



## Toxicity Assessment of Hydroalcoholic Extract of *Kelussia odoratissima* Mozaff : An Acute and Repeated Dose in Female Wistar Rat



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**T**HIS study aimed to evaluate the acute and repeated dose toxicity of hydroalcoholic extract of *Kelussia odoratissima* Mozaff as medicinal plant which is restricted to western Iran based on its potential nutraceutical applications. To determine the clinical, hematological, biochemical, necropsy and histopathological effects of the herb's extract, aerial parts were collected from Shahrekord in spring 2019. After careful evaluation and issuance of the Herbarium Code Number, herb's powder was provided and the extracted by maceration method using ethanol. Acute and repeated dose toxicity tests were performed by OECD 425 and 407 guidelines through oral gavages to female Wistar rats. Acute test didn't show any sign of toxicity in doses up to 2000 mg/kg and in delayed acute toxicity assessment no sign of toxic reaction was recordable. In repeated dose test all animals in three different dose groups (50,500 and 1000 mg/kg) survived healthy according to the clinical and necropsy observations. Biochemical, hematological and histopathological studies showed some mild to moderate congestions in the kidney and liver of high dose group of animals despite normal feature in the lung, spleen and heart of animals in all dose groups. *Kelussia odoratissima* Mozaff extract is a tolerable herbal remedy even in high doses, in female rats but dose adjustment studies should be performed in future clinical setting to provide safe and effective pharmaceutical oral dosage forms.

**Keywords:** *Kelussia odoratissima* Mozaff, *Kelussia*, Acute toxicity, Repeated dose toxicity, Hydroalcoholic Extract, Mountain celery.

### Introduction

Out of 275 genera and 2850 species of *Umbelliferae* and 119 genera and 358 species of *Apiacea* genus around the world [1], *Kelussia odoratissima* represents by only one species, *Kelussia odoratissima* Mozaff., which is endemic

to a restricted area in western parts of Iran with the popular name of “Karafse-koohi (Mountain Celery)”. Although the local people in four specific provinces of Iran and snow-covered heights of the Zagros region name this self-growing plant as *klows* [2], it is named in older traditional medicine

database as *Amircabiria odoratiaaima*, *Apium graveolens* and *Opananax* sp. [3].

Based on the detected certain phytochemicals e.g. Ferulic Acid [4], phthalide, flavonoids and terpenoids [3, 5] in *Kelussia odoratissima*, many disease protective properties or therapeutic potentials have been suggested for *this* medicinal plant. Presence of phthalides and ferulic acid may induce pharmacological benefits to *Kelussia* like anti-acid properties, antidepressant, anti-inflammatory, analgesic, sedative hypnotic, anti-anxiety and anti-allergic potentials [6] as well as some promising efficacy in improvement of brain injuries, [7], dyslipidemia [8], pulmonary hypertension [9] and a potentiating role in radiotherapy according to recent animal and cellular studies.

Besides ameliorating properties of *Kelussia odoratissima*, its different doses showed toxic effects on malignant cell lines including breast cancer (MDA-MB468 and MCF-7) [11], ovarian cancer (HeLa and SKOV3) [5], lung cancer (A549), eye cancer (Y79), and leukemia cancer cells (K562) alone or in combination with other herbs [4, 5]. In parallel to the above properties, more than 100 national studies emphasize the antioxidant, antibacterial, anti-fungal, larvicidal, insecticidal, anti tumoral, antimicrobial, anti-spasmodic, DNA Repair and fibrinolytic properties for *Kelussia odoratissima* [12] but the safety concepts of this plant has not determined yet. Based on many unknown facts and important knowledge gaps on the systemic toxicity of *Kelussia odoratissima* for its possible future clinical applications, This study aimed to evaluate evaluate the herb's oral toxicity in acute and repeated dose models according to OECD 425 and OECD 407 guidelines [13] in female Wistar rats.

## **Materials and Methods**

### *Plant collection*

The plant material was collected from the heights of Shahrekord city in Chaharmahal and Bakhtiari province in spring 2019 and authenticated by botanists at Agricultural Research, Education and Extension Organization (AREEO) of Lorestan province according to receptive standards. After careful examinations, a voucher specimen of the sample was issued and deposited at the unit herbarium under the number of 14277.

### *Extraction Method*

The collected parts of the herb were washed in running water, padded with tissue paper and dried in shade place at room temperature in the absence of light in a well ventilated room. To provide the extract, 100 g of the plant's dried powder were grounded by laboratory electric mill and the size uniformity was ensured by sieving through a stainless steel mesh (200 mm diameter) and stored in airtight containers at 20°C for further processing. The *Kelussia* extract was provided by method of maceration using ethanol in specific shaker at 500 rpm for 36 hours. In the next step the extract was filtered using Whatman filter paper grade 3 and the resulting solution was concentrated using a rotary (50 °C) and then dried at room temperature. After filtration of extract, the organic solvent was completely dried in 2 weeks. A fine powder was finally obtained in the total weight of 114 grams and used for toxicological tests after utilizing rotary evaporator [14]. The test material was prepared by solving the dried powder in distilled water at room temperature for 1h in sonicator. The required quantity of test item was weighed and diluted by distilled water as per doses for acute and repeated dose toxicity tests. The extraction efficiency of this method was considered as 6%.

### *Experimental animals and housing conditions*

After getting the Ethics committee approval (IR.IAU.TMU.REC.1398.206), 30 regularly cycling Wistar rats, aged 10-12 weeks with average initial body weights of 200±10 g were obtained from Pasture Institute of Iran. They were randomly caged in 6 groups of 5 under standard laboratory indoor conditions, with a 12-h light/dark cycle at room temperature (23 ± 2°C) and relative humidity (20%) with free access to tap water and a standard diet for all treatment groups in acute and repeated dose studies. They had free access to standard pellet diet and drinking water ad libitum but daily food and water intakes were calculated until the end of study. The cage cleaning schedule, air filtration and recirculation, health checks and facility maintenance were carried out in accordance to the Standard Operating Procedures, and recorded daily. Animals were housed and maintained according to the Ministry of Health and Medical Education of Iran for the Care and Use of Laboratory Animals and CCAC Guidelines for Care and Use of Experimental Animals.

#### *Acute toxicity test*

In acute test, we aimed to determine the LD<sub>50</sub> and to observe any abnormal sign and mortality when exposed to high dose (2000 mg/kg) of the extract over a single oral gavage of the extract in the first 24 hours to 5 female Wistar rats and compare with 5 untreated rats based on OECD 425 guideline. All animals were fasted for 1–2 hour before *Kelussia* extract administration with free water access. After the gavages, all 5 animals were fully observed, especially during the first 30 minutes up to 4 hours after extract administration, and their behavior was monitored. The rats were then monitored for 14 days and no deaths or complications were observed. At the end of 14 days, all animals underwent surgery, and the tissues of various organs such as Lung, spleen, liver, pancreas, heart, uterus and ovaries were examined for their appearance as well as their weights in comparison with 5 female Wistar rats in control group (distilled water).

#### *Repeated Dose Oral Toxicity*

To conduct repeated dose oral toxicity study, a total of 20 healthy female Wistar rats (5 female animal/group) were randomly selected and divided into 4 groups (3 treatment and a vehicle control group). Treatment groups received daily doses of 50 mg/kg (low dose), 500 mg/kg (medium dose) and 1000 mg/kg (high dose) of extract suspended in water, in a volume not exceeding than 1 ml/100 g B.W./ rat, for 7 days a week administrations. Animals from control group received water by gavage in the same volume, which was used in treatment group.

The general behavior of the animals was observed daily and before any daily administrations, the female rats were weighed at the same routine daily time. Water intake, food consumption, and body weight were measured once a day. According to OECD 407 toxicity assessment guideline (OECD guidelines, TG 407, 2008), total body weights, organ weights, macroscopic organ evaluations, hematology, serum biochemistry and organ histopathology were assessed after performing the 28 day study. At day 29<sup>th</sup>, all rats in all groups were anesthetized for 6 ml blood collections by heart puncture under light carbon dioxide anesthesia. A portion (2ml) of the collected blood was placed in bain-marie at 37 °C for 40 minutes to clot. The other 2 ml part of their whole blood was kept in acid-washed cryo-tubes at -80 °C and along with 2 ml of whole blood (in the heparin tube), was

transferred at 4 °C to the laboratory to conduct subsequent tests.

#### *Histopathological examinations*

During necropsy study, all necessary organs including lung, heart, kidney, liver, spleen, heart, uterine, ovaries and brain were dissected out. Tissues were rinsed and weighed with physiological serum to remove substances that may interfere with later stages. The tissues were then fixed in 10% formalin solution. To remove the water, the desired tissue was dehydrated with degrees of alcohol (30, 50, 70, 80, 90 and absolute alcohol). To strengthen the fixed tissues, they were placed in a paraffin block and finally 5 micron thin sections were prepared with a microtome and used for eosin-hematoxylin staining for histopathological evaluation. The sections were examined under the light microscope (Olympus BX-51; Olympus, Tokyo, Japan) by expert animal pathologist and scored.

#### *Statistical analysis*

In this analysis, treatment and control groups were compared with each other. When variances were not significantly different, data were analyzed by one-way analysis of variance (ANOVA) and the Student's t-test. A stepwise multiple comparisons procedure was used to identify the sample means that were significantly different from each other. We used post hoc test whenever a significant difference between three or more sample means has been revealed by an analysis of variance (Anova). Values were expressed as means ± SD. The level of significance was set at  $p < 0.05$ . All statistical methods were performed by SPSS version 23.

## **Results**

#### *Acute toxicity of Kelussia extract*

None of the female rats showed any behavioral or apparent changes within the first 24 hours and 14 days follow up period. All animals remained healthy without any adverse effects till day 14. This study showed that the LD<sub>50</sub> the *Kelussia odoratissima* extract was higher than 2000 mg/kg.

#### *Repeated dose 28 days toxicity of Kelussia extract*

*Clinical:* No mortality or sign of toxicity was observed in daily oral doses up of 1000 mg/kg. All animals appeared normal without any observable/recordable clinical change in their total body weights, food and water consumption. As we shown in Table 1, the mean total body weight in all dose groups remained unchanged compared to vehicle control group.

**TABLE 1. Mean  $\pm$  SD of total body weights at Day 1, 7,14, 28 and organ weights at day 29 of *Kelussia* extract oral administration compared to vehicle control group.**

Variables	<sup>A</sup> Low dose (50 mg/kg, n=5 )	<sup>B</sup> Medium dose ( 500 mg/kg, n=5 )	<sup>C</sup> High dose ( 1000 mg/kg, n=5 )	Vehicle Control (n=5)	P-value
Total Body Weight changes (g)					
Weight at day 1	163.60(5.05)	173.40(2.81)	175.67(3.78)	170.25(8.3)	NS
Weight at day 7	166.20(6.38)	174.80(4.91)	178.00(4.35)	172.1(9.48)	NS
Weight at day 14	171.40(8.56)	178.00(6.12)	183.33(3.51)	181.75(9.94)	NS
Weight at day 28	173.40(10.065)	180.40(5.55)	184.67(1.15)	183.75(13.04)	NS
<b>Organ weights(g) at day 29</b>					
Heart	0.62(0.019)	0.65(0.09)	0.67(0.06)	0.70(0.07)	NS
Kidney(left )	0.58(0.02)	0.67(0.08)	0.74(0.218)	0.69(0.07)	NS
Kidney(right )	0.60(0.05)	0.62(0.07)	0.67(0.214)	0.68(0.04)	NS
Liver	5.37(0.22)	5.68(1. 8)	5.93(0.46)	5.76(0.56)	NS
Lungs	1.24(0.18)	1.25(0.09)	1.16(0.13)	1.17(0.09)	NS
Uterine & 2 ovaries	1.018(0.39)	0.94(0.27)	0.953(0.261)	1.01 (0. 40)	NS
Pancreas	1.12 (0.23)	1.01(0.363)	1.01(0.02)	1.18(0.44)	NS
Spleen	0.74(0.14)	0.75(0.16)	0.60(0.05)	0.66(0.09)	NS

**Necropsy:** The weight of all vital organs including two kidneys, liver, lungs, spleen, heart, and ovaries plus uterine were recorded and their ratios to total body weight were calculated as shown in Table 1, no significant differences were observed between the mean organ weights of female Wistar rats compared to vehicle control group.

**Hematology:** Abnormal hematological changes was detected in low and medium dose groups of *Kelussia odoratissima* extract treated female rats .As shown in Table 2, the number of red blood cells in animals receiving the *Kelussia odoratissima* extract at doses of 50 and 500 mg were significantly decreased compared to the control group ( $p<0.05$ ). Moreover, hemoglobin, hematocrit and RDW.CV levels decreased at the dose of 500 mg/kg compared to the control group ( $p<0.05$ ). Other blood factors remained unchanged in all dose groups at day 29 of this repeated dose oral toxicity study compared to control.

**Biochemistry:** Administration of 50, 500 and 1000 mg/kg doses of *Kelussia* extract did not induce any significant change on most of biochemical parameters including glucose, Urea, Creatinine, total cholesterol, AST, ALT, ALP, Na and K compared to the control group (Table 3) but more examinations on lipid profile factors showed that oral administration of *Kelussia odoratissima* extract at 500 and 1000 mg/kg doses caused significant increase in serum levels of LDL as well as LDL/HDL ratio compared to the control group, but administration of the extract at 50 mg/kg didn't induce any significant change in these levels (Table 3).

#### **Histopathology**

**Lung:** Microscopic observations in pulmonary tissues in the group of animals that received 50 and 500 mg/kg doses of the extract ,no evidence on proliferative /nonproliferative changes was detected (Fig. 1: A1-A6). Degrees of mild atelectasis were seen in high dose group given the extract at 1000 mg/kg (Fig.1: A8).

TABLE 2. Hematological changes of female Wistar rats treated with *Kelussia odoratissima* extract .

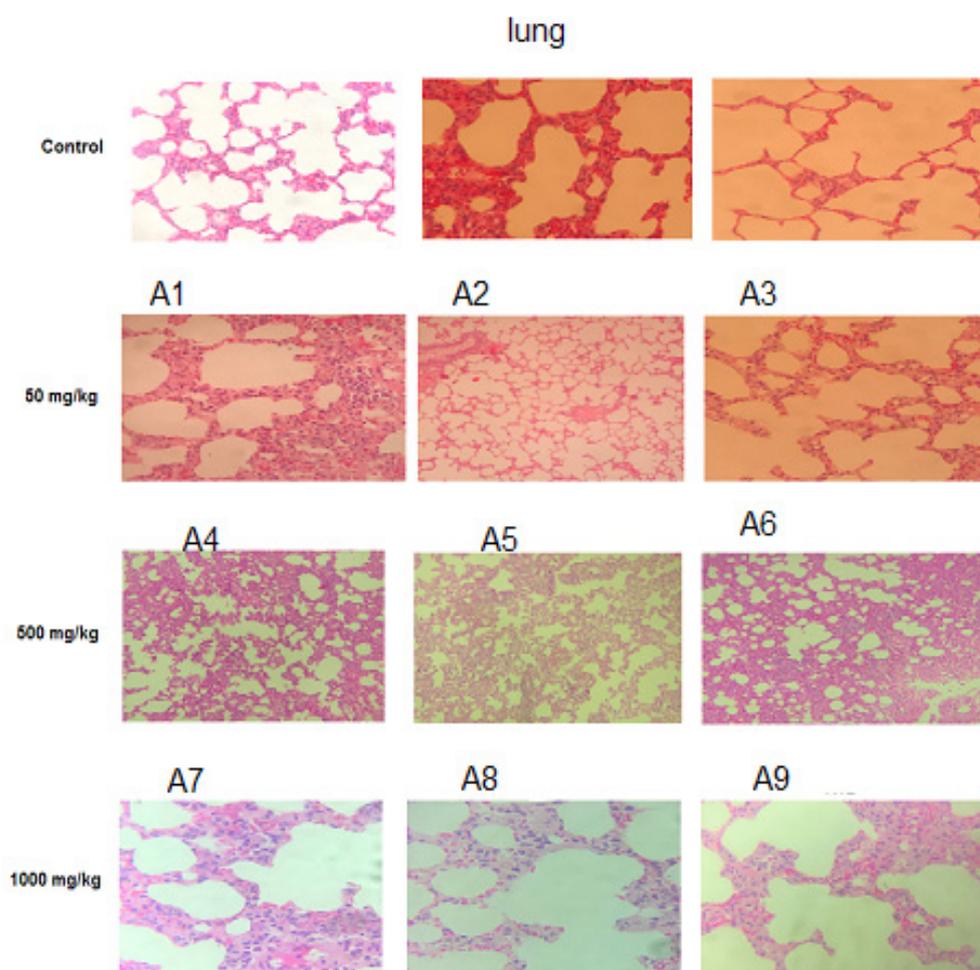
Variables	<sup>A</sup> Low dose (50 mg/kg, n=5)	<sup>B</sup> Medium dose (500 mg/kg, n=5)	<sup>C</sup> High dose (1000 mg/kg, n=5)	Vehicle Control (n=5)	P-value
WBC (10 <sup>3</sup> /μL)	5.24(2.55)	4.86(2.32)	7.86(1.85)	5.97(2.21)	NS
RBC(10 <sup>6</sup> /μL)	7.26(0.2)*	6.68(0.69)	7.73(1.45)	7.86(0.26)	<sup>A</sup> =0.007** <sup>B</sup> =0.016 <sup>C</sup> =0.898
Hb(g/dL)	13.48(0.39)	12.2(1.27)	14.16(2.14)	14.3(0.66)	<sup>A</sup> =0.05* <sup>B</sup> =0.021* <sup>C</sup> =0.926
HCT (%)	41.46(1.14)	37.9(4.28)	43.76(8.11)	44.47(2.54)	<sup>A</sup> =0.093 <sup>B</sup> =0.031* <sup>C</sup> =0.896
MCV(fL)	57.1(1.19)	56.64(1.11)	56.6(0.81)	56.57(1.47)	NS
MCH(pg)	18.6(0.63)	18.24(0.11)	18.44(0.78)	18.17(0.25)	NS
MCHC(g/dL)	27.95(1.41)	26.94(1.81)	26.28(1.04)	27.15(1.21)	NS
RDW.CV (%)	11.88(0.65)	11.58(0.46)	12.94(0.74)	12.5(0.55)	<sup>A</sup> =0.178 <sup>B</sup> =0.031* <sup>C</sup> =0.405
RDW.SD (fL)	28.18(0.51)	28.12(0.19)	29.13(0.60)	25.02(7.90)	NS
PLT(10 <sup>3</sup> /μL)	1025 (273.172)	662.2(324.477)	996.67(537.78)	1049.1(198.51)	NS

**TABLE 3. Serum biochemical parameters of female Wistar rats treated with *Kelussia odoratissima* extract .**

Variables	<sup>A</sup> Low dose (50 mg/kg, n=5)	<sup>B</sup> Medium dose (500 mg/kg, n=5)	<sup>C</sup> High dose (1000 mg/kg, n=5)	Vehicle Control (n=5)	P-value
Glucose (mg/dl)	77.25(6.89)	96.00(21.41)	84.00(7.55)	84.80(7.55)	NS
Urea (mg/mL)	53.0(3.26)	57.8(13.44)	53.67(2.517)	48.25(6.9)	NS
Creatinine	0.747(0.07)	0.76(0.05)	0.72(0.07)	0.69(0.1)	NS
AST( U/L)	163.56(35.95)	305.2(98.82)	94.67 (24.09)	277.75(153.58)	NS
ALT( U/L)	45.5 (10.8)	65.40(13.46)	33.1(2)	87.00(8.08)	NS
Alkaline phosphatase (U/L)	201.23(73.8)	192(36.82)	260.33(71.7)	178.5(70.5)	NS
LDH (U/L)	846(334.4)	1196.4(459)	484.67(142)	1097.75(343.49)	<sup>A</sup> =0.334 <sup>B</sup> =0.733 <sup>C</sup> =0.035*
Total Protein (g/dL)	7.27(0.6)	7.02(0.32)	7.2(0.6245)	7.07(0.43)	NS
Albumin (g/dL)	3.7(0.25)	3.72(0.23)	3.9(0.36)	3.77(0.22)	NS
Na (mM/L)	142.25(2.06)	140.4(1.67)	142(1)	143(2.16)	NS
K (mM/L)	6.75(1.8)	6.54(1.14)	6.9(0.2)	5.8(1.33)	NS
Calcium (mg/dl)	10.13(0.79)	10.59(0.60)	10.53(0.49)	10.68(0.80)	NS
<b>Lipid Profile</b>					
Triglyceride (mg/dl)	600.5(469.87)	608.4(462.56)	236.67(244.9)	232(153.45)	NS
Total Cholesterol (mg/dl)	68.75(8.8)	83.4(8.67)	81.00(14.73)	109(9.27)	NS
HDL(mg/dl)	37.25(2.21)	43.4(4.61)	42.67(5.13)	40.5(4.35)	NS
Non.HDL. Cholesterol (mg/mL)	31.5(6.8)	40(4.69)	38.33(10.21)	34.5(6.55)	NS
Cholesterol/HDL (Ratio)	1.84(0.14)	1.92(0.08)	1.89(0.15)	1.84(0.07)	NS
LDL.Cholesterol (mg/mL)	10(2.82)	14(1.22)	12.6(2.51)	7.50(2.08)	<sup>A</sup> =0.577 <sup>B</sup> =0.004** <sup>C</sup> =0.032*
LDL/HDL (Ratio)	0.267(0.06)	0.326(0.05)	0.30(0.06)	0.18(0.04)	<sup>A</sup> =0.204 <sup>B</sup> =0.001** <sup>C</sup> =0.031*

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase



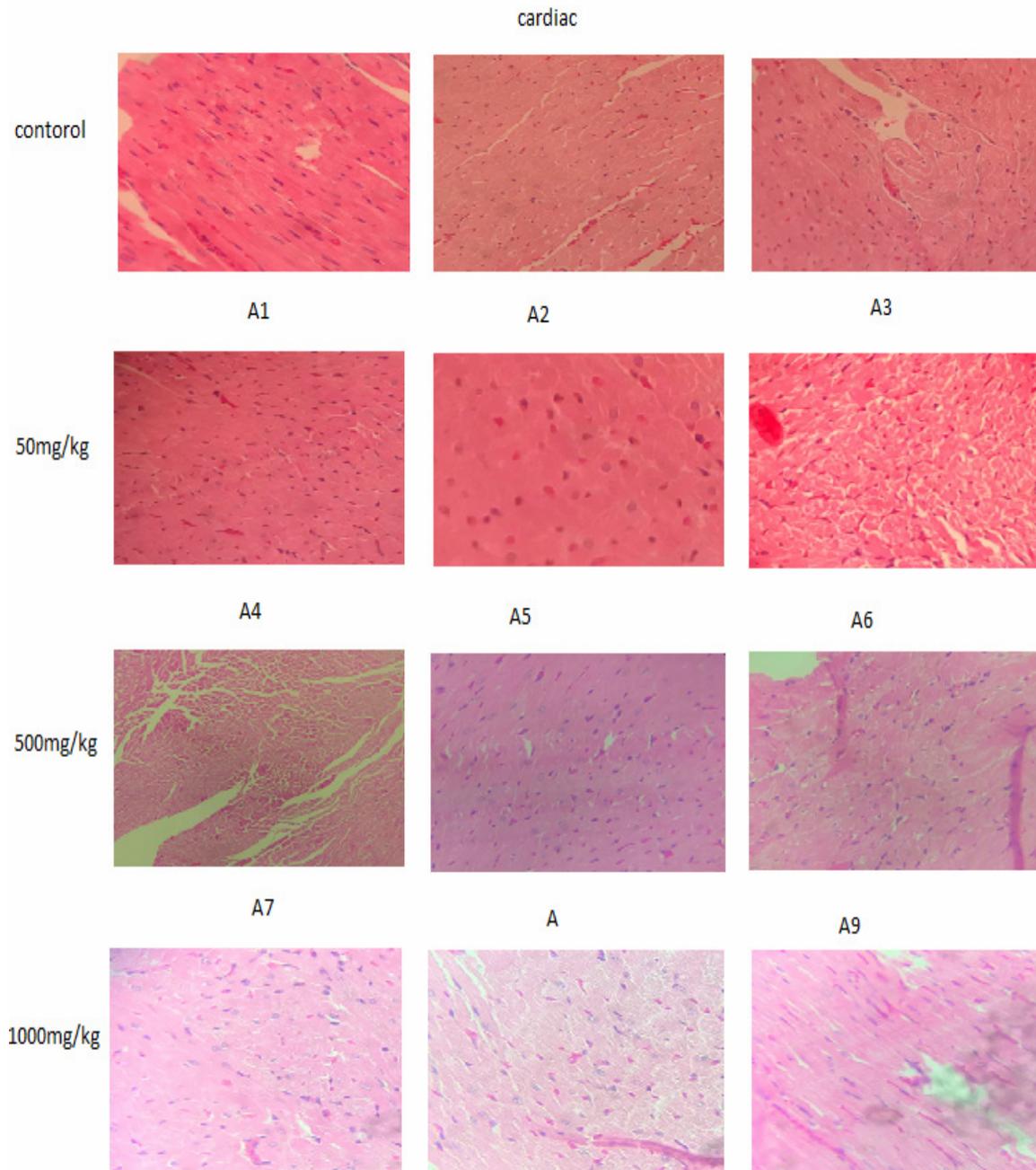
**Fig. 1.** Histopathological effects of *Kelussia odoratissima* extract different doses on lungs of female Wistar rats compared with vehicle control group. Photomicrographs of all sections stained with hematoxylin and eosin. A1: shows normal pulmonary tissue, mag: 400X - A2: shows normal pulmonary tissue, mag: 100X - A3: shows normal pulmonary tissue, mag: 400X - A4: shows normal pulmonary tissue, mag: 100X - A5: shows normal pulmonary tissue, mag: 100X - A6: shows normal pulmonary tissue, mag: 100X - A7: shows normal pulmonary tissue, mag: 100X - A8: shows pulmonary tissue with mild atelectasis, mag: 400X - A9: shows normal pulmonary tissue, mag: 400X.

**Heart:** Heart sections of all female Wistar rats in different dose groups showed normal feature (Fig. 2 A1-A8) without any sign of congestion compared with vehicle control group.

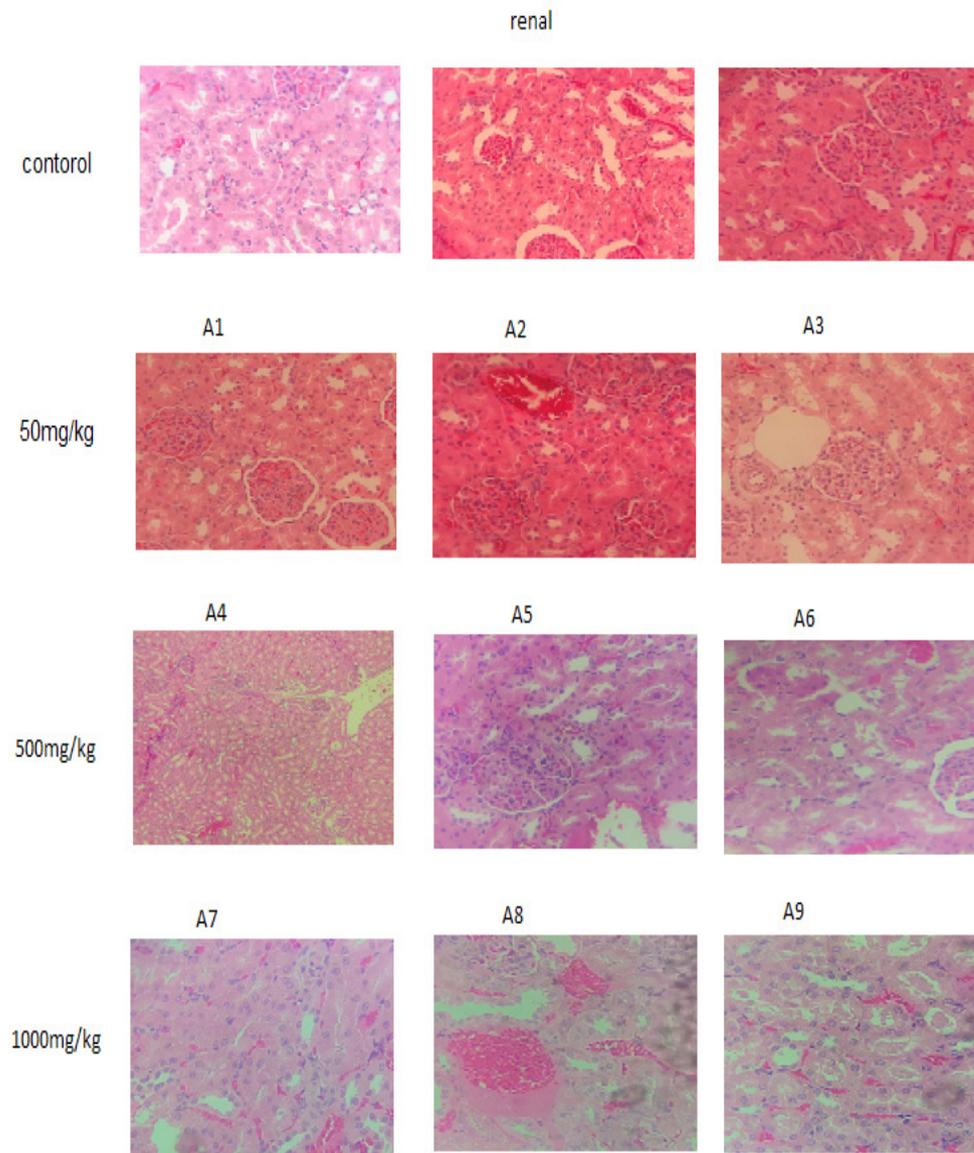
**Kidney:** The results of renal histopathology in the group receiving 50 mg/kg of the extract did not show signs of necrosis and degeneration. The overall appearance of glomeruli and capillary rings inside the glomerulus was perfectly normal and showed no signs of necrosis, hypertrophy, or congestion (Fig. 3). In the group given the extract at 500 mg/kg, mild congestion in kidney tissue was seen (Fig. 3 A6) and in the group

receiving the dose of 1000 mg/kg, renal tissue with moderate congestion and renal tubules with eosinophilic casts were observed (Fig. 3: A8-A9).

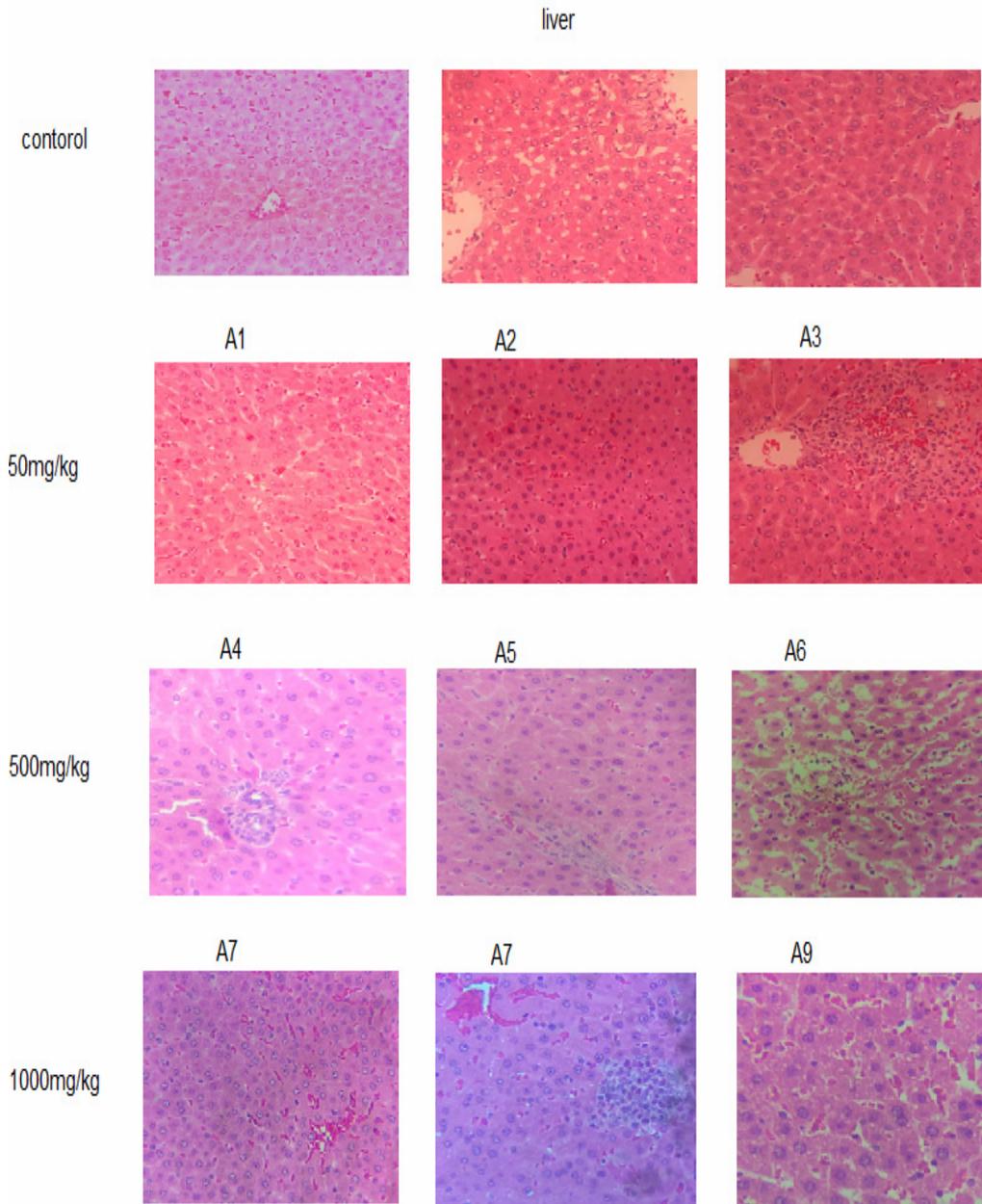
**Liver:** In the liver tissues, none of the rats receiving *Kelussia* extract showed any sign of degeneration and necrosis after 28 days. Extramedullary hematopoiesis was observed, which is normal in rodents (Fig. 4 A3). Sinusoidal dilatation was seen at 500 mg/kg (Fig. 5: A6) and a slight congestion in the liver tissue along with a few inflammatory foci was observed at 1000 mg/kg (Fig. 5: A7)



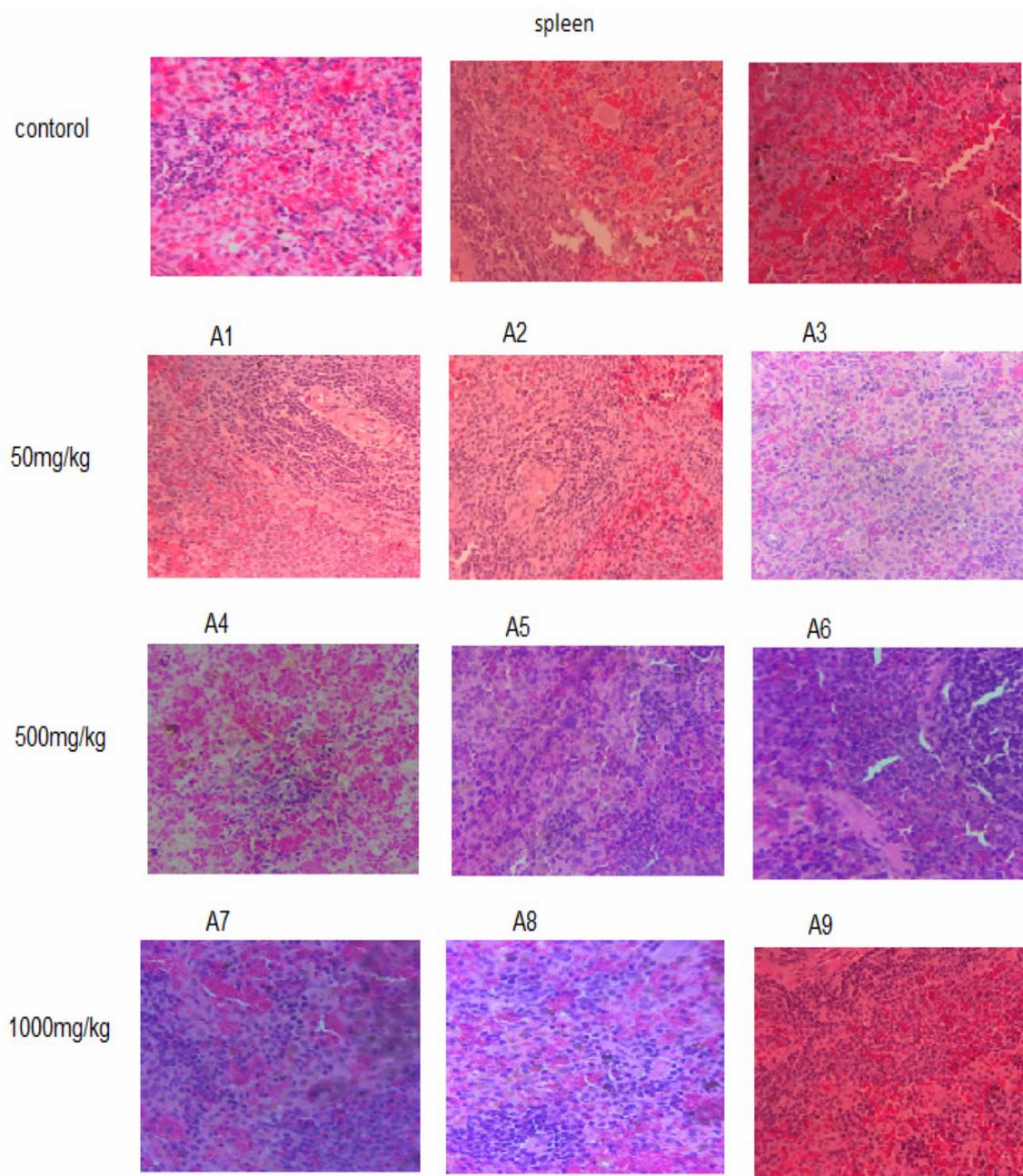
**Fig. 2.** Histopathological effects of *Kelussia odoratissima* extract different doses on cardiac tissues of female Wistar rats compared with vehicle control group. Photomicrographs of all sections stained with hematoxylin and eosin. A1: shows normal cardiac tissue, mag : 400X – A2: shows normal cardiac tissue mag: 400X – A3: shows normal cardiac tissue mag: 400X – A4: shows normal cardiac tissue mag: 400X – A5:shows normal cardiac tissue mag: 400X - A6:shows normal cardiac tissue. mag: 400X- A7:shows normal cardiac tissue mag: 400X- A8:shows normal cardiac tissue mag: 400X -A9:shows normal cardiac tissue mag:400X.



**Fig. 3.** Histopathological effects of *Kelussia odoratissima* extract different doses on renal tissues of female Wistar rats compared with vehicle control group. Photomicrographs of all sections stained with hematoxylin and eosin. A1: tissue, mag:400X shows normal renal - A2: shows normal renal tissue, mag:400X - A3: shows normal renal tissue, mag:400X - A4: shows normal renal tissue, mag:100X - A5: shows normal renal tissue mag:400X - A6: shows renal tissue with mild congestion, mag:400X - A7: shows normal renal tissue mag:400X - A8: shows renal tissue with moderate congestion, mag:400X - A9: shows renal tissue with a few eosinophilic casts in renal tubules, mag:400X.



**Fig. 4.** Histopathological effects of *Kelussia odoratissima* extract different doses on liver tissues of female Wistar rats compared with vehicle control group. Photomicrographs of all sections stained with hematoxylin and eosin. A1: shows normal hepatic tissue, mag:400X-A2: shows normal hepatic tissue, mag:400X-A3: shows hepatic tissue with extra medullary hematopoiesis, mag:400X – A4: shows normal hepatic tissue, mag:400X -A5:shows normal hepatic tissue, mag:400X - A6:shows hepatic tissue with prominent sinusoidal dilatation, mag:400X – A7:shows hepatic tissue with mild congestion, mag:400X- A8:shows hepatic tissue with focal infiltration of mononuclear, mag:400X – A9: shows normal hepatic tissue, mag:400X



**Fig. 5.** Histopathological effects of *Kelussia odoratissima* extract different doses on spleen tissues of female Wistar rats compared with vehicle control group. Photomicrographs of all sections stained with hematoxylin and eosin. A1: shows normal splenic tissue. mag: 400X – A2: shows normal splenic tissue. mag: 400X -A3: shows normal splenic tissue. mag:400X - A4: shows normal splenic tissue. mag: 400X – A5: shows normal splenic tissue. mag: 400X – A6: shows normal splenic tissue. mag:400X – A7: shows normal splenic tissue. mag:400X – A8: shows normal splenic tissue, mag:400X – A9: shows normal splenic tissue, mag: 400X.

*Spleen:* Microscopic evaluation showed normal feature in spleen sections in all animal groups (Fig. 5) compared to vehicle control group.

## Discussion

*Kelussia odoratissima* is a popular plant with nutritive and medicinal benefits; moreover, it has been mentioned in traditional literature as the top medicinal plant of Zagros mountain, the roof of Iran. It is a self-growing rare plant species that grows only in four provinces of our country without any documented study on its safety profile but based on pharmacological studies and its wide range of possible clinical applications, it should be considered for pharmaceutical formulation and applications after determination of acceptable daily intakes as an excellent candidate for disease prevention or treatment. In this study we clearly described the oral toxicity of the hydro alcoholic extract of *Kelussia odoratissima* for the first time in acute and repeated dose models. The extract didn't show any acute or delayed toxic reaction after single oral gavage of 2000 mg/kg dose in a period of 14 days. We interpret from this observation, that *Kelussia odoratissima* is a practically nontoxic herb without any risk of mortality or organ damage and without any necessary supportive care in acute exposures due to accidental or intentional human poisonings. In addition to acute test, we evaluated repeated dose oral toxicity of this medicinal herb in three dose groups compared to control using clinical, hematological, biochemical and histopathological evidence which will be discussed below.

Despite unchanged clinical and necropsy pattern of animals in all dose groups (Table 1), administration of *Kelussia odoratissima* extract increased the mean serum levels of LDL cholesterol as well as the ratio of LDL/HDL compared to the control group. Scientific database, Iranian Medicine and popular believes indicate the role of Celery phthalides on vascular smooth muscle and its antihyperlipidemic, antidiabetic, and hypotensive properties which was not in accordance of our data. Moreover in one clinical study in 2015 which was conducted on Iranian hyperlipidemic patients the role mountain celery on lipid and glucose profile in hyperlipidemia patients was rejected. The reduction rate of all lipid factors were similar in the control and intervention groups which is partially similar to our results in female Wistar rats. In hyperlipidemic patients, the mean LDL/HDL

ratio decreased during the study ( $P < 0.001$ ) [15] but this animal experiment has indicated opposite evidence in all dose groups of healthy animals; however, there was not any significant difference between the other factors ( $p > 0.05$ ) (Table 3). The mean of FBS did not change between and within groups ( $p > 0.05$ ) which was in accordance with clinical findings in hyperlipidemic patients. This part of study indicates that *Kelussia odoratissima* cannot induce any toxic or desirable effect on serum lipid profile and FBS in normal female rats but it is able to increase LDL and LDL/HDL ratio significantly in moderate and high dose groups of animals. Despite the anti-obesity properties of celery extract (ECE) in mice on high-fat diet (HFD) and anti-adipogenic properties by inhibiting lipid accumulations in adipose cells by improving adipokines levels, reducing glucose levels, and preventing insulin resistance, preventing hepatic steatosis and upregulation of liver antioxidant enzymes [16], animal weights of healthy animals in this basic toxicology model remained unchanged even in high dose groups but the role of mountain celery on the obesity and obesity related factors should be reassessed in validated animal or clinical models compared with celery for additional supplementary benefits of this herb based on its safety profile on all body organs in doses up to 500 mg/kg. Examination of ALT and AST enzymes showed that *K. odoratissima* extract at 50, 500 and 1000 mg/kg did not significantly change these enzyme levels.

To the best of our knowledge, this study is the first toxicity assessment on *Kelussia* which addressed the herb's toxicity by biochemical, hematological and histopathological data. According to the presented research outcome, no data supports any dose dependent adverse effects on hepatic, renal, pulmonary, cardiac and spleen tissue in doses up to 1000 mg/kg. Despite some minor changes in RBC related parameters in hematological study (Table 2), serum levels of Cr, BUN levels and alkaline phosphatase (ALP) remained unchanged and lactate dehydrogenase (LDH) decreased significantly in high dose groups compared with control ( $p=0.035$ ). At the same time all liver functional tests (ALT, AST) remained unchanged in doses up to 1000 mg/kg. Results of histological examinations showed did not show any degenerative changes and tissue necrosis in the liver, lung, heart and spleen of animals except mild to moderate congestions in one third of animals in high dose group.

Mountain Celery is a plant from the apiaceae family and its phenolic and antioxidant compounds of this plant [17] have been studied by several studies like celery. Although the phytochemical content of *K. odoratissima* hydroalcoholic extract is not determined yet, studied on its essential oil sowed 27 main components, which represent 99.3% of the total essential oils, the main constituent of the essential oil were phthalides including 3-butylidene-4,5-dihydrophthalide (z-Ligustilide) (85.9%), cis-3butylidene phthalide (0.4%) and 3N butyl phthalide (0.3%) [18] with excellent antioxidant activity [19]. High levels of phthalide may be considered as a possible underlying mechanisms for the safety profile of this herb .

### **Conclusion**

According to the results of present study, Kelussia extract is a practically nontoxic herb according to the acute oral toxicity study. In repeated dose toxicity model, despite some *K. odoratissima* induced minor changes in biochemical, hematological and histopathological parameters, this extract could be considered as a safe medicinal plant for long term oral administration in maximum daily doses of 500 mg/kg/day in female Wistar rats equal to 5 mg/kg/day in human uses as acceptable daily intake (ADI) . Further studies on genotoxic potentials , developmental and endocrine toxicity of this herb is necessary to determine its safety profile and to use it in future pharmaceutical dosage forms.

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### **Conflict of interest**

Authors declare no conflict of interest in present work.

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