

Research Article

Acute Toxicity Study of the Total Alkaloids of *Ruta montana* in Male Mice

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ABSTRACT

Ruta montana commonly called Fidjel is known as medicinal plant which has been used as emmenagogue, antispasmodic, rubefiant and powder echarrotic in traditional Algeria medicine. Aim of the study: For the first time, this study evaluated the lethal dose 50 and the potential toxicity of alkaloid extract from aerial parts of *Ruta montana* after acute administration in male mice. Materials and methods: Lethal dose 50 of total alkaloid extract of *Ruta montana* was determined using Litchfield and Wilcoxon method, mice were received the alkaloid extract at doses of 100, 200, 300, 400, 500 and 600 mg/kg (n=8/group) by intra-peritoneal route. Abnormal behavior, toxic symptom, and death were observed for 14 consecutive days. In acute toxicity a single intra-peritoneal administration of total alkaloid extracted from aerial parts of *Ruta montana* at 129.68 mg/kg (1/3 LD₅₀). Body weight, biochemical and hematological parameters were recorded. Histopathological examination of liver and kidney were assessed.

Keywords: *Ruta montana*, total alkaloids, mice, acute toxicity, hematological and biochemical parameters.

INTRODUCTION

Medicinal plants were one of the only sources of cure for many diseases, including more than 80 per cent of the population of the countries in the process of development and almost exclusively used in traditional medicine for their primary health needs. In addition, around 25% of the prescriptions worldwide are herbal⁴. Algeria has a vegetable flora rich and diversified, among the medicinal plants that constitute the vegetal cover, is the genus *Ruta* belongs to the family Ruttaceae, this last is widely distributed especially in the mountainous regions. Many species of this type are used in traditional medicine because they contain several molecules endowed with therapeutic activities; among the most well-known species are *Ruta montana*, commonly called Fidjel; Plant with yellow flowers, strong odor, ornamental, aromatic, known for its richness in secondary metabolites. Phytochemical studies indicate the presence of various compounds: essential oils (aliphatic ketones)⁹, coumarins (rutarin, furanocoumarins: psoralen, bergapten, xantotoxin) Furoquinolines and acridone derivatives)²⁴, flavonoids (rutoside) and tannins^{2,6}. This plant widely used as a medicinal plant for its abortifacient and anti-fertility properties¹⁵, antispasmodic, analgesic, antirheumatic, emmenagogue and antiparasitic⁵. However, the use of plants as a remedy does not mean that they are always beneficial to human health, and following the observations of Masri et al., 2015 of a case of acute poisoning by *Ruta montana* where the toxicological

analysis of the urine shows a strong presence of alkaloids, starting from their works, we try to evaluate the lethal dose 50 and the acute toxicity of the total alkaloids from the aerial parts of *Ruta montana* in male mice.

MATERIALS AND METHODS

Plant collection and identification

The aerial parts of *Ruta montana* was collected from Beni-Aziz region in North Setif (East Algeria), in the end of August, were identified by a botanist, Dr Kirouani Abd Al Razak, Department of Biology, Mohammed Al Bachir Al Ibrahimi University of Bordj Bou Arreridj, Algeria. The aerial parts were dried in air at room temperature and stored until use (See Fig. 1).

Extraction of totals alkaloids

50 g of air dried powdered of aerial parts was defatted with 200 ml of petroleum ether under reflux, and then the powder were witted with 40 ml of NH₄OH (0.5N) for 24 h, and were extracted to exhaustion with CHCl₃ using a soxhlet apparatus for 5 h. The organic extract (containing free alkaloids with lipophilic impurities) is then shaken three times with 150 ml aqueous sulphuric acid (0.5N). The acid extracts (alkaloids salts) are treated three times with 50 ml NH₄OH.(0.5N) to pH 9 to liberate the free alkaloids which are separated by extraction with 150 ml CH₂Cl₂, and then dried with Na₂SO₄ and concentrated to dryness under reduced pressure to obtain crude alkaloids⁸. The yield of this extract was approximately 0.27 ± 0.02% (w/w).



Figure1: Plant of *Ruta montana*.

Experimental animals

Male Swiss albino mice of weights 22-32g, they were purchased from the Pasteur Institute in Algiers, were used to evaluate the lethal dose₅₀, for acute studies. The mice were housed in hanging transparent plastic cages (55 × 33 × 19 cm) in the animal room. The animals were fed with a standard pellet and tap water *ad libitum*. The animals are acclimated to the conditions of the animal room for two weeks prior to the experiments.

Determination of LD₅₀

Six groups of height mice each were used. The mice were given alkaloid extract of *Ruta montana* through intra-peritoneal route at doses of 100, 200, 300, 400, 500 and 600 mg/kg body weight in each group. The extract was administered once and the treated animals are closely observed during the first 24 hour (every hour) and then daily for two weeks and changes in appearance and behavior are noted. The median lethal dose (LD₅₀) was calculated according to the Lichtfield and Wilcoxon method in 1949.

Acute

toxicity

Two groups of 12 Swiss albino mice were given single dose of 129.68 mg/kg (1/3 DL₅₀) body weight of alkaloids by intra-peritoneal route. The control group (12 mice) received saline water with few drops of methanol at the same volume. Animals were observed and recorded systematically 1, 2, 3, 4, 5, and 6 h and daily after test substance administration. The visual observations included changes in skin and fur (hair), eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system. The first group was sacrificed after 24 h of treatment; and the second group after 5 days⁷.

Blood samples were collected by cardiac puncture for the measurement of hematological (EDTA-coated tubes) and biochemical (Heparine tubes) parameters. Organs such as heart, liver, kidneys, spleen, testes, brain and lungs were excised. The organs were then weighed, and the relative weight of each organ was calculated and compared with the value of the control.

Blood analysis

The hematological components including white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean

corpuscular hemoglobin concentration (MCHC), platelet (PLT) were determined using the automated hematologic analyzer (Medonic).

Heparine tubes containing collected blood were centrifuged at 2000 rpm at 5 °C for 15 min to obtain the serum, for the measurement of biochemical parameters including alkaline phosphatase (ALP), alanine transaminase (ALAT) aspartate transaminase (ASAT), urea (UREA) and creatinine (CREA), all these parameters were evaluated using commercial Kits- Advia chemistry and were determined using a fully automatic biochemistry analyzer (Advia 1008).

Histopathological analysis

The liver, kidneys and brain were removed and fixed in 10% neutral formalin. The histological sections (5 μm) of the liver, kidney and brain tissues were assessed by haematoxylin and eosin (H and E) for evaluation through light microscopy.

Statistical analysis

Statistical comparisons between groups of acute toxicity were determined by one way analysis of variance ANOVA followed by the Dunnet's test. Data are expressed as the mean ± standard error mean (SEM). Differences were considered significant at P < 0.05.

RESULTS

Lethal Dose 50

The intra-peritoneal administration of total alkaloids extract of aerial parts of *Ruta montana* characterized by severe clinical symptoms, including decrease in locomotors activity with paralysis of the hind legs, cyanosis and tachycardia. Though there were deaths 15th minute to 24 hours; however, the animals which survived exhibited a normal behavior, similar to the animals of the control group. The toxicity was observed to be a dose-dependent phenomenon. The LD₅₀ value of the extracts was calculated to be 389.04 mg/kg (See Tab. 1, Fig. 2).

n = 4, so theoretical $\chi^2 = 9.49$ therefore experimental $\chi^2 <$ theoretical χ^2 .

LD₁₆ = 226.46 mg/kg, LD₅₀ = 389.04 mg/kg, LD₈₄ = 512.86 mg/kg

Acute toxicity

In acute toxicity studies, the intra-peritoneal administration in Swiss albino mice of single dose of 129.68 mg/kg body weight of alkaloid extract showed no mortality or abnormal behavior. As compared to control group, no biological significant effects of total alkaloids extract were noted on body weight gain (See Tab. 2) In addition, no significant pathological changes in the colors and textures of vital organs, including the liver, kidney, brain, heart, lung, spleen and testes were observed via macroscopic examination, but statistically there were significant decrease in the relative weight of the lungs of the mice sacrificed after 24h and 5 days with also significant decrease in the relative weight of liver of the mice sacrificed after 5 days as showed in Table 3.

The haematological parameters of the mice treated with total alkaloid extract are presented in Table 4. There was a statistically no significant change in the values of

Table 1: Calculus of median lethal dose of total alkaloids extracted from *Ruta montana* using Lichtfield and Wilcoxon method.

Dose mg/kg	N° of death/exp	Log dose	Observation effect		Attend effect		Difference %	χ^2
			Probit	%	Probit	%		
100	0/8	2	2.99	2.2	3.50	6.6	4.4	0.03
200	1/8	2.301	3.85	12.5	3.72	10.1	2.4	0.0058
300	2/8	2.477	4.33	25	4.49	30.5	5.5	0.014
400	4/8	2.602	5.00	50	5.07	52.7	2.7	0.0029
500	6/8	2.698	5.67	75	5.50	69.2	5.8	0.016
600	8/8	2.778	6.59	94.2	5.89	81.3	12.9	0.1

$\Sigma\chi^2 = 0.1687$, theoretical value of χ^2 for $P = 0.05$ correspond to n [Number of degrees of freedom = K (Number of doses) - 2].

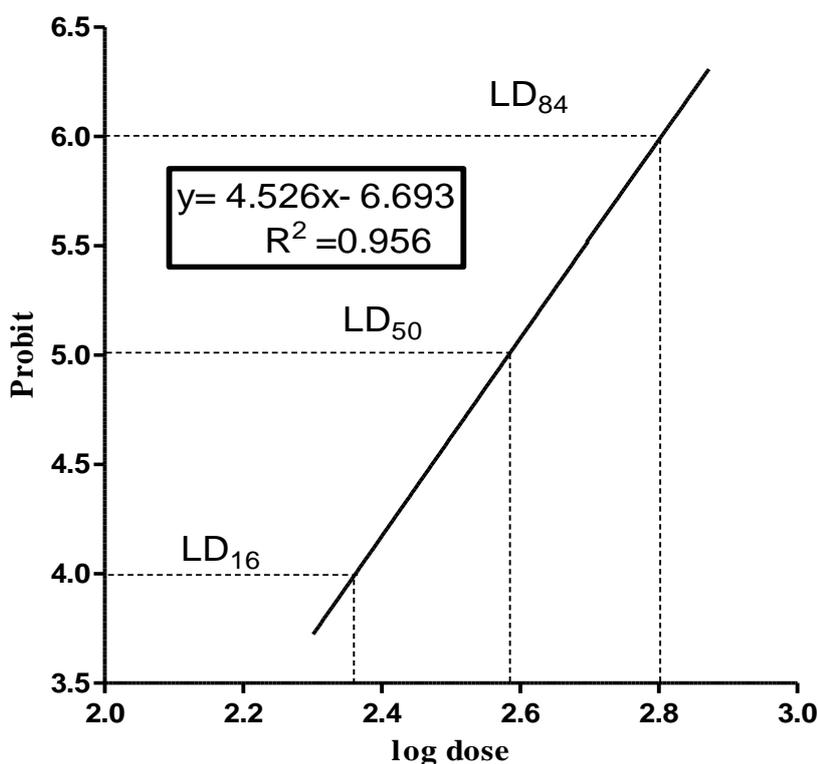


Figure 2: Curve characterizing the dose-response relationship for the determination of lethal parameters in male mice treated by simple application with the total alkaloids of *Ruta montana* ($LD_{50} = 389.04 \text{ mg/kg}$).

Table 2: Body weights of male mice treated with 129.68 mg/kg of total alkaloids extract from aerial parts of *Ruta montana*. Values are mean \pm SEM.

Groups	Body weight (g)		
	1st Day	5th Day	Difference
1	30 \pm 2.062		
2	30.28 \pm 2.036	32.45 \pm 1.054	2.1 \pm 0.352
Control	30 \pm 0.597	32 \pm 1.658	1.9 \pm 0.213

haematological parameters of treated groups when compared with control group. Biochemical analyses of the blood samples showed that the parameters, including ALAT, ASAT, and ALP, which indices of liver function did not show significant differences (See Fig. 3). As showed Figure 4, there were no changes in Urea and Creatinine.

DISCUSSION

Ruta montana has been used in traditional medicine against stomach ailment, respiratory and liver diseases¹⁶, in digestive disorders and helminthiasis, it's traditionally known for its abortive and aphrodisiac effects^{9,14}, emmenagogue¹³, as disinfectant; antipyretic and pest-destroying¹⁰. In fact, their therapeutic properties are due to the presence of thousands of secondary metabolites in particular essential oils and alkaloids². However, no study dealing with the safety assessment of this popular food and medicinal plant is reported. Therefore, the acute intraperitoneal toxicity of the total alkaloids extract of the aerial parts of *Ruta montana* was carried out in order to evaluate its safety.

Table 3: Relative organ weights of male mice treated with 129.68 mg/kg of total alkaloids extract from aerial parts of *Ruta montana*. Values are mean \pm SEM.

	Liver	Kidney	Lungs	Heart	Brain	Spleen	Testis
Control	0.0464 \pm 0.0019	0.0122 \pm 0.0008	0.0074 \pm 0.0002	0.0054 \pm 0.0001	0.0129 \pm 0.0001	0.0054 \pm 0.0003	0.0074 \pm 0.0002
Group 1st day	0.0493 \pm 0.0037	0.0135 \pm 0.0011	0.0066 \pm 0.0001*	0.0048 \pm 0.0001	0.0137 \pm 0.0001	0.0051 \pm 0.0003	0.0068 \pm 0.0004
Group 5th day	0.0255 \pm 0.0069*	0.0129 \pm 0.0005	0.0061 \pm 0.0001***	0.0116 \pm 0.0063	0.0135 \pm 0.0049	0.0067 \pm 0.0007	0.0064 \pm 0.0004

Table 4: Haematological values mice treated with total alkaloids extract of *Ruta montana* (129.68mg/kg). Values are mean \pm SEM.

Teste	RBC 10 ⁶ /mm ³	MCV μ m ³	RDW %	HCT %	PLT 10 ³ /mm ³	MPV μ m ³	WBC ₁₀ / ³ /mm ³	HGB g/dL	MCH pg	MCHC
Control	7.66 \pm 0.5187	42.75 \pm 0.6756	20.83 \pm 1.791	32.75 \pm 2.293	471 \pm 77.82	8.733 \pm 1.571	8.1 \pm 1.038	11.22 \pm 0.3177	14.98 \pm 0.9864	35.12 \pm 2.371
Group 1st day	7.863 \pm 0.6623	42.25 \pm 1.215	19.52 \pm 0.6457	33.45 \pm 3.487	443.2 \pm 59.85	7.217 \pm 0.4743	8.083 \pm 0.6188	12.18 \pm 0.6814	15.68 \pm 0.6058	37.42 \pm 2.259
Group 5 th day	7.225 \pm 0.3034	42.73 \pm 0.7775	19.85 \pm 0.4264	30.90 \pm 1.442	582.3 \pm 33.67	6.583 \pm 0.4175	11.10 \pm 1.424	10.73 \pm 0.2777	14.90 \pm 0.4740	34.97 \pm 1.393

In this study we determined the lethal dose 50 and we tested the acute toxicity of the total alkaloids extract from aerial parts of *Ruta montana* in male mice.

The LD₅₀ value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis or toxicologic classification of chemicals. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained²⁵.

The median lethal dose determined by intraperitoneal administration of total alkaloid extract from aerial parts of *Ruta montana* was 389.04 mg/kg and according to the classification of Hodge and Sterner¹¹, chemical substances with a LD₅₀ between 50 and 500 mg/kg body weight determined after a single doses in mice is considered as moderately toxic product.

An acute toxicity test can give more information about the biologic properties of a chemical compound than any other single test²⁵ so in the acute toxicity study, the alkaloid extract was administered by intra-peritoneal route at doses of 129.68 mg/kg (1/3 LD₅₀) body weight to both groups of mice. The acute treatment for 1 and 5 days indicated that *Ruta montana* alkaloid extract on intra-peritoneal route with the doses 129.68 mg/kg body weight did not produce any sign of toxicity or death in both groups of mice.

Since changes in body weight have been used as an indicator of adverse effects of drugs and chemicals, organ weights have been used as sensitive indicator to evaluate the toxic effects of drugs in toxicological studies¹⁷. In this study *Ruta montana* had no effect on the body weight of male mice. While Organ weight is one of the most sensitive drug toxicity indicators, and it changes often

precede morphological changes²¹ and can be used to indicate organ swelling, atrophy or hypertrophy¹⁸, in this study a significant changes were observed in the relative organ weight of lungs (after 24h and 5 days) and liver (after 5 days) were significantly decreased, therefore the decrease in the liver-body weight ratios in groups receiving the extract may be attributed to atrophy.

Hematological and biochemical parameters are the main diagnostic criteria in clinical practice. The values of some hematological and biochemical parameters indicate the adverse effects of drugs on organs or systems. The haematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in humans and animals²⁰. In this study, no significant differences were found in some hematological parameters at the end of the treatment as compared with control group. Thus, the results suggested that the total alkaloid administered by intra-peritoneal route at 129.68 mg/kg has no influence on the system of blood in male mice.

Alkaline phosphatases are often used to assess the integrity of plasma membrane and endoplasmic reticulum³. ALAT is a sensitive and specific marker of hepatocellular affect is exclusively present in the cytoplasm of hepatocytes and released into the circulation after cellular damage^{1,12,23}. ASAT is less sensitive and less specific than ALAT for the liver; it is also finds in other organs, especially in the skeletal muscles, heart muscle and brain while PAL, this enzyme is found especially in the liver and bones²⁶. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood²³. Therefore, no changes in ASAT and ALP activities but there are a considerable change in ALAT on first day and

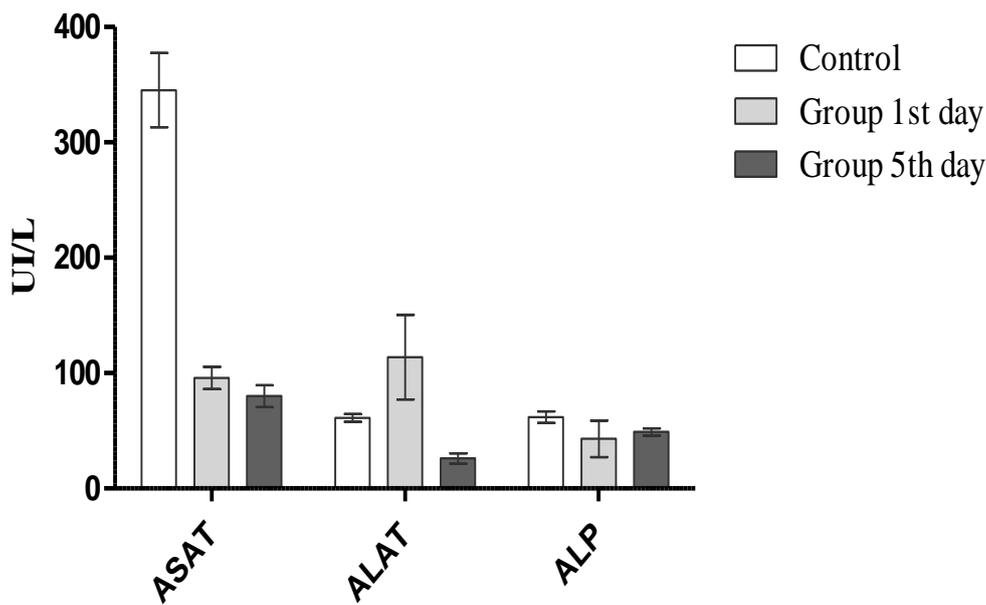


Figure 3: Effect of administration of total alkaloid extract of *Ruta montana* (129.68 mg/kg) on some biochemical parameters of liver function in male mice in acute toxicity study. Values are mean \pm SEM.

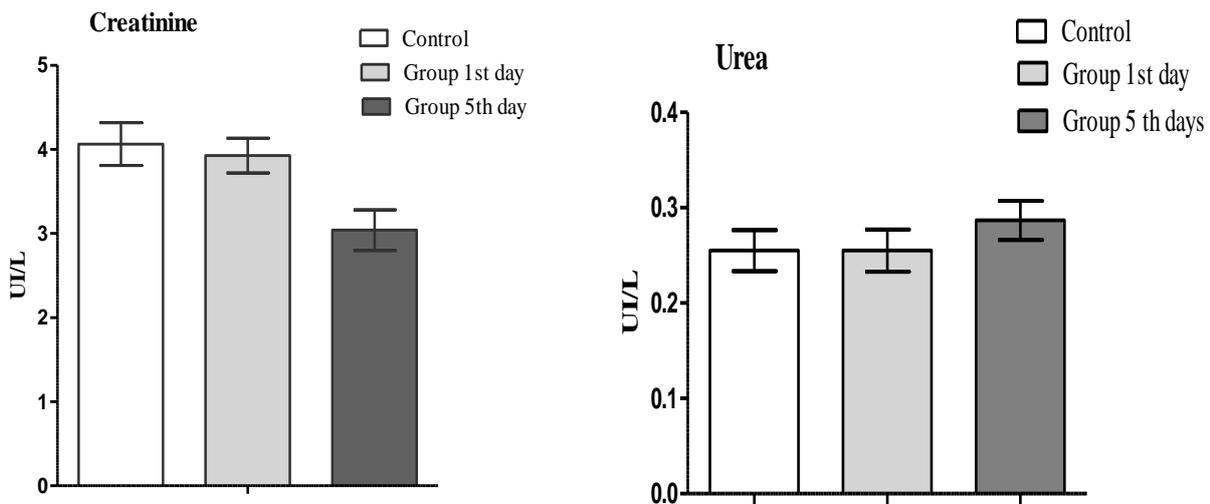


Figure 4: Effect of administration of total alkaloid extract of *Ruta montana* (129.68 mg/kg) on some biochemical parameters of kidney function in male mice in acute toxicity study. Values are mean \pm SEM.

recovers on the 5th day, as known ALT is considered the most liver-specific enzyme because it is present in higher quantities in hepatocyte cytosol. Therefore, its presence in the serum is considered a marker of hepatocellular necrosis.

The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. When there is a compromise of normal glomerular function, substances normally cleared by the kidneys such as urea and creatinine accumulate in the biological³. This study showed no significant difference between treated group by total alkaloids of *Ruta montana* and control group, this results suggest that *Ruta montana* extract did not alter the renal function.

However, the results of acute study showed no adverse effects on the usual markers of liver and kidney toxicity (ASAT, ALAT, ALP, Urea and Creatinine). It may be concluded that the alkaloid extracts of *Ruta montana* aerial parts did not induce significant damage to these organs.

The liver and kidney are sensitive organs, and their functions are known to be affected by a number of factors, such as drugs, including plant phytochemicals, ultimately leading to renal failure and liver toxicity.

The histopathological observation of the principal vital organs of the treated mice compared with control group revealed that there were no pathological changes in the kidney these confirmed by no changes in creatinine and urea so the activity and the morphology of kidney didn't

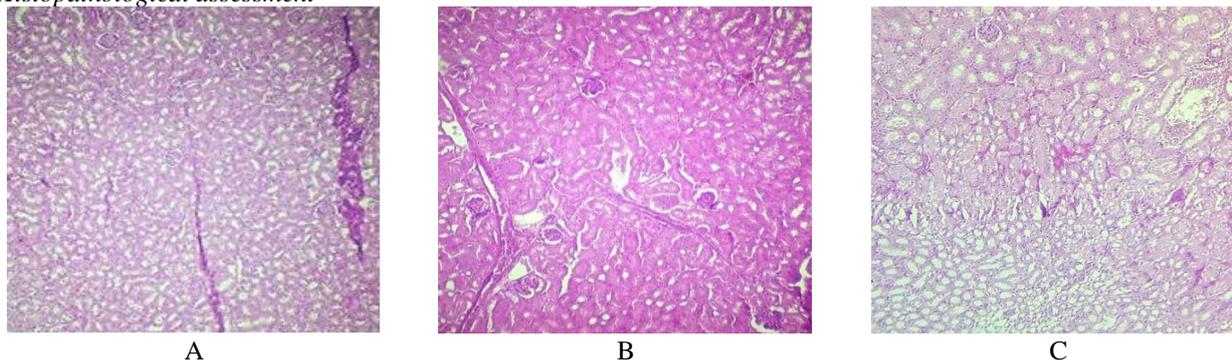
Histopathological assessment

Figure 5: Histology of the kidney (HE×10); A: Control group and treated mice with total alkaloid extract of *Ruta montana* at the dose of 129.68 mg/kg (1/3 LD₅₀) (B: After 24 hour and C: After 5 days).

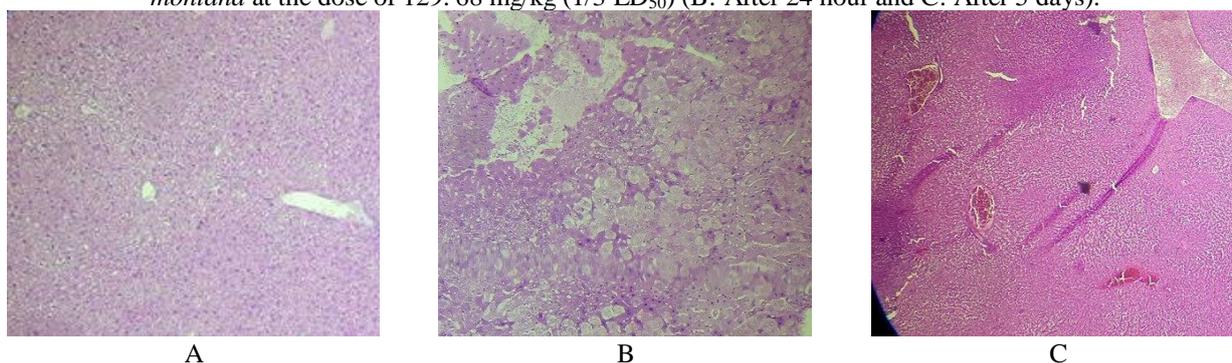


Figure 6: Histology of the liver (HE×10); A: Control group and treated mice with total alkaloids extract of *Ruta montana* at the dose of 129.68 mg/kg (1/3 LD₅₀) (B: After 24 hour and C: After 5 days). GG: Ground glass appearance of the hepatocytes, Cn: Sinusoidal and Portal congestion.

altered after the intraperitoneal administration of total alkaloids of *Ruta montana*. Although changes in the liver morphology characterized by the presence of ground glass appearance in hepatocytes after 24h, sinusoidal and portal congestion after 5 days with decrease in relative weight of liver, while the results of serum analysis showed no changes in ALAT, ASAT and ALP, that may be due to the administration of total alkaloids into organism, they may reflect the deregulation of the process of absorption of a normal substance.

CONCLUSION

In conclusion, the total alkaloids extracted from the aerial parts of *Ruta montana* were classified as moderately toxic, then the acute toxicity study with intra-peritoneal administration of *Ruta montana* alkaloids at dose of 129.68 mg/kg may not exert severe toxic effects. However, the safety of *Ruta montana* in humans needs further investigation and toxicological data should be collected and confirmed over repeated long term studies.

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