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Research Article

Acute and chronic toxicity of aqueous extract of *Ruta montana* L. in rodents

Mounira Merghem[1], Saliha Dahamna*[1], Abir Rezzagui[1], Soulef Boussahel[1], Assia Belguet[1], Khadidja Dehimi[1] and Daoud Harzallah[2]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Natural and Life Sciences, University Ferhat Abbas, Sétif 19000 Algeria
[2]Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University Ferhat Abbas, Sétif, 19000, Algeria.

ABSTRACT: The objective of this study was to evaluate the acute and chronic toxicity of aqueous extract of *Ruta montana* L. in mice and rats. For the acute study, aqueous extract of *Ruta montana* L. was administered to mice in single doses of 0–12 g/kg given by gavage. General behavior, adverse effects and mortality were determined for up to 14 days. In the chronic study, the extract was administered orally at doses of 0, 100, 300 and 600 mg/kg daily for 90 days to rats. Biochemical and hematological parameters were determined after 30 and 90 days. Enzyme activities were assayed in the plasma samples obtained. AST (GOT), ALT (GPT), cholesterol, triglyceride and glucose. The results showed a decrease in RBC and WBC after one month (sub chronic dose) of treatment (in males 75.48% and 51.47% respectively). However, an increase was registered after 3 months (in males 130% and 171% respectively). This is probably explained by the effect of this plant extract on the erythropoiesis. A decline was observed on plasma enzyme activities in both GOT and GPT on males after one month by 51.67% and 68.6% respectively. A decrease was noticed in both cholesterol and triglyceride levels. Urine analysis was negative for glucose, bilirubin, ketones, proteins, nitrite and leukocytes in the control and treatment groups. There were no significant differences in the body and organ weights between controls and treated animals of both sexes. The histopathological studies have been showed vascular congestion and lesions of interstitial nephrite.

KEYWORDS: Acute toxicity, Biochemical parameters, Chronic toxicity, Hematological parameters, *Ruta montana* L., Mountain rue

INTRODUCTION

The medicinal plant *Ruta montana* L. (Family Rutaceae), is known in Algeria as "mountain rue" or "Fidjel".[1] This plant grows in the arid regions in the Mediterranean zone, distributed in Spain, Portugal, Italy, Greece and Turkey in Europe, and in Algeria and Morocco in Africa.[2]

This plant is used in folk medicine as an antioxidant,[3] hypoglycemic,[4] antirhematic,[5] antihelmintic, antiepileptic, antipyretic.[6] It is also used in treating intestinal and hepatic diseases,[6] and in the treatment of vitiligo,[7] *Ruta montana* L. contains various active principles able to inhibit the growth of mycobacteria,[8] The antioxidative effect of plant extracts is mainly due to phenolic components, such as flavonoids,[9] phenolic acids, and phenolic diterpenes.[10] In Algerian folk medicine, *Ruta montana* is used against child fevers and as an abortive drug.[11] This plant is abortive, it can produce gastro-enteritis, hypothermia and finally coma,[1,11] the toxicity of this plant is due to the presence of methyluonylcetone,[12] and it is known that the presence of furocomarines can induce skin eruptions.[13]

*Correspondence
Laboratory of Phytotherapy Applied to Chronic Diseases,
Department of Animal Biology and Physiology,
Faculty of Natural and Life Sciences,
University Ferhat Abbas Sétif1, Sétif 19000 Algeria
E-mail: dahamna_s@yahoo.fr
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MATERIALS AND METHODS

Plant material

Ruta montana L. plant material was collected from Beni azize région, Wilaya of Sétif Northeast of Algeria during October, 2011, And identified by Professor Laouar

Hocine, Department of Biology, Faculty of Natural and Life Sciences, University Ferhat Abbas, Sétif Algeria.

Animal material

Male and female Albino Wistar mice were used for acute toxicity and Albino Wistar rats for chronic toxicity. All experimental animals were obtained from Pasteur Institute (Algiers, Algeria). These animals were kept in the animal house of the faculty of Nature and Life Sciences, University of Sétif, at a temperature of 20°C and a photoperiod cycle of 12 hours light/dark. The animals were housed in plastic cages (5 rats or mice per cage) and had free access to standard commercial diet and tap water.

Preparation of plant extract

The areal parts were washed in running water, dried and powdered. The aqueous extract was prepared by adding 1l of distilled water (100°C) to 100 g of *Ruta montana* powder at room temperature during 72 h, the resulting decoction was filtered using wattman filter paper n°3 and then evaporated in rotary vacuum evaporator.

Acute toxicity study in mice

Albino Wistar mice of both sexes weighing between 18 and 22 g, were divided into seven groups, each containing 5 males and 5 females. The animals were fasted overnight (12 h) with free access to water prior to administration of single doses (0, 2, 4, 6, 8, 10 and 12 g/kg b/w) of the extract dissolved in distilled water. Animals were gavaged by 1 ml/100 g b/w. The general behavior of the mice was continuously monitored after dosing, periodically during the first 24 h (with special attention given during the first 4 hours), and then daily thereafter, for a total of 14 days.

Chronic toxicity study in rats

Albino Wistar rats of both sexes weighing between 149 and 200 g were divided into four groups of 10 rats each (5 females and 5 males). The aqueous extract dissolved in distilled water, was administered daily by gavage for 90 days to groups I to IV (doses of 0, 100, 300 and 600 mg/kg, respectively). The animals were observed for signs of toxicity and mortality throughout the experimental period. At the end of the treatment, animals were fasted overnight, but allowed access to water and libitum. They were subsequently anesthetized with diethyl ether and blood samples were obtained by retro-orbital puncture[14] and collected in two tubes: tube 1 containing EDTA was processed immediately for hematological parameters analysis; tube 2 containing heparin was centrifuged at 4000 r/min at 4°C for 15 minutes to obtain serum (stored at −20°C until analysis). The organs (kidneys, liver, lungs, heart,) were weighed and fixed in 10% formalin for histopathological examination.

Urine samples

Urine (3–5 ml) was collected for urinalysis from rats which were kept 4 to 6 hr in metabolic cages.

Blood analysis

Hematological analysis was performed using an automatic hematological analyzer (Coulter STKS, Beckman). Parameters included: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (VGM), platelets count and mean platelet volume (MPV). For biochemical analysis, the serum analysis for cholesterol (CHOL); glucose (GLU); creatinine (CREA); aspartate aminotransferase (AST); alanine aminotransferase (ALT); triglycerides (TG); uric acid (AU); alkaline phosphatase (ALP); creatine kinase (CK); albumin (ALB). Dosages were made using automatic analyzer (Beckman).

Organ weights

After the sacrifice of all animals, the kidneys, liver, heart, lungs, spleen, brain and stomach were carefully removed and weighed individually (absolute organ weight).

Liver and kidney tissues histopathological examination

After blood collection, rats were sacrificed, and their liver and kidneys were carefully dissected out, and rinsed in 0.9% NaCl, then fixed in the formol (10%), sectioned 5 μm thickness, and embedded in paraffin and stained with hematoxylin and eosin,[15] and examined with a light microscope (Carl Zeiss Jena, Germany).

Statistical analysis

The results are expressed as the mean value ± standard deviation. One-way analysis of variance followed by the Tukey test was performed to assess differences between groups. Differences were considered significant at $p < 0.05$. Statistical analyses were performed with the aid of the software GraphPad Prism 5®.

RESULTS

Acute toxicity

No death or toxicity signs such as diarrhea and vomiting were observed in the 14 day observation period after oral administration of single doses of the aqueous extract of *Ruta montana* L. Therefore, it could not be estimated an LD_{50} could not be determined.

Chronic toxicity

No toxicity signs (diarrhea and vomiting) or death were recorded during the 90 consecutive days of treatment

via oral route with *Ruta Montana* L. at doses of 0.1, 0.3 or 0.6 g/kg b/w.

Effect of *Ruta montana* L. aqueous extract on rats body weight

Changes in body weight of control and treated rats are presented in Figure 1 and Figure 2. No significant difference in body weight changes was noted between the control and any of treated groups (1–4) at any time period.

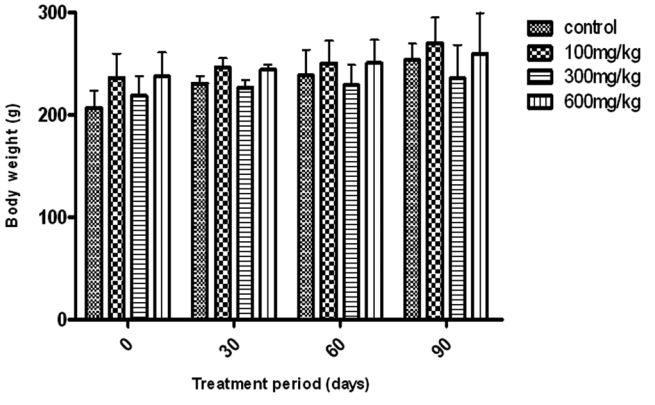

Figure 1. Alterations in the body weight of male rats after chronic oral treatment with aqueous extract of *Ruta montana* L. The values are expressed as mean ± SD (n = 5/group).

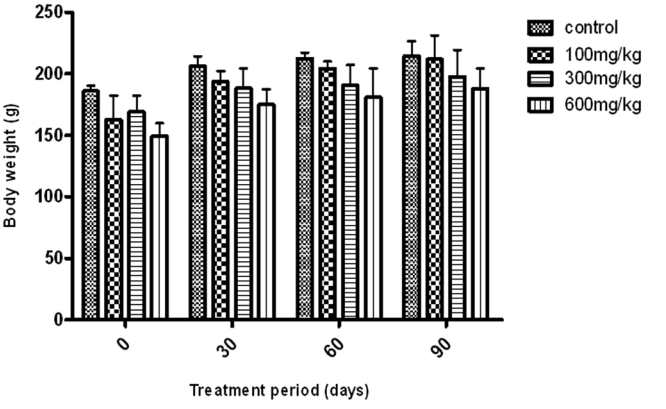

Figure 2. Alterations in the body weight of female rats after chronic oral treatment with aqueous extract of *Ruta montana* L. The values are expressed as mean ± SD (n = 5/group).

Effect of *Ruta montana* L. aqueous extract on rats blood parameters

The hematological parameters of the treated and control groups are presented in Tables 1 and 2. For female rats, no significant differences were recorded in any of the parameters examined at 30 and 90 days. In the male groups, there was no significant differences observed in any of the parameters examined at 90 days, however, a significant decrease was noticed

Table 1: Effect of aqueous extract of *Ruta montana* L. (100, 300 and 600 mg/kg) via oral route on hematological parameters in male and female Wistar rats treated for 30 consecutive days

Parameters	Control	100 mg/kg	300 mg/kg	600 mg/kg
Male				
RBC (10^6/mm^3)	9.34 ± 0.58	9.77 ± 0.51	8.94 ± 0.00	7.05 ± 2.2
WBC (10^3/mm^3)	16.63 ± 3.84	14.57 ± 3.23	14 ± 0.00	8.56 ± 2.4*
Hematocrit (%)	46.73 ± 2.4	48.27 ± 0.25	42.7 ± 0.00	31.97 ± 5.65**
Platelets	842.3 ± 145.9	971.7 ± 28.5	485 ± 0.00**	529.7.7 ± 53.13*
Hemoglobin (g/dL)	15.9 ± 0.79	16.5 ± 0.6	14.5 ± 0.00	12.53 ± 0.51**
VGM (fL)	50.07 ± 1.87	49.5 ± 2.76	47.8 ± 0.00	47.53 ± 9.0
MPV (fL)	6.1 ± 0.36	6.53 ± 0.2	8.2 ± 0.00***	8.53 ± 0.15***
MCH (pg)	17.03 ± 0.89	16.9 ± 0.75	16.2 ± 0.00	19.2 ± 6.35
MCHC (g/dL)	34.03 ± 0.56	34.23 ± 1.45	33.9 ± 0.00	39.87 ± 5.55
Female				
RBC (10^6/mm^3)	9.1 ± 0.11	8.31 ± 0.3	8.86 ± 0.48	8.88 ± 0.05
WBC (10^3/mm^3)	12.3 ± 0.51	8.6 ± 0.7	9.16 ± 2.08	12.7 ± 1.83
Hematocrit (%)	45.17 ± 3.3	39.35 ± 0.91	45.47 ± 0.55	44.95 ± 0.91
Platelets	1009 ± 257.9	1480 ± 113.1	1443 ± 75.79	1460 ± 84.15
Hemoglobin (g/dL)	15.47 ± 0.75	13.85 ± 0.49	15.73 ± 0.68	15.65 ± 0.35
VGM (fL)	49.67 ± 3.98	49.7 ± 0.42	52.77 ± 2.19	50.43 ± 0.57
MPV (fL)	6.16 ± 0.75	5.6 ± 0.14	5.86 ± 0.25	5.85 ± 0.05
MCH (pg)	17 ± 0.88	17.7 ± 0.28	17.77 ± 0.4	17.53 ± 0.23
MCHC (g/dL)	34.3 ± 1.01	35.65 ± 0.21	34.3 ± 0.65	34.8 ± 0.00

Values represent the mean ± S.D. (n = 10/group).

Table 2: Effect of aqueous extract of *Ruta montana* L. (100, 300 and 600 mg/kg) via oral route on hematological parameters in male and female Wistar rats treated for 90 days

Parameters	Control	100 mg/kg	300 mg/kg	600 mg/kg
Male				
RBC (10^6/mm^3)	6.3 ± 0.94	7.99 ± 0.56	7.76 ± 1.3	8.21 ± 0.21
WBC (10^3/mm^3)	9.7 ± 3.39	9.03 ± 1.51	9.65 ± 5.43	13.77 ± 4.25
Hematocrit (%)	32.05 ± 4.03	38.77 ± 2.41	38.4 ± 4.99	38.9 ± 1.49
Platelets	814 ± 207.9	997.7 ± 233.4	927.3 ± 115	988 ± 170.2
Hemoglobin (g/dL)	11.05 ± 1.76	13.7 ± 0.8	13.5 ± 1.64	13.43 ± 0.56
VGM (fL)	50.33 ± 1.36	56.8 ± 16.63	49.5 ± 3.12	47.33 ± 0.55
MPV (fL)	5 ± 0.55	5.5 ± 0.42	5.25 ± 0.23	5.63 ± 0.25
MCH (pg)	17.87 ± 0.55	17.08 ± 0.51	17.55 ± 0.98	16.55 ± 0.07
MCHC (g/dL)	35.53 ± 2.08	38.08 ± 5.56	35.53 ± 0.77	34.75 ± 0.21
Female				
RBC (10^6/mm^3)	8.99 ± 0.83	6.63 ± 0.58	7.89 ± 1.3	8.86 ± 0.98
WBC (10^3/mm^3)	6 ± 2.26	5.45 ± 1.06	6.33 ± 0.41	7.85 ± 0.91
Hematocrit (%)	49.35 ± 0.77	36.45 ± 6.57	40.73 ± 7.89	45.9 ± 4.95
Platelets	848 ± 128.7	596 ± 55.15	787.5 ± 174.1	697 ± 76.37
Hemoglobin (g/dL)	16.2 ± 0.7	12.5 ± 1.41	14.58 ± 2	15.8 ± 0.7
VGM (fL)	53.3 ± 4.23	55.6 ± 3.84	51.43 ± 2.87	51.67 ± 2.87
MPV (fL)	6.46 ± 0.6	6.36 ± 0.46	6.42 ± 0.22	6.13 ± 0.5
MCH (pg)	17.9 ± 0.7	18.6 ± 0.45	18.63 ± 0.86	18.53 ± 1.35
MCHC (g/dL)	33.7 ± 1.6	34.7 ± 1.7	36.47 ± 3.25	35.93 ± 3.05

Values represent the mean ± S.D. (n = 10/group).

at 30 days ($P < 0.05$) in the red blood cells (RBC), Hematocrit (Hct), Hemoglobin (Hb), Platelets (PLT) in the dose 600 mg/kg and also a significant increases ($P < 0.05$) in the mean platelet volume (VPM) in the doses 300 or 600 mg/kg when compared to control group. The results indicated that all hematological parameters measured (white blood cell, red blood cell, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, mean corpuscular volume, platelets and mean platelet volume) remained within physiological range throughout the treatment period (90 days).

The values for the biochemical parameters in treated and control rats are presented in Tables 3 and 4. Oral administration of aqueous extract did not cause any significant differences in the biochemical parameters examined of the male and female rats.

Urinalysis

The urinalysis in the control and treated groups of both male and female rats were negative for glucose, bilirubin, ketones, blood, protein, nitrite and leukocytes.

Effect of *Ruta montana* L. aqueous extract on rats organs weight

Absolute and relative organ weights of 90 days treated rats are shown in Table 5. There were no significant changes in the organs weight of the treated animals compared to the control groups.

Effect of *Ruta montana* L. aqueous extract on histomorphology of liver and kidneys in rats

Microscopic observations showed a normal liver histomorphology in control group (Figure 3A), However, the doses at 100, 300 and 600 mg/kg revealed a vascular congestion. The kidneys of control rats showed normal glomeruli and tubules (Figure 4), but the treated rats at the dose 100 mg/kg showed a vascular congestion. Chronic administration of *Ruta montana* L. extract at the other doses 300 and 600 mg/kg also showed a vascular congestion and lesions of interstitial nephrite.

DISCUSSION

This study tested the acute and chronic toxicity of the aqueous extract of *Ruta montana* L. this plant was orally

Table 3: Effect of aqueous extract of *Ruta montana* L. (100, 300 and 600 mg/kg) via oral rute on biochemical parameters in male and female Wistar rats treated for 30 days

Parameters	Control	100 mg/kg	300 mg/kg	600 mg/kg
Male				
AU (mg/L)	8.33 ± 5.57	11.97 ± 2.3	12.33 ± 2.08	10.67 ± 5.03
ALT (UI/L)	78.67 ± 7.5	80.67 ± 41.43	40.67 ± 2.3	48 ± 1.73
ALB (g/L)	30.67 ± 2.08	29 ± 13	36.67 ± 1.52	36.33 ± 5.03
ALP (UI/L)	194.8 ± 27.71	115.9 ± 6.5	118.4 ± 35.08	124.4 ± 29.9
AST (UI/L)	119.3 ± 6.02	102 ± 7.07	117.3 ± 43.89	105 ± 13.0
CHOL (g/L)	0.37 ± 0.08	0.73 ± 0.43	0.6 ± 0.13	0.52 ± 0.01
CK (UI/L)	171.3 ± 61.58	178.3 ± 70.06	107 ± 4.24	172 ± 48.5
CREA (mg/L)	5 ± 0.00	5 ± 1	5 ± 0.00	4 ± 0.00
GLU (g/L)	1.17 ± 0.07	1.25 ± 0.52	1.74 ± 0.41	1.48 ± 0.25
TG (g/L)	0.86 ± 0.12	0.64 ± 0.06	0.89 ± 0.16	0.88 ± 0.1
Female				
AU (mg/L)	6 ± 2.82	7.33 ± 2.51	6.66 ± 0.57	8.5 ± 2.12
ALT (UI/L)	46.5 ± 2.12	36 ± 4.35	34.5 ± 4.95	34 ± 2.82
ALB (g/L)	34 ± 9.89	38 ± 2.0	36.67 ± 4.04	34.67 ± 4.04
ALP (UI/L)	103.7 ± 5.23	81.1 ± 3.55	69.07 ± 2.34	86.47 ± 20.76
AST (UI/L)	96.5 ± 3.53	81.67 ± 19.35	71 ± 10	82.33 ± 5.5
CHOL (g/L)	0.76 ± 0.28	0.52 ± 0.08	0.36 ± 0.03	0.49 ± 0.13
CK (UI/L)	123.5 ± 20.51	134.3 ± 12.7	130.7 ± 51.07	169.7 ± 21.94
CREA (mg/L)	5.5 ± 0.7	5.33 ± 0.57	5.33 ± 1.15	5 ± 0.00
GLU (g/L)	1.01 ± 0.4	1.44 ± 0.01	1.15 ± 0.19	1.19 ± 0.15
TG (g/L)	0.69 ± 0.28	0.59 ± 0.03	0.45 ± 0.25	0.3 ± 0.04

Values represent the mean±S.D. (n =10/group).

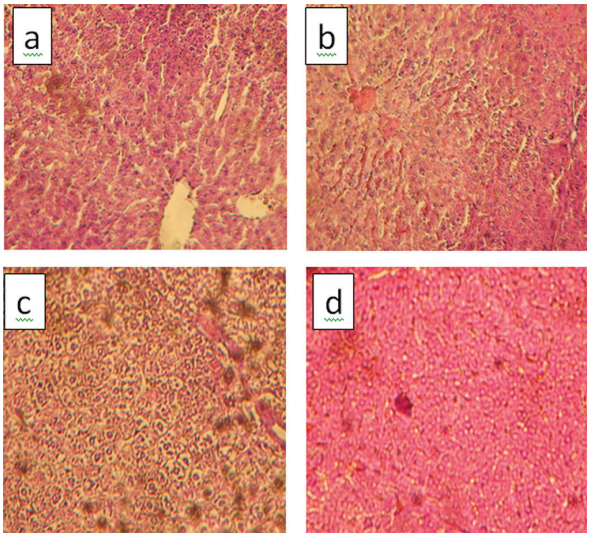

Figure 3. Effects of *Ruta montana* L. extract on liver histomorphology in rats. (a) Control, (b) treated with 100 mg/kg, (c) treated with 300 mg/kg, (d) treated with 600 mg/kg.

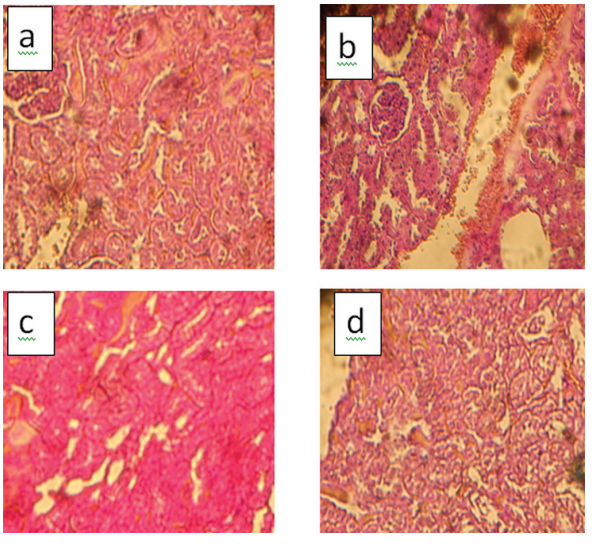

Figure 4. Effects of *Ruta montana* L. extract on kidneys histomorphology in rats. (a) Control, (b) treated with 100 mg/kg, (c) treated with 300 mg/kg, (d) treated with 600 mg/kg.

administered at doses of 2, 4, 6, 8, 10 and 12 g/kg b/w in acute and chronic toxicity tests at daily doses of 100, 300 or 600 mg/kg of body weight.

The results of the acute toxicity study indicated that *Ruta montana* L. extract via oral route with the doses from 2 to 12 g/kg body weight did not produce any sign of toxicity

Table 4: Effect of aqueous extract of *Ruta montana* L. (100, 300 and 600 mg/kg) via oral route on biochemical parameters in male and female Wistar rats treated for 90 days

Parameters	Control	100 mg/kg	300 mg/kg	600 mg/kg
Male				
AU (mg/L)	16.73 ± 0.48	18.88 ± 1.49	14.9 ± 0.00	16.27 ± 7.8
ALT (UI/L)	47.75 ± 8.61	50 ± 2.58	39 ± 5.56	41.33 ± 13.2
ALB (g/L)	42.25 ± 8.53	35 ± 4.89	36 ± 6	30.33 ± 17.01
ALP (UI/L)	389.3 ± 192.9	320.3 ± 238.9	261.3 ± 56.3	357.7 ± 243.8
AST (UI/L)	103.3 ± 13	104.5 ± 45.51	91.67 ± 20.6	108 ± 32.79
CHOL (g/L)	0.9 ± 0.18	0.78 ± 0.31	0.86 ± 0.14	0.79 ± 0.27
CK (UI/L)	85.25 ± 70.03	69.25 ± 59.42	22.67 ± 5.68	32.67 ± 2.88
CREA (mg/L)	7.5 ± 1.91	8 ± 1.82	9.66 ± 0.57	9 ± 1
GLU (g/L)	0.41 ± 0.14	0.35 ± 0.09	0.36 ± 0.0.09	0.38 ± 0.09
TG (g/L)	0.61 ± 0.25	0.43 ± 0.05	0.6 ± 0.34	0.82 ± 0.31
Female				
AU (mg/L)	13.95 ± 3.91	19.77 ± 10.07	16.63 ± 3.13	21.27 ± 9.29
ALT (UI/L)	32.5 ± 3.53	56.67 ± 17.21	60 ± 11.58	66.75 ± 23.36
ALB (g/L)	32.5 ± 3.53	31.5 ± 4.95	34.5 ± 5.74	39 ± 6.92
ALP (UI/L)	152.5 ± 74.25	142.7 ± 92.68	273.3 ± 80.66	236 ± 31.58
AST (UI/L)	102.5 ± 3.53	132.3 ± 49.92	101.3 ± 42.03	110.7 ± 41.67
CHOL (g/L)	0.87 ± 0.23	1 ± 0.00	0.69 ± 0.16	0.62 ± 0.05
CK (UI/L)	18.5 ± 9.19	108.3 ± 46.65	94 ± 24.04	175.8 ± 176.9
CREA (mg/L)	7.75 ± 3.59	8.33 ± 2.08	8.25 ± 1.5	6.66 ± 0.57
GLU (g/L)	0.36 ± 0.21	0.41 ± 0.07	0.54 ± 0.21	0.45 ± 0.34
TG (g/L)	0.55 ± 0.15	0.49 ± 0.08	0.55 ± 0.5	0.51 ± 0.14

Values represent the mean ± S.D. (n = 10/group).

Table 5: Effect of chronic oral administration of an aqueous extract of *Ruta montana* on rats organs weight

Organ (g)	Organ weight (g)				relative organ weight			
	Control (0 mg/kg)	Group I (100 mg/kg)	Group II (300 mg/kg)	Group III (600 mg/kg)	Control (0 mg/kg)	Group I (100 mg/kg)	Group II (300 mg/kg)	Group III (600 mg/kg)
Male								
Kidneys	0.78 ± 0.13	0.9 ± 0.24	0.7 ± 0.00	0.8 ± 0.1	0.31 ± 0.06	0.33 ± 0.08	0.29 ± 0.00	0.3 ± 0.37
Liver	9.26 ± 1.13	9.26 ± 1.54	7.23 ± 1.22	9.03 ± 2.67	3.68 ± 0.41	3.41 ± 0.37	3.07 ± 0.58	3.05 ± 1.15
Heart	1.02 ± 0.13	0.9 ± 0.12	0.76 ± 0.05	0.93 ± 0.23	0.4 ± 0.09	0.4 ± 0.14	0.32 ± 0.02	0.36 ± 0.09
Lungs	2.00 ± 0.23	1.88 ± 0.19	1.83 ± 0.25	2.00 ± 0.43	0.81 ± 0.2	0.69 ± 0.08	0.77 ± 0.11	0.77 ± 0.19
Spleen	1.04 ± 0.39	1.12 ± 0.43	0.86 ± 0.25	1.03 ± 0.30	0.37 ± 0.08	0.41 ± 0.16	0.36 ± 0.10	0.4 ± 0.14
Brain	1.86 ± 0.15	1.86 ± 0.20	1.56 ± 0.32	1.76 ± 0.58	0.73 ± 0.1	0.68 ± 0.04	0.66 ± 0.13	0.66 ± 0.18
Stomach	1.68 ± 0.31	1.62 ± 0.45	1.36 ± 0.23	1.86 ± 0.15	0.65 ± 0.08	0.51 ± 0.07	0.59 ± 0.12	0.71 ± 0.1
Female								
Kidneys	0.67 ± 0.12	0.92 ± 0.26	0.65 ± 0.05	0.67 ± 0.09	0.31 ± 0.04	0.43 ± 0.12	0.32 ± 0.02	0.35 ± 0.02
Liver	8.10 ± 0.77	8.7 ± 1.33	8.17 ± 1.44	6.87 ± 0.89	3.81 ± 0.39	4.09 ± 0.77	4.14 ± 0.13	3.65 ± 0.39
Heart	0.72 ± 0.09	0.8 ± 0.21	0.87 ± 0.12	0.75 ± 0.1	0.33 ± 0.02	0.37 ± 0.08	0.43 ± 0.04	0.39 ± 0.00
Lungs	1.37 ± 0.23	1.3 ± 0.29	1.2 ± 0.11	1.4 ± 0.16	0.64 ± 0.08	0.6 ± 0.11	0.6 ± 0.05	0.74 ± 0.04
Spleen	0.7 ± 0.14	0.82 ± 0.45	0.72 ± 0.09	0.75 ± 0.31	0.32 ± 0.19	0.38 ± 0.04	0.36 ± 0.1	0.38 ± 0.07
Brain	1.55 ± 0.2	1.82 ± 0.05	1.57 ± 0.22	1.65 ± 0.25	0.73 ± 0.16	0.85 ± 0.03	0.79 ± 0.09	0.87 ± 0.09
Stomach	1.46 ± 0.2	1.35 ± 0.23	1.55 ± 0.25	1.3 ± 0.14	0.69 ± 0.09	0.63 ± 0.09	0.78 ± 0.13	0.68 ± 0.02

Wistar rats (n = 10, 5 males and 5 females per group) were administered an aqueous extract of *Ruta montana* L. by daily gavage for 90 days. Data are expressed as mean ± S.D. There was no significant difference

or death in mice after 14 days of observation. According to the classification of Hodge and Sterner,[16] Chemical substances with a LD_{50} between 5000 and 15000 mg/kg body weight determined after a single oral doses in rats is considered as practically non-toxic in humans. Therefore, the aqueous extract of *Ruta montana* L. could be considered practically non-toxic.

In the chronic toxicity study in rats receiving the *Ruta montana* L. extract orally at doses of 100, 300 or 600 mg/kg during 90 consecutive days, there was a slight change in the body weight, these changes were not significantly different in the treated groups compared to the control group. Since changes in body weight have been used as an indicator of adverse effects of drugs and chemicals,[17,18] the present results suggest that at the chronic oral doses administered *Ruta montana* L. had no effect on the weight of rats. In addition, no significant changes were observed in the internal organ weight after the 90 days period. Generally, the reductions in internal organ weight are simple and sensitive indices of toxicity after exposure to toxic substances.[18,19]

The haematopoietic system is one of the most sensitive targets for toxic compounds,[20,21] and an important index of physiological and pathological status in human and animals.[22–24] The changes in the hematological system have a higher predictive value for human toxicity when data are translated from animal studies.[25–27] In this study, the data of the hematological parameters showed no significant differences between the control and the treated groups, indicating that *Ruta montana* L. extract had no effects on the circulating blood cells nor on their production.

The transaminases (AST and ALT) are well-known enzymes used as good indicators of liver function[25] and as biomarkers predicting possible toxicity.[28] Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood.[23,29] In addition, AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum.[30] Therefore, no changes in ALT and AST activities suggest that the chronic administration of *Ruta montana* L. extract did not alter the hepatocytes function and metabolism. Equally, there was also no significant change in creatinine, between the treated groups and the control group. Indeed, creatinine is known as a good indicator of renal function and any rise in creatinine levels is only observed if there is marked damage to functional nephrons.[23] Thus, the results recorded in this study suggest that *Ruta montana* L. extract did not alter the renal function. The liver is the site of cholesterol disposal or degradation and its major site of synthesis. In the same perspective, the liver also controls glucose synthesis and generates free glucose from hepatic glycogen stores. As no significant changes were observed in glucose and cholesterol levels in this study, this suggests that *Ruta montana* L. had no effects on the lipid and carbohydrate metabolism of the rats. The other biochemical parameters such as ALB, Ck and TG showed no significant changes between treated groups and control group.

CONCLUSION

In conclusion, the present study indicated that the oral doses of *Ruta montana* L. consumed may be considered as relatively safe as they did not cause death or any signs of toxicity in both the acute and chronic toxicity studies in rodents.

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