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Research Article

Evaluation of Acute Toxicity of *Ruta montana* L.

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ABSTRACT

The toxicity caused by medicinal plants using for treatment is now one of the causes of poisoning of people reported by hospitals. This study aimed to evaluate the acute toxicity of the total alkaloids of the aerial parts of *Ruta montana* L on female mice. The extraction of total alkaloids from the aerial part of *Ruta montana* L is obtained by liquid-liquid extraction. The LD_{50} was determined using Litchfield and Wilcoxon method and in acute toxicity a single intra-peritoneal administration of total alkaloid extracted at 75.23 mg/kg (1/3 LD_{50}). Body weight, biochemical and hematological parameters were recorded with histopathological examination of liver, kidneys and brain. The LD_{50} is estimated to be 217 ± 8.3 mg / kg. The acute toxicity study was showed a significant increase in the relative weight of the liver after 24h. Serum parameters (AST, ALT, PAL, Urea and Creatinine) did not show any significant change with perturbation of some blood parameters after 24 h and renal congestion with cerebral edemas are observed on histological sections of the kidneys and brain of female mice. This study revealed that the total alkaloids of *Ruta montana* L were classified as very toxic chemical products.

INTRODUCTION

Many people, especially those living in rural areas, are turning to the use of yesterday's remedies-medicinal herbs. Plants contain many active ingredients that can provoke adverse reactions when medicinal herbs are used improperly [1]. Although medicinal plants are widely used and assumed to be safe, however, they can potentially be toxic especially in pregnancy [2].

The genus Ruta (Rutaceae) mainly found in tropical and temperate regions with major centre of diversity in Southern Africa and Australia. In Algeria, this genus, known by the name of 'El Fidjel', presents three species: *Ruta graveolens* L, *Ruta chalepensis* L and *Ruta montana* L [3]. Members of this genus have been used since antiquity in traditional medicine and credited with a long list of medicinal uses [4, 5].

Ruta species are sources of different classes of natural products with biological activities; in previous studies, with *Ruta montana* L. the presence of alkaloids and coumarins were shown [6]. According to the literature the essential oil of the plant is toxic due to the presence of alkaloids, so this plant should be handled with care [7]. Indeed, the observations of Masri et al. (2015), of a case of acute poisoning by *Ruta montana* L, the toxicological analysis of the urine shows a high presence of alkaloids [8].

Alkaloids have been studied more intensively than other natural products due to their medicinal importance and their great diversity in structure and pharmacological activity. The pharmacology and biochemistry literature is replete with descriptions of alkaloid structures and of neuropharmacological activity on man and other mammals; this literature provides a vehicle for exploring toxicity [9].

The efficacy and potential toxicity of remedies employed in folk medicine do however have to be scientifically evaluated, but while toxicity assays are not available for many countries. Therefore *Ruta montana* L is the subject of several experiments on laboratory animals and rodent species are commonly used in traditional toxicology testing guidelines to predict human health toxicity outcomes [10]. Generally, mice and

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rats are models of choice but a difference has been found between rats and mice in their sensitivity to the effects of drugs, mice are more sensitive than rats. The reason why mice are more sensitive than rats, it could depend on a difference in sensitivity of the target in the liver, or on the way in which the two species distribute and metabolize the drug [11]. Thus, the current work was carried out in order to evaluate the toxicity effects of total alkaloids extracted from *Ruta montana* L in female mice.

MATERIALS AND METHODS

Plant Material

The aerial parts of *R. montana* L were collected in the Northeast Algeria in August 2017, during the period of full flowering, and the aerial parts were dried in air at room temperature and stored until use.

Preparation of Alkaloids Extract

Powdered plant material (100 g) was defatted with 250 ml of petroleum ether under reflux, After filtration, the powder was witted with 40 ml of ammonia solution (0.5 N) for 24 hours, and was extracted to exhaustion with chloroform using soxhlet apparatus and 5 to 8 cycles are necessary for a total extraction of the alkaloids. Chloroform extract is then shaken three times with a solution of 150 ml of sulfuric acid (0.5 N) and the acid extract is basified to pH 9 by adding a few ml of pure ammonia. We then exhaust the solution with 150 ml of ethyl ether, dried with anhydrous sodium sulfate and concentrated to dryness under reduced pressure to obtain crude alkaloids [12, 13].

Experimental Animals

Female *Swiss albino* mice weighing between 20 to 36 g from the Pasteur Institute in Algiers. The mice are housed in transparent plastic cages (55x33x19 cm) in the animal room of the Faculty of Sciences of Nature and Life, University Ferhat Abbas-Sétif, Algeria. The animals were fed with a standard pellet and tap water ad *libitium*. All animals were kept in standard environmental conditions two weeks prior to the experiments.

Determination of LD_{50}

Seven groups of 8 female mice have fasted for 18 h, then the alkaloids extract of *Ruta montana*. L is administered intraperitoneally at one dose to the different groups of mice (50, 100, 150, 200, 250, 300 and 350 mg/kg). After treatment with total alkaloids, the animals are observed individually every 30 minutes for 6 hours, on the first day and every day for 14 days and changes in appearance and behavior are noted. The median lethal dose (LD_{50}) was calculated according to the Lichtfield and Wilcoxon method.

Acute Toxicity

Two groups of 12 animals were given single dose of 75.23 mg/kg (≈ 1/3 LD_{50}) of total alkaloids of *Ruta montana* L by intraperitoneal route. However, the control group received only saline water. The animals are observed every hour for the first day and daily for the second group, to note the behavior and the clinical symptoms. At the end of all experimental periods, the first group was sacrificed after 24 hours of treatment and the second group after 5 days.

Blood Analysis

Two blood samples were obtained by cardiac puncture: a sample for hematology with the automated hematologic analyzer (ABX micros 6-Français) and sample for serum were used for measurement of biochemical parameters in 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 (Integra Roche-Français).

Histopathological Analysis

After dissection, all tissues were observed macroscopically and major organs (liver, brain, heart, kidneys, spleen, testicles, and lung) were weighted, and 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

All data were expressed as mean ± standard error of mean (SEM), the comparison was performed using one-way analysis of variance (ANOVA) followed by the Tukey's test using Graphpad Prism version 5.00 and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for *Ruta montana* L, because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems [14]. It should, in addition to the use of historical documentation

on *Ruta montana* L, also have a formal toxicological evaluation of this plant to optimize it's safe use as a medicine.

Yield of the Total Alkaloids

The extracted total alkaloids appear as sticky paste, with a dark brown color, characterized by a strong odor. After liquid-liquid extraction, the yield of total alkaloids from aerial parts of *Ruta montana* L was 0.26 ± 0.05 g/100 g of the vegetable powder. This low yield can be explained by the temperature of soxhlet for several hours which can degrade some sensitive compounds as polyphenols [15].

Median Lethal Dose

The LD_{50} value (precise or approximate) is currently the basis of the toxicological classification of chemicals and is therefore required by government authorities in different situations [16].

The effects of intraperitoneal single doses of total alkaloids of *R. montana* on mortality and LD_{50} values in female mice are summarized in Table 1. LD_{50} value was determined using Lichtfield and Wilcoxon method (1949) [17]. All doses administered, caused immediate agitation and behavioral perturbations with sedation, decrease in locomotor activity with paralysis of the hind legs, convulsions, cyanosis and heart rhythm disturbance. Generally, the animals died 1 hour after the administration of alkaloids. The surviving animals quickly recovered their normal activity and growth, after a period between 2 to 5 days, depending on the dose.

Depending on the literature, quinoline alkaloids and furocoumarins are the active constituents of *Ruta montana*. L extracts [18]. A furoquinoline alkaloid (skimmianine), is a common compound in Rutaceae, inhibits vasopressin responses induced by 5-hydroxytroptamine (5-HT) and produces a non-specific bloc of cardiovascular functions and at high concentrations results hypotension [19]. However, convulsions and paralysis are explained according to Tamokou and Kuete (2014), by the action of the toxic substance on receptors which lead to biochemical effects such as the disruption of metabolism and the disruption of exchanges through the membranes cells resulting in changes in behavioral and physiological responses [20]. The symptomatology observed is consistent with the symptoms described by Jouglard (1977) and Bellakhdar (1997) with the same plant [21, 22].

The results obtained show the increase in the mortality rate with the increase in the dose of the total alkaloids of *R. montana*. This made it possible to deduce a dose-response effect. So from the data presented in the table, we draw the curve probit = f (log dose) (Fig. 1).

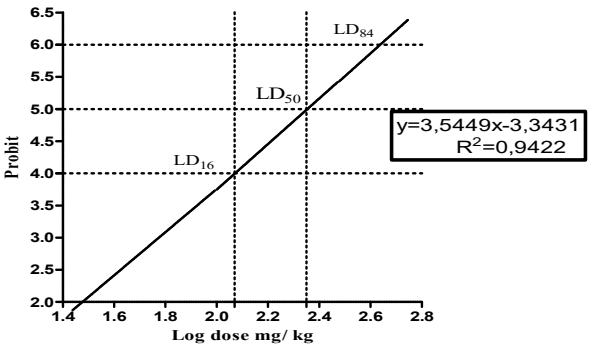

Figure 1: Median lethal dose (LD_{50}) of the total alkaloids of *R. montana*. L in female *Swiss albinos* mice (LD_{16} = 117.88 mg/kg, LD_{50}= 225.70 mg/kg, LD_{84}= 432.16 mg/kg)

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

Dose mg/kg	No of Death/exp	Log Dose	Observation Effect		Attend Effect		Difference %	χ2
			Probit	%	Probit	%		
50	0/8	1.698	2.35	0.4	2.76	1.3	0.9	0.0063
100	1/8	2	3.85	12.5	3.74	10.5	2	0.0044
150	2/8	2.177	4.33	25	4.36	26.2	1.2	0.0005
200	3/8	2.301	4.68	37.5	4.84	43.8	6.3	0.017
250	4/8	2.398	5.00	50	5.15	56	6	0.015
300	6/8	2.477	5.67	75	5.41	66	9	0.038
350	8/8	2.544	6.47	92.9	5.68	75.3	17.6	0.17

$\Sigma\chi_2$ experimental = 0.0385. Theoretical value of χ2 for P= 0.05 correspond to n[Number of degrees of freedom = K (Number of doses) -2]. N= 4, **so theoretical χ_2 = 11.1. Therefore experimental χ2 < theoretical χ2,** so the work is acceptable.

The calculation of the LD_{50} by the methods of Leichtfeld and Wilcoxon (1949) gave an LD_{50} of 225.7 mg/kg and based to the classification of Hodge and Sterner (1949), the total alkaloids of the aerial parts of *Ruta montana* L is considered as a moderately toxic product and as a very toxic product according to Gosselin *et al*. (1957) [17, 23, 24].

Acute Toxicity

An acute toxicity test can give more information about the biological properties of a chemical compound than any other test [16]. The results of the study of animals administered with a single dose of 75.23 mg/kg (1/3 LD_{50}) of alkaloids did not show any signs of toxicity or death such as immediately after injection or at the end of 5 days.

1. Effects on Body Weight and Weight of Organs

Various parameters are important in the evaluation of the toxicity of medicinal plants; these include body weight, absolute and relative organ weight with their respective biomarkers and hematologic profile [25, 26]. The Table 2 shows that the total alkaloids of *Ruta montana* L has no effect on body weight gain in treated females compared to controls.

Table 2: Body weights of female mice treated with 75.23 mg/kg of total alkaloids of *Ruta montana* L.

	Body weight (g)		
Groups	**1st day**	**5th day**	**Difference**
1	31.64±0.6039		
2	29.06±0.2085	28.18±0.4487	0.8783±0.4948
Control	27.64±0.3631	28.24±0.7288	0.5925±0.8142

Values are mean ± SEM

The effects of total alkaloids of *Ruta montana* L on relative organs weights are presented in Table 3. Female treated mice and sacrificed after 24 hours showed a significant increase in the relative weight of liver by 12.28% and a significant decrease in the relative lungs weight of 10.76% in as compared to control group.

Table 3: Relative weights of organs of female mice treated under the conditions of acute toxicity with total alkaloids of *Ruta montana* L.

	Liver	Kidneys	Lungs	Heart	Brain	Spleen
Control	0.0464±0.0019	0.0093±0.0003	0.0065±0,0001	0.0048±0.0002	0.0143±0.0004	0.0039±0.0002
Group 1st day	**0.0521±0.0008****	0.009±0.0003	**0.0058±0.0001***	0.0042±0.0001	0.0136±0.0003	0.0039±0.0004
Group 5th day	0.0455±0.0009	0.0087±0.0004	0.006±0.0002	0.0044±0.0001	0.0147±0.0004	0.0038±0.0004

The values are expressed in M ± SEM. * P <0.05 comparing to controls.

Organ weight is one indicator of drug toxicity [27] and can be used to indicate atrophy or hypertrophy [28]. In this study the significant increase in the relative weight of the liver, while hypertrophy of liver can be induced by a large number of compounds and some hypolipidemic agents cause liver enlargement in mice. Typically, the hepatocytes are hypertrophied throughout the liver lobule and exhibit a ground glass appearance of their cytoplasm. Electron microscopy in these cases reveals a marked increase in peroxisomes [29].

2. Effect on the Hematological Parameters

The hematopoietic system is one of the most sensitive targets for toxic compounds [30] and an important index of physiological and pathological status in man and animals [31]. The hematological parameters of the female mice treated with the total alkaloids of *Ruta montana* are presented in Table 4. Statistically, a significant increase in WBC, CCMH, Lymphocytes and Granulocytes was recorded of 96.12%, 4.34%, 93.20%, and 125.62% respectively with a significant decrease in mean globular volume of 8.03% in the blood after 24 hours from treatment compared to the control group.

An increase of white blood cells number following administration of an extract reveals the stimulating effect of the extract on the immune system according to Shah *et al*. (2008) [32], Yakubu and Afolayan (2009) also indicates a proliferative activity of the cells of the bone marrow which can be attributed to the alkaloids constituting the plant [33]. Kumer *et al*. (2005), explain the increase in basophils after administration of a plant extract by an inflammatory activity of the extract, this could be related to the increase in the relative weight of the liver [34]. However, the decrease in MCV after exposure to the plant product reveals the

antihematic potential of the extract [35], the decrease in MCV in mice according to Fox *et al*. (2007), is also considered as an indicator of microcytic anemia [36], these changes are not observed in female mice treated and sacrificed after 5 days, which is probably explained by an immediate effect of the total alkaloids of *Ruta montana* L and which disappears after 48 hours.

Table 4: Hematological parameters of female mice treated under conditions of acute toxicity with a dose of 75.23 mg/kg (≈ 1/3 LD_{50}) of total alkaloids of *Ruta montana* L.

Test Groups	Control	Treated 1st day	Treated 5th day
RBC $10^6/mm^3$	8.943±0.2025	9.33±0.1726	8.934±0.2392
MGV Fl	49.91±0.7089	**45.9±0.7684****	48.61±1.007
HCT %	44.67±1.341	42.81±0.7288	43.33±0.7174
PLT %	575.1±55.92	586.7±60.43	536.9±66.84
MPV Fl	7.3±0.2507	7.314±0.3997	7.043±0.2918
IDP Fl	10.83±0.3932	11.27±0.7269	10.30±0.4515
PTC %	0.4157±0.0399	0.4314±0.0604	0.3743±0.0456
WBC $10^9/l$	6.2±0.414	**12.16±1.478*****	8.843±0.6535
HGB g/dl	14.27±0.2533	14.27±0.1848	13.94±0.2935
MTCH Pg	15.96±0.2543	15.27±0.2925	15.63±0.2296
MCCH g/dl	32±0.6016	**33.39±0.5721*****	32.17±0.3029
LYM $10^9/l$	4.843±0.3206	**9.357±1.277****	6.943±0.453
GRAN $10^9/l$	0.6143±0.0799	**1.386±0.1299****	0.9429±0.1901

The values are expressed in M ± SEM.

3. Effect on the Biochemical Parameters

The transaminases (AST and ALT) are well known enzymes used as biomarkers predicting possible toxicity [37]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [38]. The lack of significant alterations in the levels of ALT, and cholesterol and creatinine, good indicators of liver and kidney functions, respectively [39].

Biochemical analysis of markers of hepatic and renal function (ASAT, ALAT, PAL, Urea and Creatinine) evaluated in the serum of female mice treated with total alkaloids from the aerial parts of *R. montana*, showed a low increase in ALAT during the 1st day, which regains its level around the 5th day (Fig. 2). Urea and creatinine showed no significant change compared to the control groups (Fig. 3).

The lack of significant alterations in the levels of ALT and creatinine, good indicators of liver and kidney functions, respectively [39], suggests that acute ingestion of total alkaloids of *R. montana* did not alter the hepatocytes and kidneys of the mice and furthermore, the normal metabolism of the animals.

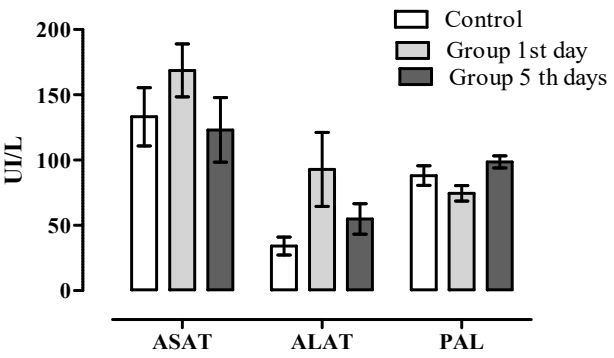

Figure 2: Effect of total alkaloids of aerial parts of *Ruta montana* (75.23 mg / kg ≈ 1/3 LD_{50}) on some biochemical parameters of liver function in female mice under conditions of acute toxicity.

4. Histopathological Examination

The histological sections of hepatic, renal and cerebral tissues of female mice treated and sacrificed after 24 hours shows no changes in comparison with the control group, however renal congestion and cerebral edema were observed under the conditions of acute toxicity after the 5th day of treatment, it is probably due to a disturbance of cerebral cellular exchanges. (Fig. 4-6)

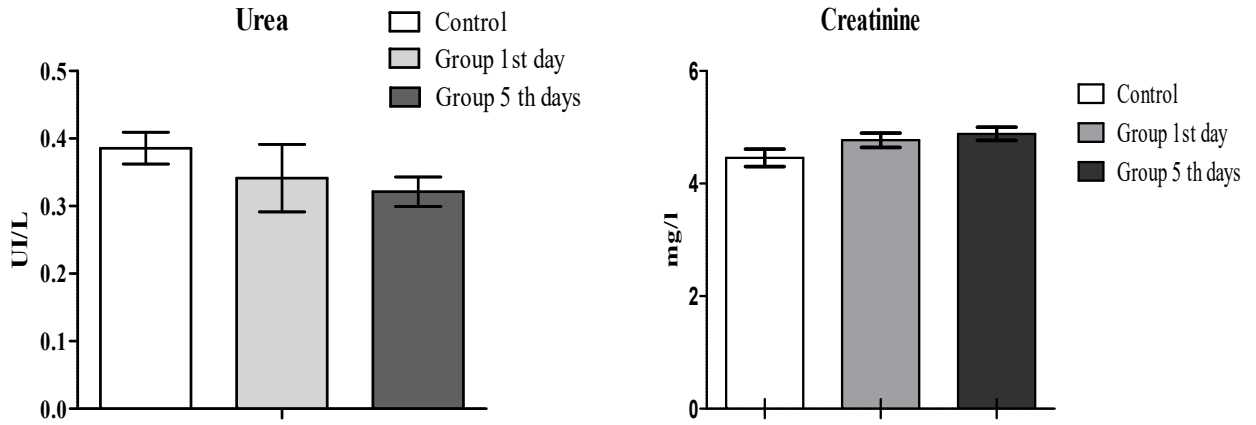

Figure 3: Effect of total alkaloids of *Ruta montana* (75.23 mg / kg ≈ 1/3 LD_{50}) on some biochemical parameters of renal function in female mice under conditions of acute toxicity.

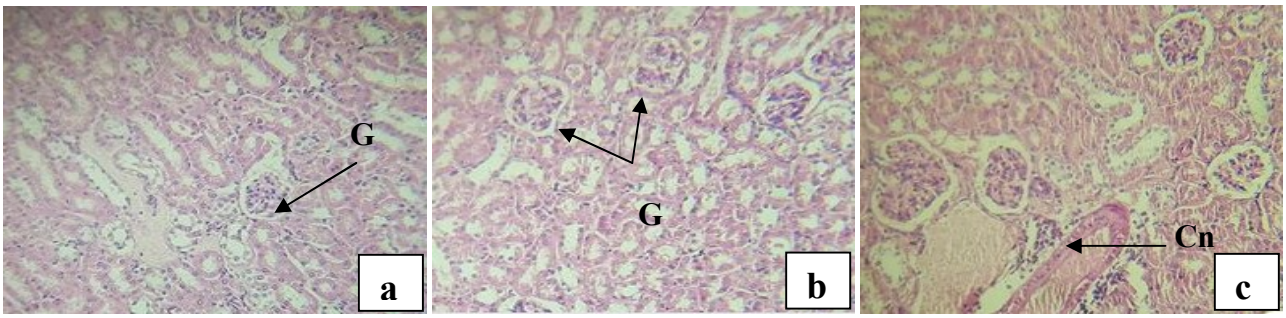

Figure 4: Histological section of the renal tissue of control female mice (a) and treated groups under conditions of acute toxicity with a dose of 75.23 mg / kg of total alkaloids of *Ruta montana* (b: after 24 hours and c: after 5 days). Eosin hematoxylin stain × 40. Cn: Renal congestion., G: Glomeruli

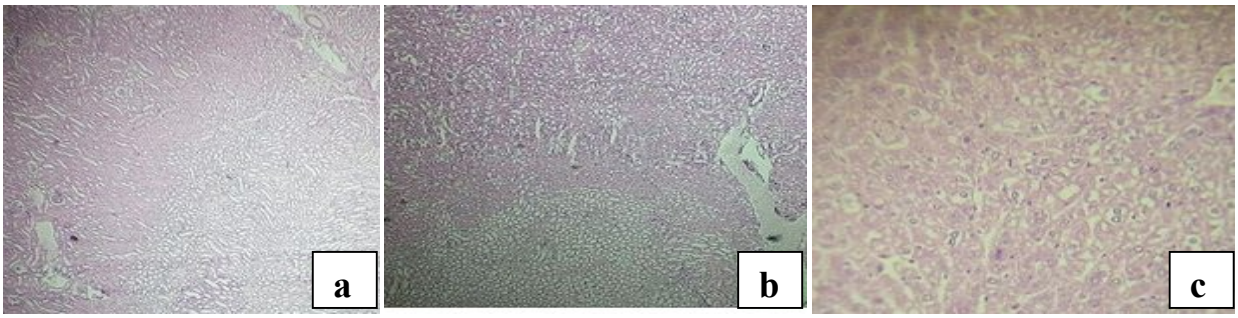

Figure 5: Histological section of the liver tissue of control female mice (a) and treated groups under conditions of acute toxicity with a dose of 75.23 mg / kg of total alkaloids of *Ruta montana* (b: after 24 hours and c: after 5 days). Eosin hematoxylin stain × 10 and × 40.

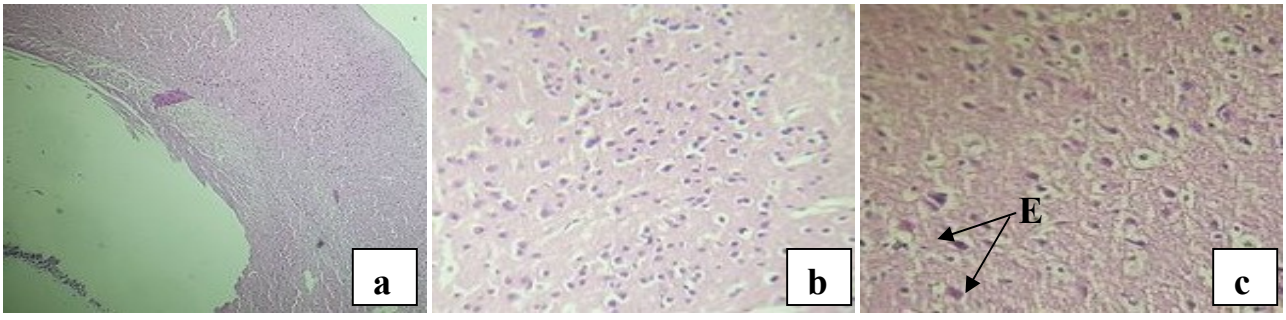

Figure 6: Histological sections of brain tissue of control female mice (a) and others treated under conditions of acute toxicity with a dose of 75.23 mg / kg of total alkaloids of *Ruta montana* (b: after 24 hours and c: after 5 days). Eosin hematoxylin staining × 10 and × 40. E: Cerebral edema.

CONCLUSION

The determination of the LD_{50} in female mice allowed classifying the total alkaloids of *Ruta montana* in the category of very toxic chemical products according to Gosselin (1957) [24]. The changes observed in the behavior on the treated animals during the study of acute toxicity called for a thorough biochemical and histological study of the brain. The acute toxicity study showed overall the absence of significant changes in biochemical parameters with the disturbance of some hematological parameters. The histological observation was characterized by some structural changes. The results obtained in this study can be deepened by a subacute and chronic toxicity study of total alkaloids to assess the cumulative toxicity of the substances.

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