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Original article

Safety Assessment of *Arctium lappa* L. Fruit Extract in Female Wistar Rats: Acute and Repeated Oral Toxicity Studies

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Abstract

Background and objectives: *Arctium lappa* belonging to the Compositae (Asteraceae) family has been used as a medicinal and nutritional supplement in the world. The fruits, leaves and roots of the plant are well-known for their pharmaceutical effects. Toxicity of the fruit's extract in female rats was investigated in the present study. **Methods:** To assess the toxicity profile of *Arctium lappa* fruit extract (ALFE), it was administered to rats by gavage in acute and repeated models. The animals were divided into two groups: control and test groups**.** In the acute toxicity model, 1000 and 5000 mg/kg ALFE were administered to the animals. Toxic symptoms, body weight, death and abnormal behaviors were observed for 14 days. In the repeated toxicity model, ALFE (300 mg/kg) was daily administered for 4 weeks. Biochemical and histopathological changes were assessed and compared with the control group. Statistical significance was determined by one-way analyses of variance, followed by the Tukey test using GraphPad Prism 6. **Results:** No mortality was noticed in the acute test; therefore, the oral LD_{50} value determined in the female rats was greater than 5000 mg/kg. In the repeated test, the animals received ALFE (300 mg/kg) and no mortality was observed. The hematology and serum chemistry parameters showed no statistically significant changes. The histopathological studies revealed evidences of microscopic lesions in two main organs lungs and small intestine. **Conclusion:** The results indicated that the oral acute toxicity of ALFE in the rats was of a low order with LD_{50} being more than 5000 mg/kg. Moreover, they revealed slight tissue damage to several organs when sub-chronically administered at a dose of 300 mg/kg.

Keywords: acute; *Arctium lappa* L.; subchronic; toxicity test; Wistar rats

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Introduction

Arctium lappa L. (greater burdock) belongs to the Compositae (Asteraceae) family. It is locally known as "Baba-Adam" and its roots have been used in Iranian traditional medicine for gout, rheumatoid arthritis and inflammatory diseases of skin [1]. The plant's root, leaves and seeds have several medicinal effects [2], including anti-inflammatory [3], anti-bacterial [4], antiviral [5], anti-diabetic [6] anti-cancer activities [7] and hepatoprotective effects [8,9]. *Arctium lappa* has also been reported to have side-effects. The most commonly reported side effect has been the induction of contact dermatitis [10]. There was also a case of development of anaphylaxis due to

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burdock consumption. A Japanese man had developed urticaria after consuming boiled burdock as food; with redness occurring over his entire body and other symptoms and he was diagnosed to be in anaphylactic shock [11].
There are a few studies on the toxicity and safety of *A. lappa* [12-15] while no study on the total vital organs and blood biochemical parameters was observed. In the present study, the acute and repeated toxic effects after oral administration of *A. lappa* fruits extract was investigated for the first time in female Wistar rats.

Material and Methods

Ethical considerations

All experiments were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by Research and Ethics Committee of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS). (IR.IAU.PS.REC.1397.012).

Plant collection and preparation of extract

Dried fruits of *A. lappa* L.were obtained from Jahad Keshavarzi Institute of medicinal plants, Karaj, Iran. The fruits were identified by Dr. Gh. Amin and the voucher specimens were kept under No. IAUF 50-PMP/A at the herbarium of Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran. The fruits were ground and extracted with ethanol 70% and then filtered, dried and stored in a proper container until use.

Animals

Female Wistar rats (150-200 g) were bought from Shahid Beheshti University of Medical Sciences, Tehran, Iran. The experiments were conducted between 9 a.m. and 13 p.m. with normal room light (12 h regular light/dark cycle) and temperature (22 ± 1°C). All procedures were carried out in accordance with the local guidelines for the care of laboratory animals of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Iran.

Acute toxicity study

Acute toxicity was evaluated according to guideline No. 423 of OECD [16] with a little modification. Six female Wistar rats were used (3control/3 test). The first dose of the extract was determined as 1000 mg extract/kg body weight. Since no mortality was observed 24 h after the extract administration, it was decided to increase the dose to 5000 mg extract/kg body weight which also resulted in no mortality 24 h after the administration. The animals of both groups were observed for general behavior changes (i.e., appearance of feces, urine color, sensitivity to sound and touch, mobility, aggression, convulsions, ataxia, hypo activity and ventilation disorders). Physical examination included death, hair coat, mucus membrane/eye/skin color, body temperature, respiratory rate, lacrimation, salivation amount, eye prominence and body weight. Blood samples and main organs were collected on the 15th day of experiment for biochemical assays and pathological studies.

Sub-chronic toxicity study

Sub-chronic oral toxicity test was performed in compliance with the OECD guideline No. 407 [17]. Female Wistar rats were divided into controls (normal saline) and test (*A. lappa* extract) groups each consisting of five animals. The test group was daily treated with oral administration of the plant extract at dose of 300 mg/kg for 28 days. The control group was also treated with normal saline for 28 days. Behavioral parameters, body weight and physical changes were observed during the experimental period. Blood samples and main organs were collected on the 29th day of the experiment for biochemical assays and pathological studies.

Biochemical assays

At the end of acute and sub-chronic toxicity studies, the experimental animals were fasted for 12 h. The animals were then sacrificed and their blood heart was collected into dry tubes and centrifuged at 3000g at 4 °C for 15 min. The serums were sent to lab for biochemical analysis including creatinine, urea, CBC, electrolytes, ALT, AST and ALP.

Pathological studies

The major organs focused in this study were heart, kidney, liver, lungs, stomach, small intestine, spleen, uterus and ovary. These organs were removed for histopathological studies at the endpoint of both acute and sub-chronic toxicity studies. The tissues were fixed in 10% buffered formalin and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Multiple sections from each block were prepared in 5 µm and stained with

haematoxylin and eosin (H&E) for histopathological studies.

Statistical analysis

Data were expressed as the mean ± SEM. The statistical significance was determined by one way analysis of variance followed by the Tukey as the post-test using GraphpadPrism6 software. Differences were considered significant at $p<0.05$.

Results and Discussion

At the dose of 1000 mg/kg, *A. lappa* extract showed no adverse effects on the behavioral responses of the tested rats up to 14 days observation. Physical observations indicated no signs of change in the skin, fur, eyes mucous membrane, behavior patterns, tremors, salivation and diarrhea of the rats. No mortality was also observed at the tested dose and after the oral administration of the extract (1000 mg/kg) in the acute test; the body weight did not change (figure 1A). Since no mortality was noticed at 1000 mg/kg, the dose of the extract was increased to 5000 mg/kg. At this dose too, no mortality, no adverse effect on behavioral responses and no physical changes were observed in the tested rats up to 14 days of observation. Similarly, no change was noticed in the body weight after oral administration in the acute test (figure 1A).

Daily oral administration of *A. lappa* extract at the dose of 300 mg/kg for 28 days did not induce any obvious symptom of toxicity in the animals. No deaths or obvious clinical signs were found in the test group throughout the experimental period. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioral changes, diarrhea, tremors, salivation, sleep, and coma. Like in the acute test, no changes were noticed in the body weight of the rats after oral administration of the extract (300 mg/kg) in sub-chronic test (figure 1B)

The effects of acute and sub-chronic administration of *A. lappa* extract on some biochemical parameters have been presented in table 1. Blood AST level in treated female rats (1000 mg/kg) was significantly ($p<0.05$) higher than the control group. In the test group treated with 5000 mg/kg of the extract, WBC level was significantly ($p<0.05$) less than the control group. Moreover, the extract had no effect on other biochemical parameter.

Though Cl anion was significantly ($p<0.001$) higher than the control group, biochemical analysis was normal in sub-chronic administration of *A. lappa* extract.

Histopathological study of the control group showed normal structure and absence of any gross pathological lesion in organs. Macroscopic examination of the vital organs of tested animals revealed no abnormalities in the color or texture, compared with the control group. Chemically-induced histological changes were not observed in the heart, spleen, stomach, kidney and ovary of test group animals (figures 4-6, 9,10). However, some microscopic damages were present in the liver (figure 2A), lungs (figure 3A), small intestine (figure 7A) and uterus (figure 8A) in acute toxicity test. Macroscopic examination of the vital organs in sub-chronic test also revealed no abnormalities in the color or texture, compared with the control group. Chemically-induced histological changes were not observed in the heart, spleen, stomach, kidney, liver, ovary and uterus of test group animals. However, some microscopic damages were present in the lungs (figure 3B) and small intestine (figure 7B).

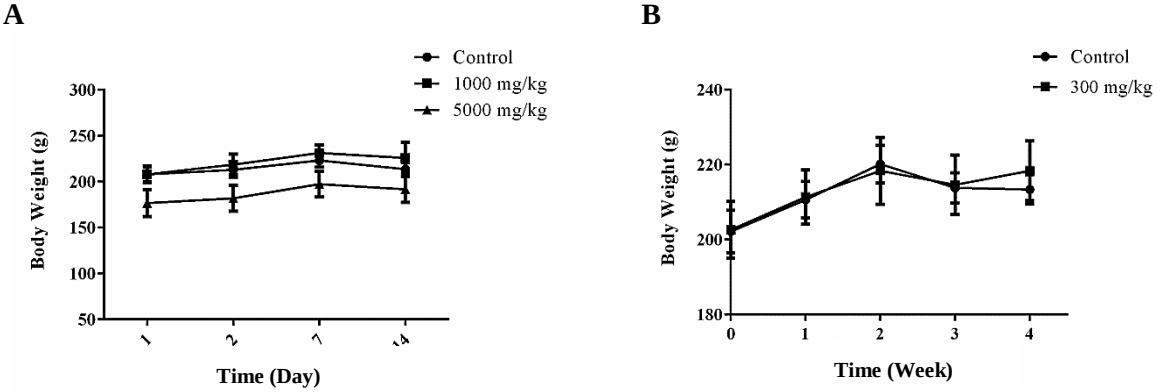

Figure 1. Effects of acute oral toxicity (A) and sub-chronic oral toxicity (B) of *Arctium lappa* fruits extract on the evolution of body weight

Table 1. Effects of *Arctium lappa* L. fruits extract on some serum biochemical parameters in the acute and sub-chronic toxicity in female Wistar rats

Parameter	Acute			Sub-chronic	
	Control	1000 mg/Kg	5000 mg/Kg	Control	300 mg/Kg
AST (units/L)	137.00 ± 4	**172.00 ± 10***	125.00 ± 12	159.70 ± 22.8	174.70 ± 12
ALT (units/L)	31.00 ± 8	55.00 ± 4	31.00 ± 3	33.70 ± 5.3	39.70 ± 5.8
ALP (IU/L)	411.00 ± 4	615.50 ± 97.5	273.50± 43.5	355.70±55.4	334.70±35.7
Urea (mg/dL)	46.50 ± 3.5	47.00±6	37.00±4	47.33±2.2	48.67±2.8
Cr (mg/dL)	0.85±0.25	0.50±0	0.35±0.05	0.77±0.17	0.57±0.14
Na^+ (mmol/L)	143.00±4	175.50±3.5	175.50±2.5	143.30±2.3	157.00±4.6
K^+ (mmol/L)	3.80±0.1	4.35±0.65	3.15±1	3.60±0.2	4.43±0.28
Cl^- (mmol/L)	94.50±2.5	121.50±2.5	136.50±12.5	94.00±1.5	**121.70±1.8*****
WBC (cells /mcL)	5650±950	5500±1700	**3500±900***	6200±777	5067±722
RBC (cells/mcL)	5.3×10^6±665000	7.6×10^6±45000	6.3×10^6±250000	5.9×10^6±669386	5.1×10^6±150148
HGB (g/dL)	16.00±0.7	16.20±0	13.90±0.5	16.00±0.4	15.27±0.6
HCT (%)	31.45±2.5	43.95±0.05	36.70±0.8	31.37±1.5	30.60±1.5
MCV (fL)	59.20±2.6	57.45±0.45	58.35±1.05	58.47±1.7	59.37±1.4
MCH (pg/cell)	30.25±2.45	21.25±0.15	21.90±0.3	30.87±1.5	29.63±0.3
MCHC (g/dL)	51.01±1.9	36.80±0.1	37.45±0.15	51.10±1.1	49.91±1.0
PLT (cells/mcL)	810500±12500	741500±31500	825500±23500	810333±7219	850333±3180

Data have been expressed as mean±SEM; n=3. *p<0.05 **p<0.001 ***p<0.0001, compared to the control group; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; Cr: creatinine ; Na^+ : sodium ion ; K^+: potassium ion ; Cl^-: chlore ion; WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit ; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet count

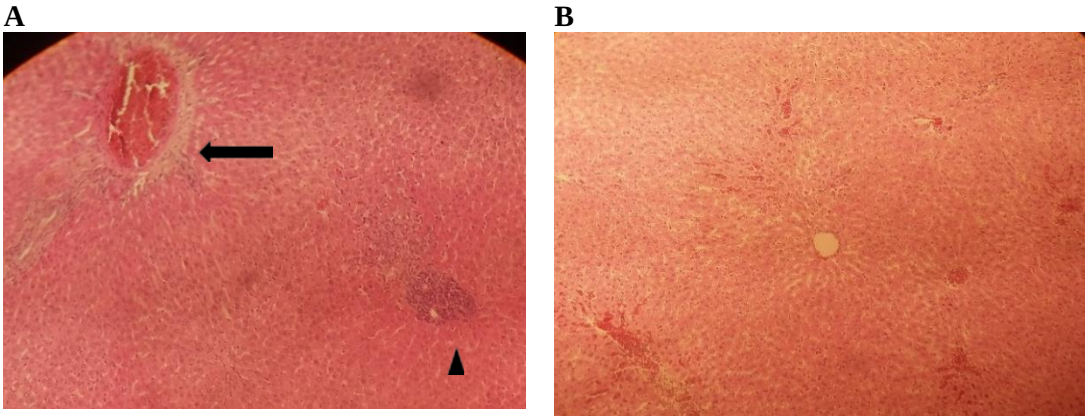

Figure 2. Histopathological structure of liver in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. Hepatic cord arrangement changed a little (arrow). The portal vein was full of leucocytes (arrowhead) (A)

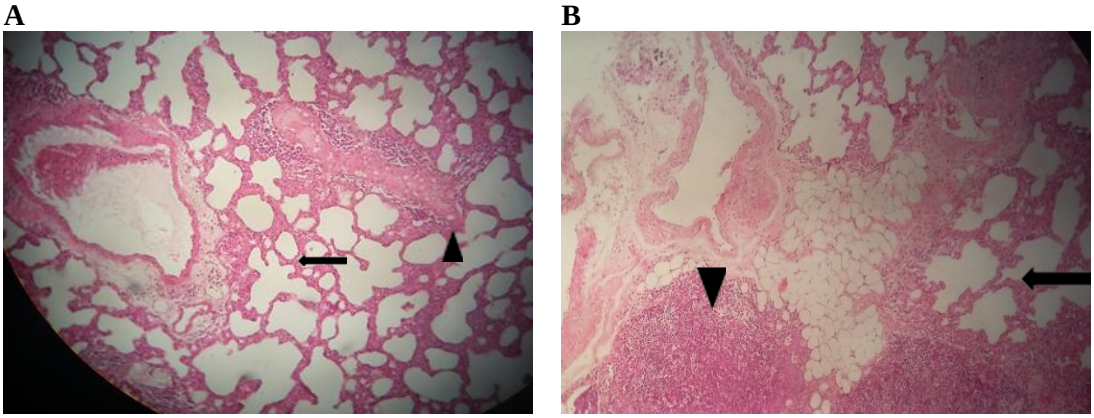

Figure 3. Histopathological structure of lung in acute (A) and sub-chronic (B) toxicity test groups of *Arctium lappa* L. fruit extract toxicity experiments. The alveolar wall thickened very much (arrow) and hyalinized areas (arrowhead) were seen in lung after acute administration of *A. lappa* extract (A). The alveolar thickened very much (arrow) and there was an accumulation of

leucocytes (arrow head) (B)

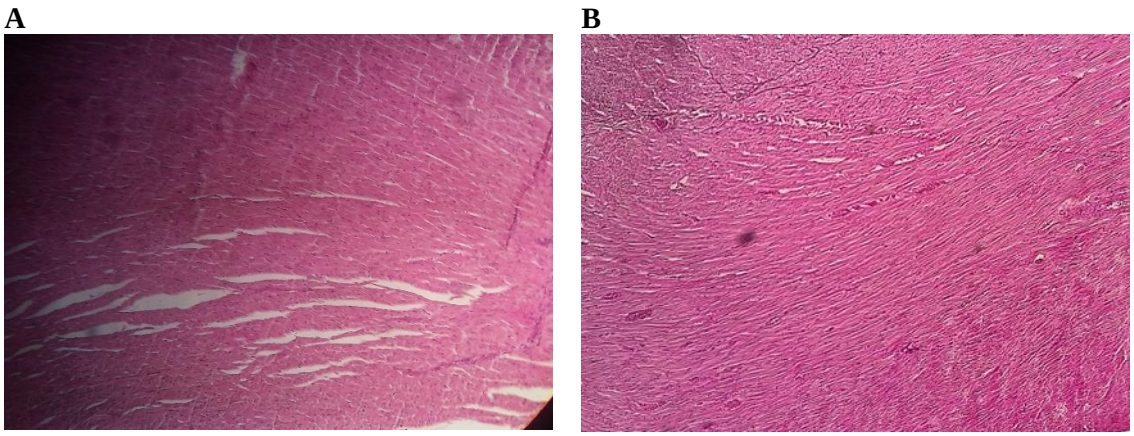

Figure 4. Histopathological structure of heart in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. There was no structural change in the shape and orientation of the muscle cells.

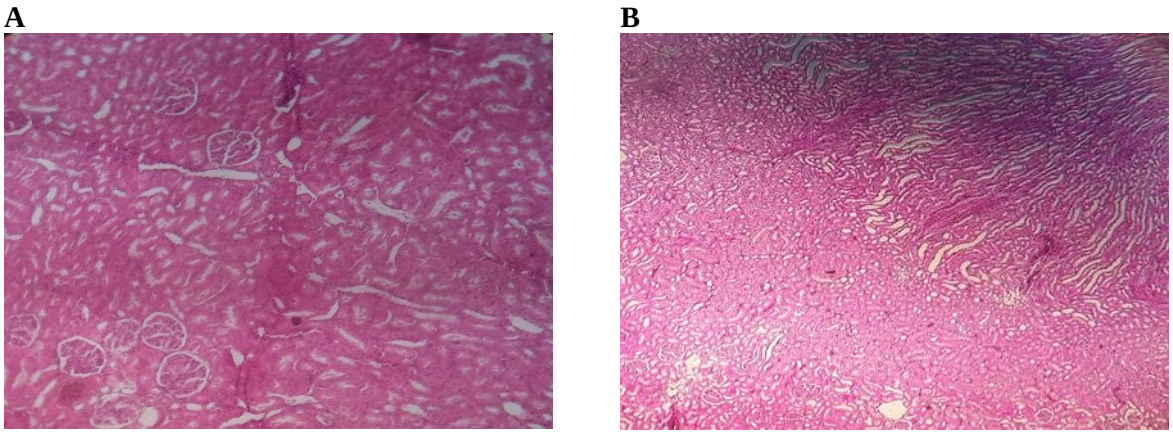

Figure 5. Histopathological structure of kidney in acute (A) and sub-chronic (B) toxicity test group of *A. lappa* fruit extract toxicity experiments. There was no structural change in the shape of renal corpuscles and convoluted tubules.

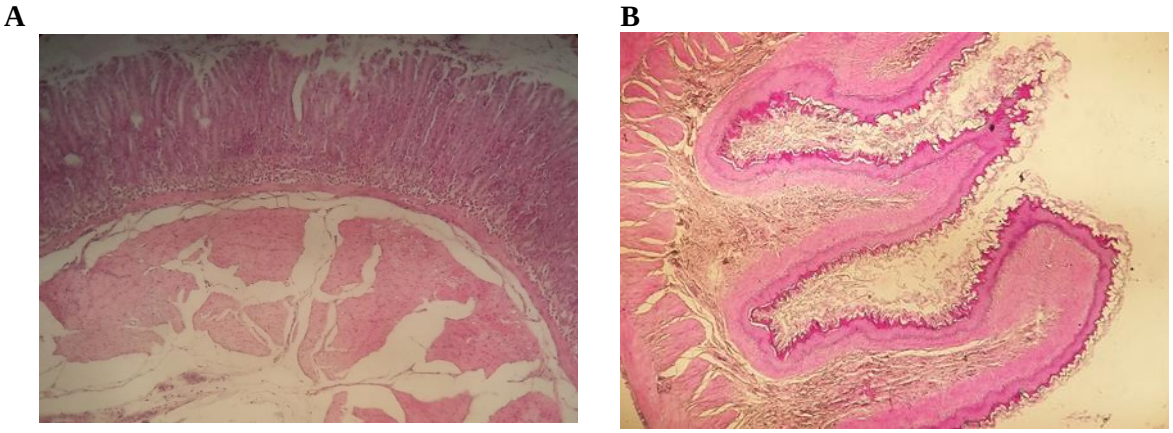

Figure 6. Histopathological structure of stomach in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. There was no structural change in different layers of stomach wall.

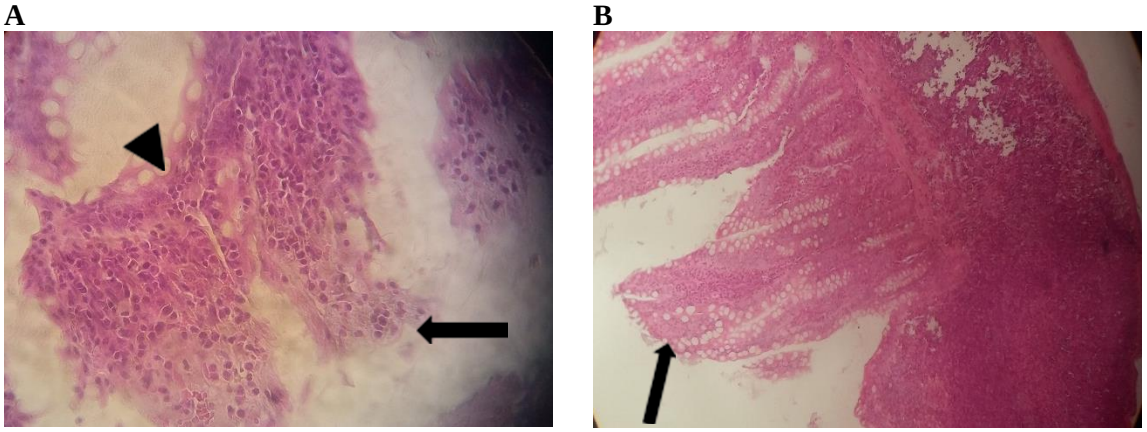

Figure 7. Histopathological structure of Small intestine in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. The number of goblet cells reduced (arrowhead) and mucosa layer was destroyed (arrow) (A). The columnar cells of mucosa layer were destroyed a little (arrow) (B).

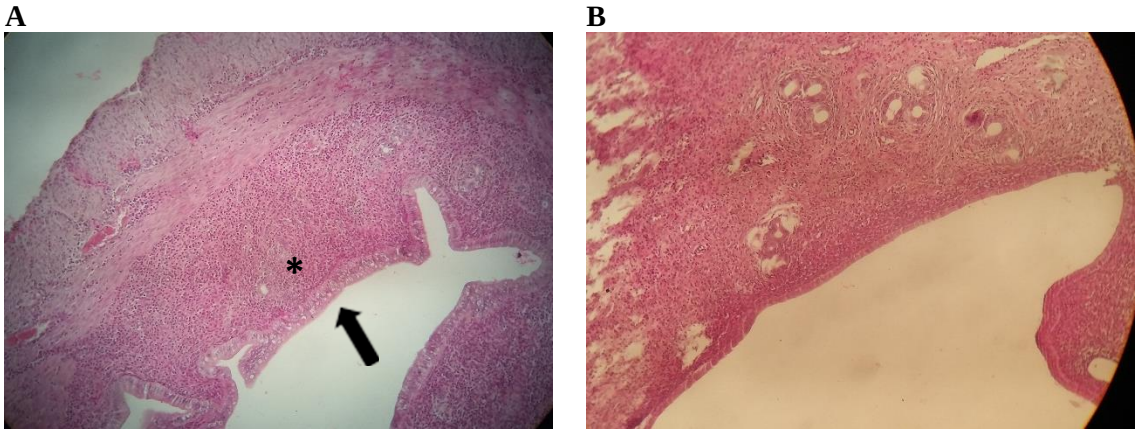

Figure 8. Histopathological structure of uterus in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. Columnar epithelium arrangement of endometrium changed (arrow). There was an extra layer between endometrium and myometrium full of leucocytes (star) (A).

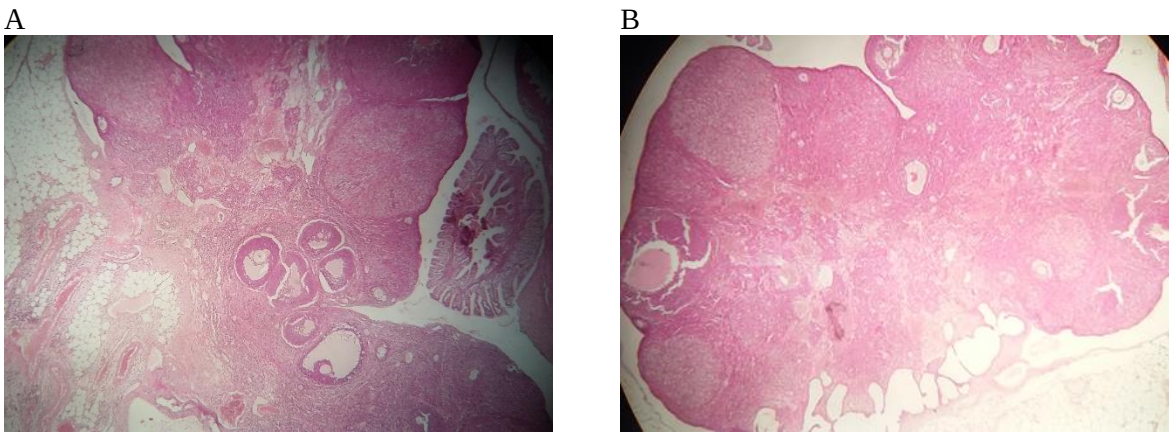

Figure 9. Histopathological structure of ovary in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. There was no structural change in cortex and medulla.

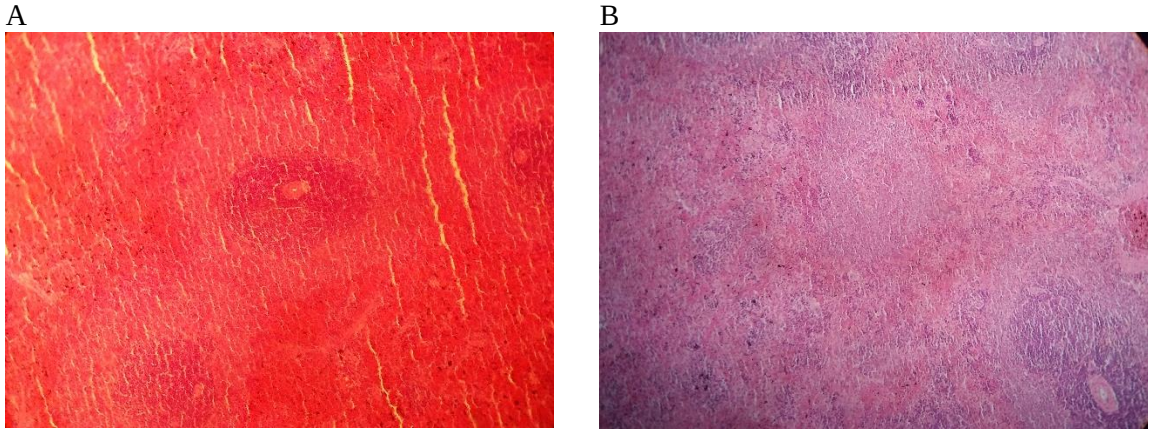

Figure 10. Histopathological structure of spleen in acute (A) and sub-chronic (B) toxicity test groups of *A. lappa* fruit extract toxicity experiments. There was no structural change in white and red pulps.

The main objective of the present study was to examine the acute and repeated toxicity of *A. lappa* fruit extract in female rats. The results showed that the administrated acute dose of *A. lappa* extract did not result in any mortality; however, the repeated administration of the extract had a few toxic effects on two main organs lungs and small intestine.

Arctium lappa is commonly used in Iranian traditional medicine for its potent therapeutic effects. Regarding the safety profile of the plant in acute and repeated tests, the present study undertook and succeeded to demonstrate its safety profile in both models of toxicity assessment. Given that no death and signs of toxicity was observed in the first 24 h following *A. lappa* fruit extract administration at doses of 1000 mg/kg and 5000 mg/kg, the extract can be classified as practically nontoxic according to Hodge and Sterner scale [18]. In the acute toxicity study, there were no changes in the body weight, physical changes and behavioral patterns. In addition, blood AST level in treated female rats (1000 mg/kg) was significantly higher than the control group which could be due to liver damage; however, this increase was not observed at higher doses and needs to be further investigated.

In the group test treated with 5000 mg/kg of the extract, WBC level was significantly less than the control group which could be due to accumulation of leucocytes in the organs; however, the extract had no effect on other biochemical parameters. In histological analysis of the acute toxicity test, some microscopic damages were present in liver, lungs, small intestine and uterus.

In another study on the "extraction, quantification, formulation and evaluation of oral capsules from *burdock* fruits", the researchers aimed at using the capsule for its hepatoprotective effects. Moreover, according to other studies in which the hepatoprotective effect of *A. lappa* extract was determined by administrating the dose of 300 mg/kg [8,9], the same dose was decided to be used in sub-chronic toxicity test. In repeated treatment, administration of the extract at 300 mg/kg did not cause significant changes in body weight in the test group. The sub-chronic administration of the extract for 28 days increased the Cl ion level which could be due to the thickening of alveolar walls that caused respiratory alkalosis. The histological analysis of different organs showed slight microscopic tissue damage in lungs and small intestine.

Reports on the toxicity of this plant were not comprehensive. In 2017, the safety of 8 weeks oral administration of *A. lappa* root extract was conducted. The result showed that the plant was safe for repeated administration but there was no report on acute toxicity [14]. In 2016, the therapeutic effect of *A. lappa* seeds in *Schistosoma haematobium* in association with kidney disturbance was evaluated. The maximum non-lethal dose of *A. lappa* seed extract was found to be 3000 mg/Kg, but only the kidney tissue and its biochemical parameters were studied [15]. In a study conducted in 2012, the results indicated the LD_{50} of seeds was 9.3 mg extract/Kg body weight but the method and study duration was not mentioned [12]. In 2002, a study about hepatoprotective effects of *A. lappa* on liver injuries induced by chronic ethanol consumption and potentiated by carbon

tetrachloride showed the LD_{50} of the plant higher than 2000 mg/kg, but only the liver tissue and its biochemical parameters were studied [9]; However, in the present study, both the acute and sub-chronic toxicity were evaluated along with changes in biochemical parameters and vital organs.

The current study suggests a daily oral dose of less than 300 mg/kg for the long-term administration of *A. lappa* fruit extract. Hence, it is necessary to establish a scientific basis for the therapeutic actions because it may serve as the source for the development of more effective drugs.

The fruits of *A. lappa* contain arctiin, arctigenin, chlorogenic acid, caffeic acid, coumarin and cynarin [12,13]. Previous studies have reported that arctiin and arctigenin are major constituents in *A. lappa* fruit [13]. These constituents have been reported to show a variety of biological activities and a number of important pharmacological properties such as anti-proliferative, cytotoxic, anti-inflammatory, calcium antagonist and anti-carcinogenesis activities [19-23]. However, little is known about its main toxic ingredients and underlying mechanisms. The study of in vivo toxicity of arctiin and arctigenin is recommended.

Tannin is one of the most common active compounds found in the root of burdock. It inhibits tumor growth, induces macrophage responses, and possesses immuno-modulatory activity [24]. Though, tannin is potentially toxic in nature. It may reason stomach upset and at high concentrations it has some dangerous side effects such as nephrotoxicity and hepatic necrosis [24]; therefore, the use of tannin should be carefully monitored.

In the present study, ethanol extract of *A. lappa* fruit showed histopathological changes in lungs and small intestine. Moreover, it is important to note that the ethanol extract contains mainly polar compounds. It is well known that the type of solvent used in the extraction process determines the chemical composition of the resulting extracts and consequently its biological properties. Thus, considering that in traditional medicine *A. lappa* extract is medicinally consumed as tea it is needed to be the future studied for probable toxic compounds.

In conclusion, the findings of the present study demonstrated that *A. lappa* fruit extract was practically nontoxic with the LD_{50} greater than 5000 mg/kg. However, the repeated administration of *A. lappa* fruit extract (300 mg/kg) over a period of 28 days and at a relatively lower dose induced some organ damages in the rats. Thus, the tested dose is not safe and further investigation should be performed to determine NOAEL (no-observed-adverse-effect level**)** for *A. lappa* Fruit Extract. These results confer additional evidence that the extract, if applied at non-recommended doses, can cause functional damage to critical organs such as liver, lung, small intestine and uterus in animals and probably in humans. This is an ongoing study and further work is being carried out to investigate its toxic activities. Overall, in this study, the female rats appeared to tolerate *A. lappa* fruit extract at the acute dose of 5000 mg/kg of body weight well.

Acknowledgments

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Author contributions

Maedeh Yaghoubi did all tests, analyzed the data and wrote the manuscript; Zahra Mousavi designed the animal studies and edited the manuscript; Tayebeh Rastegar interpreted the pathological results; Gholamreza Amin helped in identifying *A. lappa* Fruits. All authors have read and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

NOAEL: no-observed-adverse-effect level; ALFE: *Arctium lappa* fruit extract; LD_{50}: median lethal dose