 ± 0.12 *(14.81)*	0.35 ± 0.12 *(14.81)*	0.46 ± 0.25 *(16.75)*	0.35 ± 0.12 *(19.63)*	0.606
	Degeneration ***(Mean rank)***	0.45 ± 0.12 *(16)*	0.53 ± 0.18 *(16)*	0.53 ± 0.50 *(18)*	0.75 ± 0.25 *(19)*	0.87
	Infiltration ***(Mean rank)***	0.46 ± 0.25 *(17)*	0.35 ± 0.12 *(15)*	0.46 ± 0.25 *(17)*	0.46 ± 0.16 *(17)*	0.91

Scoring was done as follows for each microscopic field: none (0), low (1), mild (2) and severe (3).

Discussion

Toxicological assessments in experimental animals usually were categorized into four classes: acute, subacute, subchronic and chronic. Acute toxicity test is defined as a single exposure for less than 24 h, subacute toxicity refers to repeated exposures for 1 month or less, subchronic toxicity refers to repeated exposures for 1 to 3 months and chronic toxicity assay refers to repeated

exposures for more than 3 months.[24] The oral route of drug administration is the most convenient and commonly used method for toxicity evaluations in pre-clinical animal models.[25,26] In the current study, in addition to acute study, subacute toxicity assay was performed to obtain data on the toxicity of the three doses of AH (75, 150 and 300 mg/kg) after 28 days repeated oral administration. The main purpose of subacute toxicity was to establish the lowest level of adverse effects and identify the specific affected organ/s by the AH after repetitive administration.

The results of acute toxicity assay showed that AH at dose of 2000 mg/kg did not cause death and behavioural changes in the animals. Therefore, it can be concluded that according to OECD guidance,[20] the ethanolic extract of AH may be assigned to be the lowest toxicity class 5 (LD50> 2000 mg/kg).

In both of acute and subacute evaluations, no significant difference observed in food consumption, water intake and weight change between treated and untreated animals. These findings indicate normal metabolism and health status of AH treated animals.[27,28] Moreover, insignificant difference in vital organs' weight between the control and AH treated animals clearly demonstrates that AH at the all doses (75-300mg/kg) did not cause any sensitivity, alteration and acute organ damage.[29]

The biochemical parameters evaluation regarding liver function (AST, ALT, Bil.t and albumin) showed no significant difference between the control and AH treated groups. Plasma levels of AST and ALT are the first and foremost indicators in assessing liver injuries.[30] The enzymes normally present in the cytosol and are leaked out into the blood stream, when the hepatocyte plasma membrane is damaged.[31] The results of some *in vitro* studies have proven AH hepatoprotective activity and beneficial effects on liver damages.[17,32] Similar to biochemical parameters, histological evaluation of liver tissue revealed no alteration regarding hepatic toxicity in AH treated animals.

Monitoring plasma levels of glucose, cholesterol and triglyceride are of utmost importance in toxicological studies due to their direct link with devastating ailments like diabetes, hypertension and cardiovascular diseases.[33] Compared to the control group, AH treating did not cause any significant changes in FBG and TG levels; however, at the maximum dose (300 mg/kg) significantly decreased total cholesterol levels in both male and female rats. To best of our knowledge, there is no study regarding AH effects on lipid profile either in clinical or animal studies. However, the cholesterol lowering activity of AH may be due to its high amount of flavonoids and phenolic compounds which are able to reduce TC levels by inhibiting 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase (HMG CoA reductase), the rate-regulatory enzyme of cholesterol biosynthesis.[34]

Kidneys play pivotal role in excretion of metabolites and drugs, and regulating blood flow and many metabolic activities, hence they are highly susceptible to damage by drugs or herbs.[23,35] One of the important functions of kidneys is electrolytes balance. Sodium is the main extracellular cation; in contrast, potassium has considered the main intracellular cation. Sodium regulates the amount of body's water and also plays crucial role in body function by its movement across the cell membrane. Along with sodium, potassium regulates water and acid-base balance in the blood and tissues. Moreover, potassium helps the muscles and heart to work properly. Also, it participates in protein synthesis from the amino acids in cells. Therefore, inadequate of electrolytes (too much or too little) can cause cell malfunction.[4,36] The results of present study showed subacute administration of the AH extract at the all doses did not alter plasma concentrations of sodium and potassium in rats. However, the maximum dose (300mg/kg) of AH caused significant elevation in plasma concentrations of Cr and uric acid in both male and female rats. Numerous conditions cause elevation of Cr and uric acid in the body. The main causes for elevated plasma Cr and uric acid are higher synthesis and lower excretion due to renal dysfunction or both.[37]

Haematological parameters are highly sensitive markers of drug-induced toxicity.[38] The results of haematological study showed that AH at the highest dose (300 mg/kg) significantly decreased RBC counts only in male rats. The bone marrow has been considered as the most sensitive target for toxic compounds.[39] However, this finding regarded as toxicologically irrelevant because these values are within the normal range.[40]

This is the first study that presents acute and subacute toxicological assays of AH seedpod ethanolic extract in rats. The results of this investigation explain absence of acute toxicity of the ethanolic extract of AH; however, subacute study showed that AH at the high dose may cause renal dysfunction and anemia.

Conclusion

The results of present study showed that AH seedpod has considerable cholesterol lowering activity in rat. Acute and subacute assays suggested that AH has lower toxicity. However, in subacute study and highest dose, AH revealed some signs regarding renal dysfunction as well as RBC count decreasing effects. It could be concluded that despite the many beneficial effects of AH, we should not be unaware of its unwanted effects particularly in renal function and hematopoietic system.

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Conflict of interests

The authors claim that there is no conflict of interest.

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