Journal of Reports in Pharmaceutical Sciences 2015, 4(2), 125-134

Evaluation of Acute and 8-Week Sub-chronic Toxicities of Aromatic water of *Rheum Ribes* in Rats

Mahdi Mojarrab[a], Kiumarth Ghanbari[a], Mohammad Shakeri[b], Zahra-Minoosh Siavash-Haghighi[c], Leila Hosseinzadeh[a*]

[a]Pharmaceutical Sciences Research Center, Faculty of pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[b]Students Research Committee, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[c]Pathology Department, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2015-06-02
Revised: 2015-07-15
Accepted: 2015-07-25
ePublished: 2015-08-10

***Keywords*:**
Acute toxicity
Rat
Rheum ribes
Subchoronic toxicity

A B S T R A C T

In Iranian folk medicine, especially in west of the country, the aromatic water of *Rheum ribes* has been used for several purposes such as fever reduction. Despite the widespread use of this plant as food or as a medicinal plant, there is still lack of information on its toxicity profile. In the present study we aimed to evaluate the acute and sub chronic toxicities of aromatic water of *R. ribes* in Wistar rat. In the acute toxicity study, single doses of aromatic water were administered orally, and the rats were then monitored for 14 days. In the subchronic toxicity study, aromatic water was administered to rats for 60 days. Results of acute toxicity study indicated that the LD50 of *R. ribes* aromatic water is higher than 5000 mg/kg. Biochemical analysis showed aromatic water of *R. ribes* increased significantly Creatine Phospho Kinase (CPK) and Lactate De-Hydrogenase (LDH) levels. Moreover some significant abnormality of heart organ such as coagulated necrosis of cardiac muscle cells associated with hemorrhage, hypertrophy and infiltration of inflammatory cells were observed. Based on the result of this study, no observed adverse effect level (NOAEL) of aromatic water from *R. ribes* considered to be 250 mg/kg/day for male rats and 500 mg/kg/day for female rats.

*Corresponding Author: Leila Hosseinzadeh, E-mail: lhoseinzadeh@kums.ac.ir, lhosseinzadeh90@yahoo.com

Introduction

The genus *Rheum* (English name: Rhubarb) belongs to the family of Polygonaceae, which includes about 103 species. This family of plant grows mainly in North America, Europe and eastern Mediteranean regions including Iran, Turkey, Afghanistan and Pakistan [1, 2]. Only four species of this genus, *Rheum ribes, R. turkestanicum, R. persicumand R. khorasanicum* were distributed in Iran and the last two are endemic [1, 3].*Rheum ribes L.* is a hardy perennial branching herb that grows in mountainous areas of Iran under the common Persian name "Rivas" [4]. The roots of *R. ribes* are used as medicinal plant in diseases like diabetes, hypertension, obesity, diarrhea [5, 6], hemorrhoids, ulcers [7], constipation, hypercholesterolemia and psoriasis[8, 9]. The young shoots and petioles of this plant are consumed as a popular vegetable and also used for improving digestion and appetite [10]. *R. ribes* has been reported to contain mainly anthraquinones such as emodin, aloe-emodin, rhein, physcion, chrysophanol and their glycosides [10, 11]. In addition to the monomeric anthraquinones, dianthrone and stilbeneglucosides [12] as well as flavonoids [10] have also been obtained from this species. In various studies, aqueous extracts of *R. ribes* were reported to show hypoglycemic activity in different in vivo and in vitro tests [13-16]. Likewise, the antioxidant property of the chloroform [5] and ethanol [17] extracts of this plant was confirmed using DPPH, ferric thiocyanate and β-carotene bleaching methods. In other investigations, ethanol and methanol extracts of *R. ribe s*exhibited acetylcholinesterase [18] and urease [7] inhibitory activities as well as antibacterial [11, 19], antifungal [20], antiviral [21] and antiulcer [22] properties. In two different clinical trials, hydroethanol extract of this plant, were shown to have beneficial effects in treatment of major depressive disorder [23] and obsessive compulsive disorder [24]. In Iranian folk medicine, especially in west of the country, the aromatic water of *R. ribe* has widely been used for reducing fever. Despite the prevalent use of this plant as food and medicine there is still lack of information on its toxicity profile. In this paper, we report the results of a study aimed to evaluate acute and sub-chronic toxicity of aromatic water of *R. ribes* in male and female wistar rats.

Material and methods

Plant Material

The aerial parts of *Rheum ribes* L. were collected from Gavrood, Songhor, Kermanshah province, Iran, in April 2013. The identity of the plant was confirmed by Kermanshah Center for Research of Agricultural Science and Natural Resources (voucher numbers: 297, 681), Kermanshah, Iran.

Preparation of aromatic water (Distillation Method)

The fresh and coarsely ground aerial parts of the plant (3 × 1 kg) were submitted to a water distillation method for 3h using a specially designed stainless steel distiller. The plant material was entirely immersed in distilled water and then slowly heated to the boiling temperature. Water-soluble volatile components were transferred by steam via a hose to the condenser where the aqueous solution, saturated with volatile material was collected as aromatic water. Then it was stored at 4^0C until tested and analyzed.

Animals

Four-week-old Wistar rats of each sex were obtained from the animal house of the Kermanshah University of Medical Sciences (Iran) and kept under controlled temperature (22± 2^0c) and a 12 h light/dark cycle. Rats were divided in different groups of 5 animals and acclimatized for one week before starting the experiment. After one weeks of acclimatization animals were randomly assigned to a control or treatment group, and fasted for 3 h before treatment dosing. Study was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Publication no. 85–23, revised in 1985).

Single dose acute toxicity study

Rats were divided into 3 groups (5males and 5 females per each group). The first group (control) received sterile water, given orally. Groups 2 and 3 were orally treated with test compounds in doses of 2.5 and 5 g/kg, respectively. Animals were visually observed to change in the skin, fur, eyes and mocous membrane for irritation and dryness. Also the body weight changes, hazardous symptoms, and mortality were recorded for a 14-day period after treatment. All of the animals were sacrificed on day 15 and the relative organ weight (weight of organ as a proportion to the total body weight of each rat) was calculated in the highest dose group.

8- Week sub-chronic toxicity study

Group assignment and the treatments

Dosing was initiated when the rats were five weeks old, when the males and females weighed 180±16 g and 171±13 g, respectively. They were divided into one control group and three test groups of 5 animals each. The first group was given 1 ml distillated water and used as the control. Each animal received, for a period of 60 days, water (control group) or aromatic water of *R. ribes* at doses of 250, 500, and 1000 mg/ kg by gavage. All of the solutions were prepared just prior to dosing and were kept chilled and tightly capped. Mortality rate, abnormalities such as stereotype activities, hyperventilation, locomotion, aggressiveness, touch sensibility and tremor were monitoreddduring the treatment period. Besides, the body weights of animal were recorded at the end of the first day and at weekly intervals throughout the course of the study.

Laboratory test

After 60 days, 12 h-fasted animals were anesthetized with ether and their blood was collected by retro-orbital puncture using capillary tubes. One blood sample was placed in test tubes containing EDTA as an anticoagulant, and another in a dry tube for separation of serum. The hematologic evaluations included Red Blood Cell(RBC) count, red cell distribution width (RDW), total and white blood cells(WBC) counts, hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count and Platelet indices such as plateletcrit (*PCT*), *mean platelet volume* (*MPV*), *platelet distribution width* (*PDW*). Blood samples for biochemical analyses were centrifuged at 3,000 × g for 5 min, and the plasma was collected and analyzed for glucose, albumin, globulin, total protein, cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydro-genase LDH, creatinine phospho kinase, phosphorus, K, Na, Ca and phosphate using a COBAS Mira S chemistry analyzer (Roche Diagnostic Systems, West Sussex, England).

Histopathological analysis

Following blood collection, the rats were sacrificed by decapitation, and the liver, heart, lung and spleen were removed. Then, organ weight per 100 g body weight (relative weight) was calculated based on the fasted animal's body weight. All above tissues were preserved in 10% neutral phosphate-buffered formalin then they were embedded in paraffin, sectioned at approximately 5 mm, stained with hematoxylin and eosin, and examined with an optical microscope.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the differences between means separately for each sex. Data were analyzed using statistical methods and values were presented as the mean ± standard errors (S.E.M).A probability value of <0.05 was considered statistically significant. Because no treatment-related animal deaths were observed, the LD50 values could not be calculated.

Results

Acute toxicity studies

In the acute studies, the oral LD50 of aromatic water of *R. ribes* was greater than 5000 mg/kg body weight suggesting that the aromatic water of *R. ribes* is practically non-toxic. No signs of toxicity were observed at any dose level throughout the 14 days period. No treatment-related changes were noted at the post-mortem examination in the group that received the highest dose (Table 1).

Table 1. Effect of *R. ribes* on mean organ weights adjusted to overall body weight (% body weight) in male and female rats (g % body weight) for acute toxicity test.

Sex	Dose mg/Kg	Liver (%)	Kidney (%)	Spleen (%)	Heart (%)
Male	Control	5.095±0.158	0.44±0.012	0.281±0.023	0.479±0.053
	5000	4.69±0.211	0.397±0.024	0.269±0.031	0.578±0.045
Female	Control	4.127±0.152	0.374±0.026	0.326±0.067	0.524±0.069
	5000	4.86±0.320	0.459±0.020	0.411±0.085	0.488±0.061

Subchronic toxicity test

No treatment-related mortality or behavioral signs of toxicity were observed throughout the subchronic toxicity study: No statistically significant changes were found in body weight (Figure 1), food consumption ($p>0.05$), or water intake ($p>0.05$) (data not shown), regardless of sex or treatment. No treatment-related changes were noted at the post-mortem examination (Table 4).

Table 2. Hematological parameters of Wistar rats after 60 days of treatment with R*. ribes.*

Sex	Dose mg/kg	Hb (g/dL)	WBC (10^3/µl)	HCT (%)	MCV (Fl)	MCHC (g/dL)	MCH (pg)
Male	Control	13.97±0.46	10.45±0.8	43.92±1.58	56.125±1.156	31.85±0.68	17.87±0.44
	250	14.22±0.085	9.47±0.78	45.77±0.48	58.4±0.4	31.07±0.27	18.15±0.11
	500	10.075±3.364	11.5±1.56	32.5±10.79	55.4±1.254	30.57±0.96	17.37±0.56
	1000	13.725±0.39	11.5±1.56	44.52±1.71	55.825±0.65	32.85±1.043	18.3±0.44
Female	Control	12.85±0.47	6.77±69.4	40.85±1.34	60.75±0.69	31.45±0.21	19.125±0.3
	250	14.1±0.27	10.45±1.5	43.32±0.52	59.64±0.67	32.55±0.75	19.45±0.48
	500	13.93±0.15	8.02±3.31	42.7±0.41	56.85±0.27	32.7±0.15	18.57±0.12
	1000	13.95±0.12	8.87±1.19	43.52±0.77	57.7±0.76	32.1±0.45	18.52±0.36

Continued (table 2).

Sex	Dose mg/kg	RBC (10^3/ µl)	Platelets (10^3/µl)	RDW (%)	MPV (fL)	PCT (%)	PDW (%)
Male	Control	7.82±0.12	889.5±118.34	11.37±0.23	2.47±0.11	0.15±0.02	16.26±0.43
	250	7.84±0.08	961.5±63.74	11.5±0.22	2.73±0.06	0.15±0.01	16.15±0.31
	500	5.79±1.94	849.5±286.86	12.87±1.39	3.63±0.83	0.16±0.01	16.23±0.31
	1000	7.52±0.4	917.75±53.62	11.25±0.14	2.81±0.23	0.14±0.03	16.41±0.61
Female	Control	6.72±0.22	876.85±119.15	11.52±0.16	2.67±0.17	0.17±0.006	16.52±0.35
	250	7.26±0.15	845.5±151	11.45±0.21	3±0.31	0.14±0.01	16.65±0.23
	500	5.59±1.85	648.5±236.26	11.25±0.16	3.4±0.16	0.037±0.17	16.4±0.55
	1000	7.54±0.15	1021±153.96	11.17±0.21	2.82±0.11	0.17±0.01	16.3±0.33

No relevant changes were observed on hematological parameters in both male and female rats at 60th day of treatment (Table 2). Some statistically significant differences were noted in biochemical parameters when the control and treatment groups were compared(Table 3). Aromatic water of *R. ribes* had no effects on serum electrolytes, such as calcium, potassium, sodium, and phosphate.

Table 3. Biochemical parameters of Wistar rats after 60 days treatment with *R. ribes*.

Sex	Dose mg/kg	blood sugar (mg/dl)	Cholesterol (mg/dl)	LDL (10^3/µl)	Triglycerdes (mg/dl)	HDL (10^3/µl)
Male	Control	89±4.637	87±6.36	3.367±1.68	43.25±3.42	34±0.4
	250	73.75±4.23	88.75±3.705	3.862±1.931	43±4.34	34.75±0.48
	500	73±4.34	98.75±2.52	3.873±1.936	61±7.56	35.5±1.04
	1000	66.25±3.68	92.5±2.59	1.732±0.86	49.75±2.62	34.75±0.25
Female	Control	114.5±7.94	142.75±16.675	10.25±1.031	55.75±0.62	38.25±2.42
	250	111.25±5.92	116.75±5.03	9.5±0.28	55±1.47	37.75±0.25
	500	90.75±6.57	97.5±13.46	9±1	45.25±5.154	35±1.225
	1000	98.75±9.81	111±7.69	9±1.08	52.75±5.21	35.75±0.75

Continued (Table 3).

Sex	Dose mg/kg	blood sugar (mg/dl)	Cholesterol (mg/dl)	LDL (10^3/µl)	Triglyceride (mg/dl)	HDL (10^3/µl)
Male	**Control**	89±4.637	87±6.36	3.367±1.68	43.25±3.42	34±0.4
	250	73.75±4.23	88.75±3.705	3.862±1.931	43±4.34	34.75±0.48
	500	73±4.34	98.75±2.52	3.873±1.936	61±7.56	35.5±1.04
	1000	66.25±3.68	92.5±2.59	1.732±0.86	49.75±2.62	34.75±0.25
Female	**Control**	114.5±7.94	142.75±16.675	10.25±1.031	55.75±0.62	38.25±2.42
	250	111.25±5.92	116.75±5.03	9.5±0.28	55±1.47	37.75±0.25
	500	90.75±6.57	97.5±13.46	9±1	45.25±5.154	35±1.225
	1000	98.75±9.81	111±7.69	9±1.08	52.75±5.21	35.75±0.75

Sex	Dose mg/kg	D.Bili	CPK (mg/dl)	A LT (IU/L)	AST (IU/L)	Globulin (g/dL)
Male	**Control**	**0.05**±0.02	341 ±26.51	70.5±6.513	237.5±109.48	4.28±0.11
	250	0.005±0.0	429±50.946	45.25±4.785	147±20.482	4.57±0.14
	500	0.033±0.01	633±71.890***	70±3.97	166±17.828	4.57±0.19
	1000	0.044±0.02	622.75±63.2456***	66.75±6.836	178.25±12.257	4.35±0.15
Female	**Control**	0.08±0.01	235.25±37.104	49.25±3.66	110.5±9.215	5.02±0.17
	250	0.087±0.01	229±37.530	60±7.036	114.75±15.451	4.75±0.30
	500	0.125±0.0	471.75±123.17	67.25±9.393	149.25±4.837	4.67±0.11
	1000	0.175±0.04	214±33.124	54.75±3.425	134.75±16.080	4.80±0.183.35

Sex	Dose mg/kg	Albumin (g/dL)	Total. Protein (g/dL)	LDH (IU/L)
Male	**Control**	4.075±0.40	7.65±0.09	1289.33±166.6
	250	3.375±0.02	7.35±0.05	1553.25±592.16
	500	3.5±0.07	7.6±0.16	1986.75±138.14
	1000	3.62±0.06	7.97±0.21	3304.75±25.662***
Female	**Control**	3.82±0.09	8.75±0.02	1023.5±181.47
	250	4.185±0.19	8.525±0.2	913.23±271.23
	500	3.95±0.05	7.4±0.32	1393.5±371.66
	1000	3.55±0.15	9.15±0.52	1735.5±333.03

Data presented as mean ± S.E.M. for N=5.Significantly different from control: ***p<0.001.

No statistically significant differences in liver function parameters (ALT, ALP) were seen. However, the activity of CPK was significantly increased in mid(250 mg/kg) and high dose (500 mg/kg)males ($P< 0.05$). Additionally, an increase in LDH at 1000 mg/kg in male rats were also observed. No similar changes in CPK and LDH were found in the female groups. No significant changes in total protein, globulin, albumin, glucose, triglycerides, or cholesterol were noted. Macroscopic analysis of target organs of the treated animals (liver, heart, lung, kidneys and spleen) did not show significant changes in color and texture when compared with the control group. Also, the results of organ weights showed a large but not significantly decrease in mean organ weights in the lung and kidney of male rats that received highest dose of *R. ribs* in the subchronic study (Table 4).

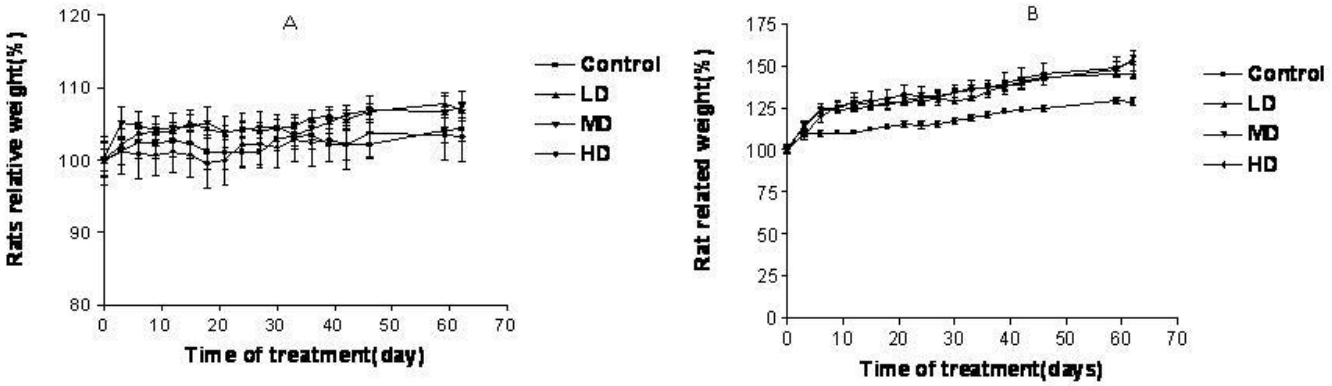

Fig. 1. Changes in females (A) and males (B) rat body weight with duration of subchronic treatment. Each point represents mean ± SEM, (LD: Low Dose, MD: Medium Dose, HD: High Dose).

Histopathological analyses

Figure2 exhibits photomicrographs of the heart (Figure. 2, A-C). Histological features of the heart of control rats showed normal structures (P1). Male and female rats treated sub-chronically with 1000 mg/kg dose of *R. ribes* showed cardiac damages including coagulated necrosis of cardiac muscle cells associated with hemorrhage, hypertrophy and infiltration of inflammatory cells. No treatment related lesions were shown in other organs**.**

Table 4. Relative organ weight at termination of the subchronic treatment (g % body weight).

Sex	Dose (mg/kg)	liver%	kidney%	Heart%	Spleen%	Lung%
Male	Control	3.64±0.16	0.73±0.03	0.36±0.04	0.20±0.008	0.66±0.10
	250	3.95±0.13	0.75±0.04	0.33±0.07	0.25±0.03	0.81±0.12
	500	3.60±0.65	0.70±0.08	0.35±0.04	0.22±0.06	0.64±0.11
	1000	3.70±0.18	0.47±0.16	0.30±0.01	0.24±0.04	0.48±0.16
Female	Control	3.40±0.31	0.72±0.09	0.36±0.03	0.21±0.04	0.78±0.14
	250	3.38±0.23	0.75±0.04	0.36±0.01	0.28±0.07	0.81±0.16
	500	3.24±0.48	0.72±0.11	0.41±0.03	0.24±0.02	0.96±0.07
	1000	3.37±0.25	0.74±0.69	0.39±0.05	0.26±0.06	0.88±0.08

Data presented as mean ± S.E.M. for N=5.

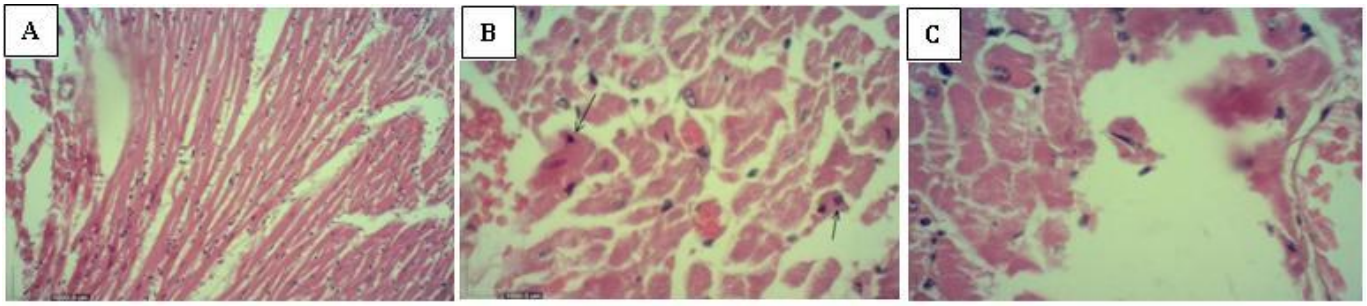

Fig. 2. Selected microphotographs of heart hematoxylin and eosin of A) control) female (myocardium, pycnotic nucleus (arrow) and C) male rats that received the highest dose of *R. ribes* (scale enlargement is 100x).

Discussion

To evaluate the safety of drugs and plant products for human use, conventional toxicity studies is carried out in various experimental animals to provide indications of the appropriate dosage range, the probable adverse effect, and the affected organ [25]. Usually, toxicity found in animal studies occurs with similar incidence and severity in humans. It must be noted that clinical tests with humans are needed to confirm the results of non-clinical laboratory studies [26]. Determination of LD50 is a first step which provides information on health hazards likely to arise from short-term exposure to compounds [27]. In a previously published study, the toxicity of the essential oils of *R. ribes L* was evaluated by the brine shrimp lethality bioassay. The obtained results showed that essential oil of *Rheum ribes L.* exhibited moderate toxicity effect against *Artemia salina* with LC50 value of 20.02 µg/mL [28]. In this study the oral LD50 value *of aromatic water* of *R. ribes* is higher than 5000 mg/kg which falls in the class of non-toxic substances. Plants or plant products with LD50 higher than 2–3 g/kg are considered free of any toxicity [29, 30]. The oral administration of aromatic water of *R. ribes* to male and female Wistar rats for 60 days was not associated with any mortalities and abnormalities in general conditions, behavior and growth of animals. Body weight gain and/or organ weight were similar in both control and treated animals. Analysis of blood parameters plays an important role in risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal

studies [31]. Certain medicinal herbal preparations or conventional drugs/chemicals adversely affect various blood components [32]. Sub-chronic treatment showed that 60 days administration of aromatic water of *R. ribes* did not produce any significant difference in the hematological profile of rats. The result of biochemical parameter analysis showed a significant increase in CPK values in males in the two highest dose groups. The source of the CPK was considered to be from either skeletal or heart muscles. Another finding was a significant increase in LDH, an initial factor in myopathy, in the 500 and 1000 mg/kg male groups. Minimal heart lesions were also found on microscopic examination in the highest dose group. Thereby, remarkable elevation in CPK and LDH in males represents potentials for muscular damage. These two enzymes have diagnostic value in myocardial or skeletal muscle injuries [33]. Hence, it could be suggested that aromatic water of *R. ribes* may cause muscular damage in males. Liver function was evaluated by means of serum proteins, SGOT and SGPT. These markers usually help to detect chronic liver disease [29]. In the present study no indicators of liver injury were observed. No pathological changes of the liver, spleen, lung and kidneys were discovered in both test groups during the study period.

Conclusion

On the basis of current study, it can be concluded that heart is possible main target organs of *R. ribes* although the mechanism of toxic effect on heart is unclear and additional clinical toxicological evaluations need to be performed. The no-observed-adverse-effect-level [NOAEL] definition is an important part of the non-clinical risk assessment. NOAEL is the highest dose that is without observed effects in properly designed toxicology studies [26]. In the current study it was indicated that no observed adverse effect level (NOAEL] of the *R. ribes* is less than 250 mg/kg and 500 mg/kg for male and female rats, respectively.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

Acknowledgment

The authors appreciate the Research Council of Kermanshah University of Medical Sciences for the financial supports. This work was performed in partial fulfillment of the requirement for Pharm. D. of M. Shakeri.

References

[1] Jafari A, Taheri G, Baradaran B, Bahrami AR. *Rheum khorasanicum*(polygonaceae), a new species from Iran. Ann Bot Fenn. 2012;49:255-258.
[2] Davis PH Flora of Turkey and East Egean Islands. Vol 2, Edinburgh University Press, Edinburgh; 1985.
[3] Mozaffarian V. A Dictionary of Iranian Plant Names. FarhangeMoaser.Tehran; 2005.
[4] Dehkardy Q. Rheum palmatum L. Iranian herbal pharmacopoeia. 1st ed. Tehran: Health Ministery press; 2002.
[5] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant Species. Food Bioprod Process. 2011;89:217-233.
[6] Abu-Irmaileh BE, Afifi FU. Herbal medicine in Jordan with special emphasis on commonly used herbs. J Ethnopharmacol. 2003;89:193-197.
[7] Nabati F, Mojab F, Rezaei MH, Bagherzadeh K, Amanlou M, Yosefi B. Large scale screening of commonly used Iranian traditional medicinal plants against urease activity. DARU J Pharm Sci. 2012;20:1-9.
[8] Najafpour B, Imanpour MR, Shabani A. Effects of *Rheum rebis*Extract on the Blood Parameters and Responses of *Rutilusfrisiikutum*under Heat Stress. Global Veterinaria. 2012;8:222-228.
[9] Cakilcioglu U, Khatun S, Turkoglu I, Hayta S. Ethnopharmacological survey of medicinal plants in Maden (Elazig-Turkey).J Ethnopharmcol. 2011;137:469-486.
[10] Tosun F, kizilay CA. Anthraquinones and flavonoids from *Rheum ribes*. J Fac Pharm Ankara. 2003;32:31-35.
[11] Alaadin AM, Al-Khateeb EH, Jager AK. Antibacterial activity of the Iraqi Rheum ribes root. Pharm Biol. 2007;45:688–690.
[12] Mericli AH, Tuzlaci E. Constituents *of Rheum ribes.* Fitoterapia1990;61:375.

[13] Naqishbandi AM, Josefsen K, Pedersen ME, Jager AK. Hypoglycemic activity of Iraqi *Rheum ribes*root extract. Pharm Biol. 2009;47:380-383.

[14] Kasabri V, Afifi FU, Hamdan I. *In vitro* and *In vivo* acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. J Ethnopharmacol. 2011;133:888-896.

[15] Kasabri V, Abu-Dahab R, Afifi FU, Naffa R, Majdalawi L, Shawash H. *In vitro* Modulation of Pancreatic MIN6 Insulin Secretion and Proliferation and Extrapancreatic Glucose Absorption by *Paronychia argentea*, *Rheum ribes* and *Teucriumpolium* Extracts. Jordan J Pharm Sci. 2012;5:203-219

[16] Ozbek H, Ceylan E, Kara M, Ozgokce F, Koyuncu M. Hypoglycemic effect of *Rheum ribes* roots in alloxan induced diabetic and normal mice. Scand J. Lab AnimSci2004;31:113-115.

[17] Oktay M, Yildirim A, Bilaloglu V, Gulcin I. Antioxidant Activity of Different Parts of Isgin (*Rheum ribes* L.). *Asian J Chem* 2007;19:3047-3055.

[18] Gholamhoseinian A, Moradi MN, Sharififar F. Screening the methanol extracts of some Iranian plants for acetylcholinesterase inhibitory activity. Res.Pharmaceutic. Sci. 2009;4:105-112.

[19] Fazly Bazzaz BS, Khajehkaramadin M, Shokooheizadeh HR. *In vitro* antibacterial activity of *Rheum ribes*extract obtained from various plant parts against clinical isolates of gram-negative pathogens. Iran J Pharm *Res.* 2005;2:87-91.

[20] Sardari S, Shokrgozar MA, Ghavami G. Cheminformatics based selection and cytotoxic effects of herbal extracts. Toxicol in Vitro. 2009;23:1412–1421.

[21] Hudson JB, Lee MK, Sener B, Erdemoglu N. Antiviral activities in extracts of Turkish medicinal.plants Pharm Biol. 2000;38:171–175.

[22] Sindhu R, Kumar P, Kumar J, Kumar A, Arora S. Investigations into the antiulcer activity of Rheum ribes Linn leaves extracts. Int J Pharm Pharm Sci. 2010;2:90-93.

[23] Sayyah M, Boostani H, Pakseresht S, Malayeri A. Efficacy of hydroalcoholic extract of *Rheum ribes*L. in treatment of major depressive disorder. J Med Plants Res 2009;3:573-575.

[24] Sayyah M, Boostani H, Malayeri AR, Siahpoosh A. Efficacy of hydroalcoholic extract of Rheum ribes L. in treatment of Obsessive compulsive disorder: A double blind clinical trial. J Pharm Biomed Sci. 2011;1:57-61.

[25] Frank C. Lu (1996): Basic Toxicology. Taylor and Francis Press, Washington.

[26] Klaassen CD, Casarett & Doull's Toxicology, McGraw Hill; 2007.

[27] Abubakar A, Ogbadoyi EO, Okogun JI, Gbodi TI, Tifin UF. Acute and sub chronic toxicity of *Tridaxprocumbens*in experimental animals, J Environ. Sci. 2012;6:19-27.

[28] Nikbakht MR, Esnaasharib S, Heshmati AF. e Chemical Composition and General Toxicity of Essential Oil Extracted from the Stalks and Flowers of *Rheum Ribes*L.Growing in Iran -reJ Rep Pharma Sci. 2013;2:165-170.

[29] Mojarrab M, Khan Mohammadi A, Hosseinzadeh L, and MinooshSiavash-Haghighi Z, Antioxidant activity and safety assessment of Tragopogon buphthalmoides hydroethanolic extract: Acute and subchronic toxicities. Res Pharma Sci, October 2014;9:359-366.

[30] Mirghazanfari SM, Hosseinzadeh L, Shokoohinia Y, Aslany M, Kamali-Nejad. M. Acute and subchronic toxicological evaluation of *Echinophoraplatyloba* DCA piaceae; total extract in Wistar rats, CLINICS. 2012;67:497-502.

[31] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken Concordance of the toxicity of pharmaceuticals in humans and in animals Regul Toxicol Pharmacol. 2000;32:56-67.

[32] Nyarkoa AK, Okineb LKN, Wedzib RK, Addoa PA, Ofosuhenea M. Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat: absence of organ toxicity and modulation of cytochrome P450. J Ethnopharmacol. 2005;97: 319–325.

[33] Todd GC, Novilla MN, Howard LC, Animals Comparative Toxicology of Monensin Sodium in Laboratory J Anim Sci. 1984;58:1512-1517.

