Turkish Journal of Agriculture - Food Science and Technology, 11(10): 1862-1870, 2023
DOI: https://doi.org/10.24925/turjaf.v11i10.1862-1870.5768

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Acute and Subacute Toxicity of *Ruta Montana* Extract to Female Rats: Effect on Liver, Kidneys and Ovaries

Nadia Mahdeb[1,a*], Khadidja Attafi[1,b], Souha Bouhouhou[1,c], Allouni Rima[1,d], Abdelouahab Bouzidi[1,e]

[1]*Department of Biochemistry, Faculty of natural sciences and life, University Ferhat Abbas, Setif 1 – 19000 Setif, Algeria*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 15-11-2022
Accepted : 24-09-2023

Keywords:
Ruta montana L.
Toxicity
Liver
Kidneys
Ovaries

ABSTRACT

Ruta montana L. is an annual aromatic plant of the family rutaceae. Quantitative analysis of the methanolic crude extract of *Ruta montana* L. yielded 8.43%, whereas the qualitative analysis revealed the presence of alkaloid or coumarin. The Litchfield and Wilcoxon method calculated the LD_{50} of the crude methanolic extract of *Ruta montana* L. in Wistar albino female rats at 393.18 mg/kg. This allows the plant to be classified as moderately toxic. The subacute toxicity study of the methanolic crude extract of *Ruta montana* L. in female Wistar albino rats treated with 100 mg/kg (≈ 1/4 LD_{50}) and intraperitoneally showed a significant increase in body weight of the rats treated at the 4th week. Animals treated and sacrificed after 30 days showed a disturbance of the relative mass of the organs. Biochemical parameters of hepatic function assessment showed a significant increase in PAL with elevation of AST and ALT, whereas those of renal function revealed a significant decrease in creatinine with an increase in urea. Hematologic parameters recorded a decrease in RBC, HGB and HCT. The histological sections of the treated rats reveal the existence of blood congestion in the central veins and liver tissues, foci of necrosis and steatosis in the liver, blood congestion and some glomerular atrophy in the kidneys, as well as blood congestions and developed follicles without oocytes in the ovaries.

[a] *nmahdeb@yahoo.com* *https://orcid.org/0000-0003-1568-8752*
[c] *souhabiologie@yahoo.com* *https://orcid.org/0009-0003-7734-8216*
[e] *bouzidiab@yahoo.fr* *https://orcid.org/0000-0002-4370-0600*
[b] *attafik@yahoo.com* *https://orcid.org/0009-0004-7036-4683*
[d] *rym87@yahoo.fr* *https://orcid.org/0000-0001-6098-5399*

Introduction

Plants and their extracts are indispensable sources of life, used for the prevention and treatment of human diseases in many countries (Selamoglu, 2018). Among these plants are those of the Rutaceae family, which includes the annual aromatic plant Ruta montana L. (Quezel and Santa 1963). Rutaceae are frequently woody plants having secretory pockets of a sort not found in any other so-called schizolysigenic family (Ozenda, 2000). *Ruta montana*, *Ruta chalepensis*, *Ruta tuberculata*, and *Ruta latifolia* are the four species found in Algeria. They differ from one another in terms of the morphology of their leaves, fruiting clusters, bracts, and sepals (Quezel and Santa, 1963).

Ruta montana L. thrives in warm and temperate climates. It naturally thrives on arid slopes and rocks. It is common in the Mediterranean basin's calcareous soils (Quezel and Santa, 1963), as well as in arid regions of southern Europe and North Africa. It can be found in desert grasslands and mountainous regions in Algeria (Clevely and Richmond, 1997).

This plant contains above the phytochemicals compounds such as alkaloids, flavonoids, essential oils, coumarins and phenols, which make it widely used in traditional medicine (kara et al., 2016). *Ruta montana* L. was employed in ancient Greece and Egypt to induce abortions where it is used as an emmenagogue, an aphrodisiac, abortive (Masri et al., 2015; Polio et al., 2008). Root decoction is used to treat liver disorders, pulmonary diseases and stomach ailments (Lahsissene et al., 2009). To induce abortion, it is ingested in the form of paste or decoction (Bellakhdar, 1997). The objective of this work is to assess the acute toxicity of the methalonic extract through the determination of LD_{50} and the effect of subacute toxicity on liver, kidney and ovaries.

Materials and Methods

Plant Material

The plant *Ruta montana* L. was collected in the northern region of the Setif City, north-east Algeria, when it reached maturity in late spring and early summer during the flowering and fruiting period. The identification of the plant was done on the basis of its morphological characteristics and confirmed by Dr. Nouiwa Wafa

(Department of Plant Biology and Ecology, Faculty of Nature and Life Sciences, Setif 1 University, Algeria).

A specimen has been deposited at the Faculty of Natural and Life Sciences. The aerial components were cleaned, then dried at ambient temperature and shielded from light in a ventilated area (Figure 1).

Figure 1. The plant Ruta montana L. (2019)

Plant Extraction

Using an electric grinder, the aerial parts of *Ruta montana* L. are ground. A good extraction yield by maceration is possible by increasing the surface area of the powder obtained with the extraction solvent. 711 g of the plant powder of the aerial part is macerated in 1000 mL of methanol (99.7%).

A vacuum filter is used to filter the heterogeneous mixture after it has been mechanically stirred for 72 hours at room temperature. The methanol in the filtrate is allowed to evaporate naturally in the open air. The recovered *Ruta montana* L. crude methanolic extract (EBMRM) is kept in the fridge until usage.

Thin layer chromatography (TLC).

Before experimenting with EBMRM in animals, we checked whether or not active ingredients such as alkaloids which have a strong toxic action in the extract. A ready-to-use TLC plate made of Macherey-Nagel 60 F254 silica gel, 20 x 20 cm in size, was used after drying. The mobile phase prepared is a mixture of methanol/chloroform/ammonia: 78.5/20/1.5 (V/V/V) (Kurt, 1971).

After dissolving the 1g of EBMRM in 1 mL methanol, a drop is placed 1 cm from the bottom of the TLC plate and then dried in an oven. The plate is immersed about 0.5 cm in the mobile phase contained in a conventional glass tank whose atmosphere will have previously been saturated with vapors of the mobile phase, which then progresses by capillarity along the stationary phase, and entrains the compounds contained in the EBMRM according to their weight and their solubility. When the front of the solvent reaches 3 cm from the upper edge, the plate is removed from the tank, then dried and revealed by spraying with Dragendorff's reagent to reveal the presence of alkaloids which are known to be toxic.

Acute Toxicity

Animals

Adults female rats (albinos Wistar) weighing about 210–245 g were purchased from the Pasteur institute (Algiers-Algeria). The animals were acclimatized for 3 weeks to the conditions of the animal room of the faculty of Nature and Life Sciences, Setif 1 University before the commencement of the study. They were fed a standard diet and tap water *ad libitium;* however the litter was changed two to three times a week. For easy identification, the rats were marked on their body by a solution of picric acid (2%).

In the absence of an ethics committee on the use of animals for scientific purposes in our University, Ethical approval for the study was sought from the scientific council of the Faculty of Natural and Life Sciences-Ferhat Abbas University Setif 1. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals.

Determination of the lethal dose (LD_{50})

Lethal dose 50 (LD_{50}) is the amount of a chemical that, when given to laboratory animals, results in the death of 50% of them (Diallo, 2005). The LD_{50} makes it easy to gauge a substance's toxicity and define toxicity classes (Oduola et al., 2007). By marking the rats, female rats weighing are separated into five groups of six each. Prior to the experiment, the animals fasted for 24 hours. One dose of 250, 400, 600, 1000, or 1100 mg/kg of rat weight of the test substance is injected intraperitoneally to the treated rats after being dissolved in a few drops of methanol and diluted in physiological water. A saline solution with a trace amount of methanol was given to the control group. The animals are individually monitored for the first day and every day for 14 days following the administration of EBMRM. The number of dead rats as well as the behavior and clinical symptoms of the animals are noted throughout the experiment.

The LD_{50} and its confidence interval are calculated using the Litchfield and Wilcoxon method (Litchfield and Wilcoxon, 1949).

Subacute toxicity

20 rats were divided into two groups of 10 animals each: the treatment group and the control group. The second batch received 0.5 mL of saline solution (0.9% NaCl), while the first batch received 100 mg/kg (≈1/4 LD50 mg/kg) of EBMRM intraperitoneally. For a total of 30 days, animals receive daily treatment under subacute toxic circumstances.

Study of Some Hematological and Serum Biochemical Parameters

The animals were sedated by chloroform inhalation under a bell at the conclusion of the experiment, and after 30 days of intraperitoneal administration of EBMRM and physiological saline, blood samples were obtained, conducted with hematocrit tubes at the level of the orbital vein of the animal's eyes.

Blood from each animal was collected in heparinized tubes for the measurement of biochemical parameters and ethylene diamine tetra-acetic acid (EDTA) tubes for the determination of hematological parameters namely RBC (red blood cells), WBC (white blood cells), HCT (hematocrit), HGB (hemoglobin), PLT (platelets), MGV (mean corpuscular volume), MPV (mean platelet volume), MCHC (mean corpuscular hemoglobin concentration) which were performed using a α Swelab Coulter blood cell counter.

The heparinized tubes were spun at 3200 rpm for 5 min in order to measure the biochemical factors glucose, urea, and creatinine using the Spinreact clinical diagnostic reagent kits - Barcelona Spain. For aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (PAL) were used the Tunisian Society's Biomaghreb enzyme kits (BIOMAGHREB) - Tunis Tunisia. The animals are sacrificed, dissected, and their organs macroscopically examined *in situ* after blood collection; after that, they are removed and weighed on a precision scale.

Histological Study

The liver, kidneys, and ovaries of the control and treatment rats were rapidly removed and stored in 10% formalin. Fixation, dehydration, incorporation in paraffin, creation of 5µm slices, staining with hematoxylin-eosin (HE), and observation under an optical microscope are the many stages of microscopic examination.

Statistical analyzes

The "Sigma Stat 3.5" program was used to conduct the statistical analysis. The one-way ANOVA test was used to statistically assess the data, which are represented by the mean and standard deviation. *P values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Ruta montana L. aerial components were extracted by maceration, and the resulting 8.43% yield crude extract took the form of a dark green sticky paste. After being revealed by Dragendorff's reagent, the alkaloids or coumarins that appeared in the form of an orange-colored spot could be separated using thin layer chromatography of the crude methanolic extracts of the aerial portions of *Ruta montana* L. (Figure 2).

Acute Toxicity

Observation of animal behavior and clinical symptoms

From the beginning of treatment with various doses of crude methanol extract from the airborne portions of *Ruta montana* L. administered intraperitoneally to female rats, toxic effects were visible. The clinical map displayed by the animals was marked by severe symptoms, including convulsions and tremors, tachycardia, hair smoothing, and abdominal intussusception. The animals begin to become less active, move more slowly, and end up lying on their belly, with their back legs spread out.

Following the injection of a dose of 1100 mg/kg of EBMRM, the female rats died within the first few minutes.

The Rats that managed to survive in the treated groups did not eat and remained in a severe condition, but they started to improve gradually and eventually recovered from the second day.

Determination of the LD_{50} by the method of Litchfield and Wilcoxon.

Following the intraperitoneal administration of EBMRM to different groups of rats at different doses ranging from 250 mg/kg to 1100 mg/kg, the percentage and dose-dependent mortality of female rats in Probit units is presented in Table 1 and Figure 3. The sum of the various contributions to $\chi 2$: $\Sigma\ \chi 2$ experimental = 0.46 $\chi 2$ experimental = $\Sigma\ \chi 2$ experimental. N/K = 0.46.30/5 = 2.76 (N: total number of animals, K: total number of doses. The theoretical $\chi 2$ value for the probability threshold p = 0.05 for a degree of freedom n = 5-2 (the dose-2 numbers) is 7.82. It is therefore acceptable to work. From the data presented in the Table 1, we plot the Probit curve as a function of the logarithmic dose presented in Figure 3. The toxicity was observed to be a dose-dependent phenomenon.

From the line drawn, we have determined:
The LD_{16} = 145.26 mg/kg.
The LD_{50} = 393.18 mg/kg.
The LD_{84} = 1064.20 mg/kg.

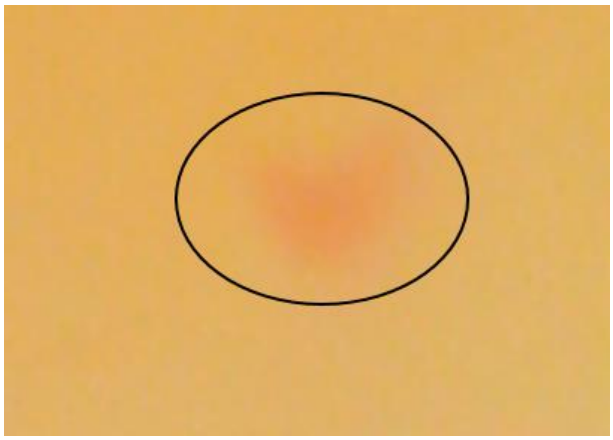

Figure 2. Chromatogram of the crude methanolic extract of *Ruta montana* L.

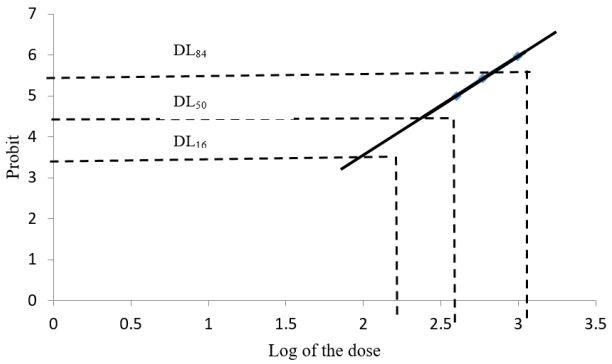

Figure 3. Curve characterizing the dose-response relationship for the determination of lethal parameters in female rat treated by simple application with the EBMRM of *Ruta montana.*

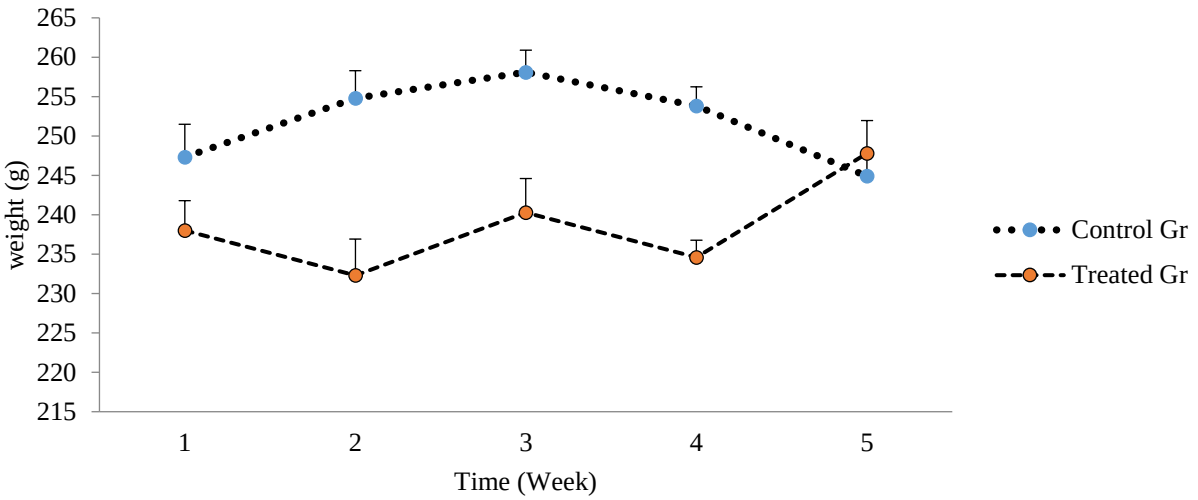

Figure 4. Changes in body weight (g) between the control and treatment rats after exposure to 100 mg/kg of EBMRM under subacute toxicity conditions

The values are shown as mean ± standard deviation, * significantly different, P<0.05. Time: 1- Initial weight; 2- 1st Week; 3- 2nd Week; 4- 3rd Week; 5- 4th Week.

Table 1. Calculus of median lethal dose of the EBMRM from *Ruta montana* using Litchfield and Wilcoxon method.

Dose mg/kg	Number of deaths/group	Log dose	Observed effect		Expected effect		Difference de	χ^2
			%	Probit	%	Probit	%	
250	0/6	2.39	7.8	3.58	27.9	4.41	20.1	0.23
400	3/6	2.60	50	5.00	43.3	4.82	6.7	0.02
600	4/6	2.77	66.6	5.43	56.9	5.17	9.7	0.04
1000	5/6	3	83.3	5.97	74.1	5.64	9.2	0.05
1100	6/6	3.04	93.9	6.55	79.7	5.82	14.2	0.12

Table 2. The relative mass of the organs of the control and treated rats under the conditions of subacute toxicity by the dose of 100 mg/kg of EBMRM.

Group	Lungs	Heart	Liver	Spleen	Kidneys	Brain
Control	0.00703±0.000735	0.00335±0.000292	0.0327±0.00356	0.00433±0.000623	0.00724±0.000676	0.00717±0.00109
Treated	0.00617±0.000454 *	0.00324±0.000217	0.0349±0.00169	0.00410±0.000773	0.00679±0.000829	0.00723±0.000681

The values are shown as mean ±standard deviation, * significantly different, P≤ 0.05.

Weight fluctuation

The difference in body weight between the treated and control rats is seen in Figure 4. Weight changes were minimal in the first and third weeks, somewhat positive in the second, and significantly positive in the fourth for the treated group. The first and second weeks saw typical weight gain for the controls, followed by a modest reduction in the third and fourth weeks.

The relative mass of the organs

By comparing EBMRM-treated rats with macroscopically observed controls, the organs showed no visible morphological changes. With the exception of a noticeable reduction in the relative mass of the lungs, no significant difference was seen between the relative masses of the treated rats' organs and those of the control group presented in Table 2.

Study of hematological parameters

The hematological results obtained are illustrated in Table 3. A significant increase in mean blood cell volume (MMV), red blood cell distribution index (IDRa) and a significant decrease in mean corpuscular hemoglobin concentration (CCMH) have been registered. Values for the other parameters showed no significant difference presented in Table 3.

Study of serum biochemical parameters

Rats given EBMRM showed slightly elevated levels of ASAT and ALAT as well as a considerable increase in PAL when blood biochemical parameters were examined to assess the condition of the liver and kidneys which are presented in Figure 5. With a slight increase in urea and glucose shown in Figures 6 and 8, serum creatinine concentrations in treated rats decreased significantly in Figure 7.

Histopathological study

It became possible to see some glomerular atrophy and blood congestion in the kidneys of rats treated with EBMRM under subacute toxic circumstances compared to the control rats by looking at histological sections of the kidneys presented in Figure 8.

In contrast to the control rats, the treated rats' livers showed foci of necrosis, hepatic steatosis, and blood congestion in the central veins and hepatic tissues presented in Figure 9. It was able to see thrombus, developed follicles without oocytes, and others with oocytes with a very low quantity of oocytes in the ovaries of the treated rats through histological sections presented in Figure 10.

Table 3. Hematological parameter values of control and treated rats when exposed to subacute toxicity at a dose of 100 mg/kg EBMRM.

Parameters	RBC 10^{12}/l	VGM fl	IDR %	IDRa Fl	HCT %	PLT 10^9/l	VPM Fl	IDP fl	PTC %	LPCR %
Control	7.470 ± 0.231	56.083 ± 1.165	14.400 ± 0.410	39.117 ± 1.105	41.917 ± 1.808	678.333 ± 118.867	5.700 ± 0.245	9.183 ± 0.279	0.388 ± 0.0741	3.550 ± 0.860
Treated	7.128 ± 0.347	58.383 ± 1.286*	14.767 ± 0.734	43.067 ± 1.665*	41.650 ± 2.468	690.333 ± 93.712	5.667 ± 0.216	9.033 ± 0.234	0.390 ± 0.0623	3.483 ± 0.866
Parameters	GB10^9/l	HGBg/dl	TCMHpg	CCMHg/dl	LYM10^9/l	GRAN1$0^9$/l	MID10^9/l	LYM %	GRA %	MID %
Control	5.917 ± 1.516	13.400 ± 0.473	17.917 ± 0.204	31.967 ± 0.463	4.117 ± 1.023	1.167 ± 0.561	0.633 ± 0.367	70.600 ± 9.725	20.167 ± 7.256	9.233 ± 3.552
Treated	5.550 ± 1.719	12.817 ± 0.722	17.950 ± 0.524	30.750 ± 0.481*	3.783 ± 0.868	1.133 ± 0.592	0.633 ± 0.432	69.650 ± 8.734	20.533 ± 6.287	9.817 ± 3.205

Les valeurs sont présentées en moyenne ± écart type, * significativement différent, P<0.05.

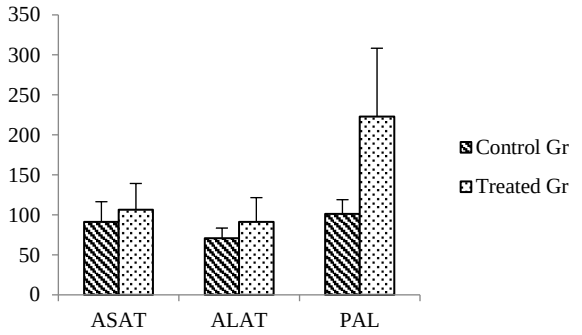

Figure 5. Serum level of "ASAT, ALAT and PAL" of control and treated rats in the conditions of subacute toxicity by the dose of 100 mg/kg of EBMRM.
Values are expressed as mean ± standard deviation, * significantly different, P < 0.05.

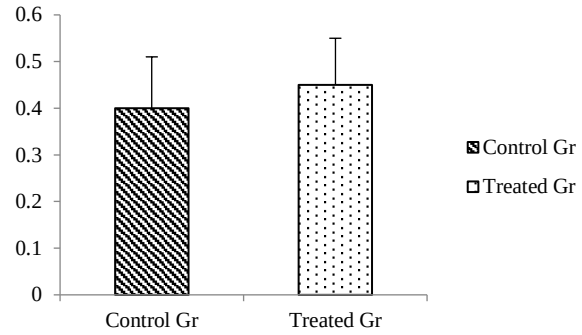

Figure 6. Serum "Urea" levels of control and subacute treated rats under conditions of subacute toxicity conditions with 100 mg/kg EBMRM.
Values are expressed as mean ± standard deviation.

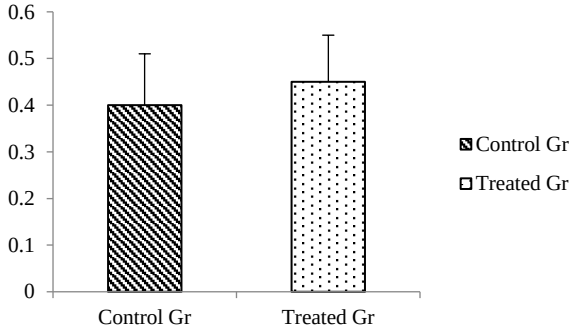

Figure 7. Under subacute toxicity circumstances, serum "creatinine" levels of untreated and treated rats received a dose of 100 mg/kg of EBMRM.
Values are presented as mean ± standard deviation, * significantly different, P < 0.05.

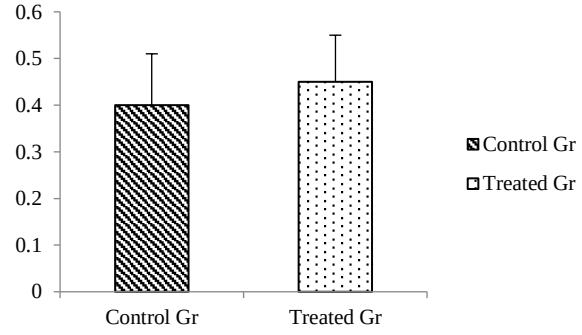

Figure 8. Serum "glucose" levels of control and treated rats were measured under subacute toxic circumstances with a dosage of 100 mg/kg EBMRM.
Values are expressed as mean ± standard deviation

Discussion

The Rutaceae family includes the plant *Ruta montana* L. (Ozenda, 2000). It is well known for its pharmacological effects (Da Silva et al., 2006; Pelletier, 1983), as well as its toxic and particularly anti-fertilizing effects, an emmenagogue, anaphrodisiac and abortive (Masri et al., 2015;Ulubelen et al., 1994).

An 8.43% yield was obtained by extracting the dry powder from the *Ruta montana* L. plant by macerating it in methanol. This yield is lower than that obtained by Ghadjati *et al*. whose raw extract yield was 12.42% (Ghadjati et al., 2022).This variation is likely due to several possible causes, including the maceration period, the chemical composition of the plant, which may vary

depending on the location and time of collection, the age of the plant parts, the nature of the soil, the climate (Brown et al., 2012; Özcan and Chaichat , 2005), as well as the extraction solvent used (Kalt et al., 2001).

The TLC allowed for the separation of several EBMRM constituents, and the Dragendorff reagent revealed the presence of an orange spot, which is likely a coumarin or an alkaloid because coumarins can also produce a weak, non-specific reaction with the reagent (false positive Dragendorff reaction) because of the α,β unsaturated lactone structure (Wagner and Bladt, 1996). This result is not in agreement with the work of Ghadjati et al. where they separated three alkaloids from an extract of total alkaloids of *Ruta montana*, and Touati et al. where they identified 6 alkaloids from *Ruta montana* collected in Morocco using more developed methods like Heteronuclear multiple quantum coherence (HMQC), Heteronuclear Multiple Bond Correlation (HMBC) and MS spectral (Touati et al., 2000). This could be mainly explained by the more developed methods used, the more humid climate and the nature of the soil in Morocco.

Female rats treated with EBMRM under acute toxic conditions displayed a clinical profile that was marked by an accelerated heart rate, which was most likely brought on by the blockage of muscarinic M2 receptors, which resulted in the suppression of vagal tone. The central nervous system (CNS) has likely been damaged by preventing the generation of acetylcholine in the synapses of the CNS, which results in respiratory distress, leg paralysis, and convulsions (Goulle et al., 2004). The majority of the survivors regained a normal appearance up to day 14, while the probable causes of death were respiratory arrest and convulsions. These results are in agreement with those obtained by Ghadjati (Ghadjati *et al*., 2022). The LD_{50} of treated female rats is equal to 393.18 mg/kg following treatment with EBMRM using the Litchfield and Wilcoxon technique in acute toxicity settings. The plant *Ruta montana* L. can be categorized as moderately poisonous using Hodge and Sterner's toxicity categorization system (Frank, 1991).

The first symptoms of a toxic substance are changes in body weight, food consumption and general behavior (Almança et al., 2011). Rats treated with a dose of 100 mg/kg under circumstances of subacute toxicity showed a minor loss of body weight in the first and third weeks, followed by a considerable gain in the fourth. The adverse effects of chemicals and drugs are related to changes in body weight. However, many experts agree that these changes in body weight are due to fat accumulation and physiological adaptation to the plant extracts rather than the toxic effects of the chemicals or drugs causing the animal to eat less because it has no appetite (Kifayatullah et al., 2015).

The relative mass of the organs is a good parameter for indicating whether the organ has been targeted by a drug or not (Hor et al., 2012). The calculation of the relative mass of the organs of the treated and control rats made it possible to detect a significant decrease of 12.58% in the lungs of the treated rats. According to Girish et al., a decrease in the relative mass of the lungs is probably due to pulmonary necrosis caused by the administration of chemical substances (Girish et al., 2009).

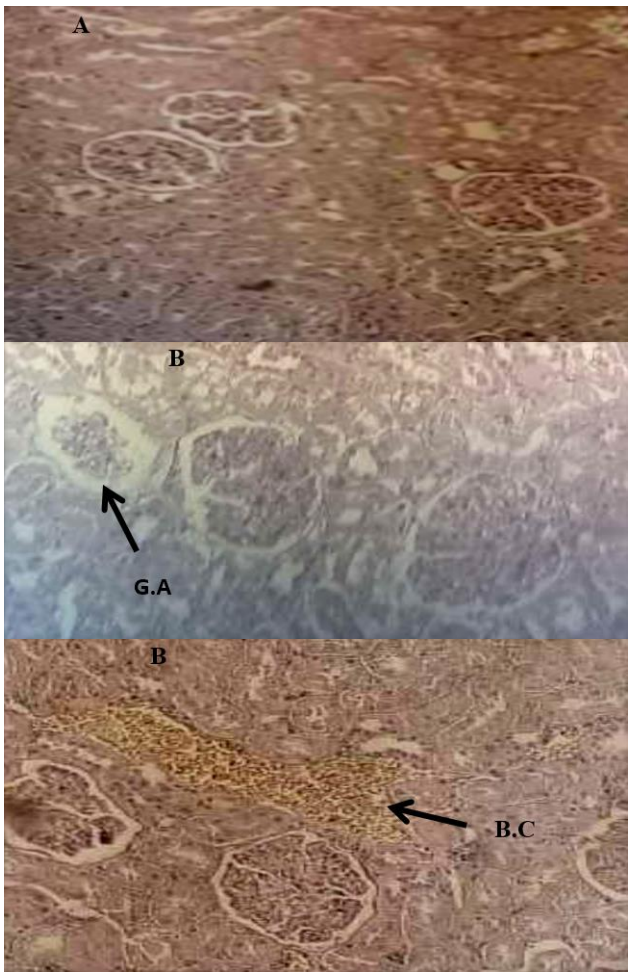

Figure 9. Histological sections of kidney tissue from control (A) and treated (B) rats under subacute EBMRM (100 mg/kg) toxicity conditions.
Glomerular atrophy (G. A) and blood congestion (B.C) (x100). Coloring HE

One of the most vulnerable systems to the effects of toxic substances is the hematopoietic system, which also serves as a crucial indicator of both pathological and physiological health in both humans and animals (Kulkarni et al., 2012). When data are extrapolated from animal research, analysis of blood parameters is important for risk assessment since any alteration in the hematological system is a great indicator of human toxicity (Chandra et al., 2012). MCV and IDRa levels in hematological parameters significantly increased whereas mean corpuscular hemoglobin concentration significantly decreased (MCHC). For the diagnosis of anemia, MCHC and MCV are crucial RBC indices (Voigt, 2000). Additionally, the MCHC parameter indicates the hemoglobin concentration of red blood cells while the VGM parameter gives details on the size and quality of erythrocytes (Nussey et al., 1995). However, the impact of EBMRM on RBCs, may contribute to the decrease in RBC, HGB, and HCT values. If the dosage (>100 mg/kg) or the frequency of the treatment are increased, it can be concluded that EBMRM may have an anemic effect on the blood system. The leakage of cellular enzymes into the plasma is definitely an indication of liver damage. A number of enzymes that are typically found in the cytoplasm of hepatocytes are released into the blood when their plasma membranes are damaged, and their detection in serum serves as a helpful indicator of the kind and severity of hepatotoxicity (Kumar et al., 2004).

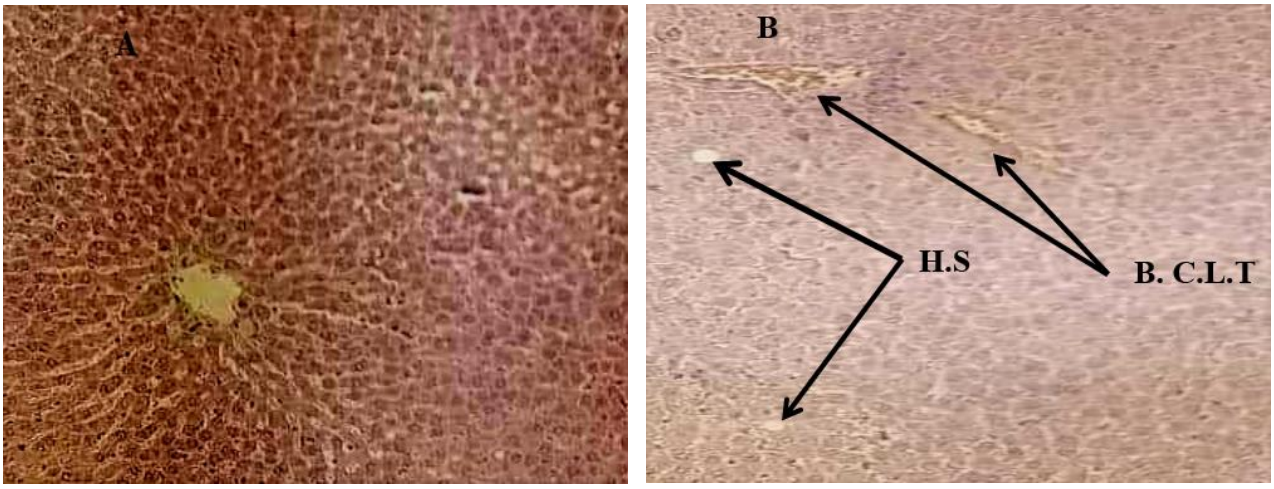

Figure 10. Histological sections of hepatic tissue from control rats (A) and rats treated (B) under the conditions of subacute toxicity by EBMRM (100 mg/kg).
B. C.L.T: Blood congestion in liver tissue. H. S: Hepatic steatosis. (x100). Coloring HE

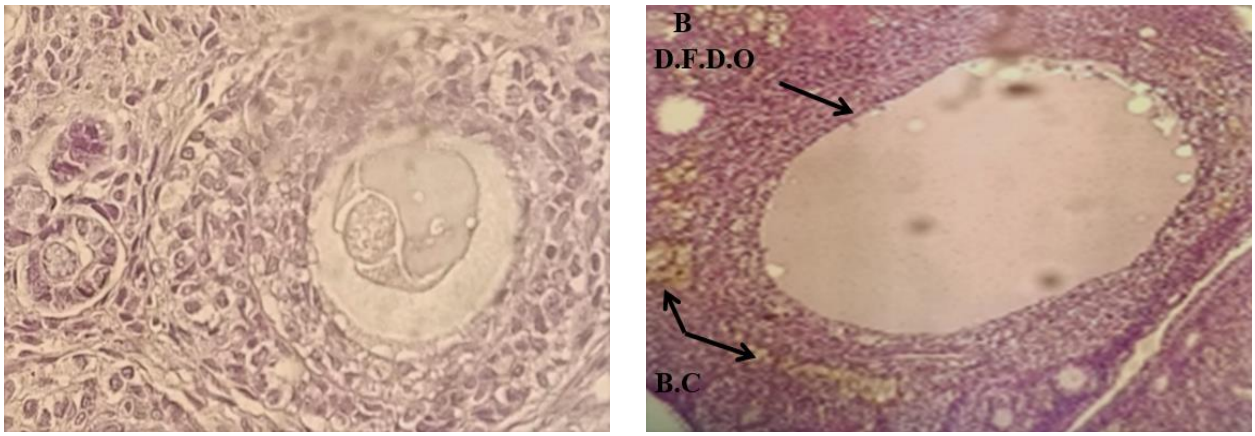

Figure 11. Histological sections of ovarian tissue from control (A) and treated (B) rats in the conditions of subacute toxicity by EBMRM (100 mg/kg).
D.F.D.O: Developed follicle devoid of oocyte. B.C: blood congestion. (x100). Coloring HE

Transaminases (ALAT and ASAT) are enzymes that have significant metabolic activity inside of cells; hence, an increase in their serum levels indicates cell damage, especially in the liver. The cytoplasmic enzyme ALAT is present in the liver in very high concentrations, and an increase in serum levels of this enzyme may indicate hepatocellular damage. But ASAT is an enzyme that is abundantly found in the mitochondria and cytoplasm of numerous organs, including the liver, heart, skeletal muscle, kidney, and brain (Magdy, 2013). Enzymes known as alkaline phosphatases (PAL) are found throughout the body, but are concentrated in the liver, bone, gut, kidneys, and white blood cells. Alkaline phosphatase is released as a result of damage to these organs (Lazare et al., 2011).

The assay of the biochemical parameters in the serum of the treated and control rats made it possible to detect a significant increase in the rate of PAL in the group of treated animals compared with the controls. The increase in PAL can be explained by damage to the bile ducts, i.e. cholestasis (Aragon and Younossi, 2010).

The existence of some hepatic necrosis seen in the histological sections can be used to explain a minor elevation in the serum levels of ALAT and ASAT. Hepatic steatosis, blood clots in the veins and liver tissues, and foci of necrosis were also found during a histopathological analysis of the liver sections. The latter are most probably brought on by an imbalance of lipid metabolism induced by intraperitoneal administration of EBMRM. Additionally, the biochemical analysis allowed for the validation of an increase in blood sugar. Although this could be a result of the stress-related increase in metabolic performance, it does not necessarily mean that there is a problem with glucose metabolism (Landray et al., 2002).

Concentrations of urea, creatinine and electrolytes in serum or urine are used to measure renal function. Serum creatinine is a reliable indicator of renal function (Atsamo et al., 2011).

When comparing treated and control rats, it was shown that treated rats had higher serum urea levels and significantly lower creatinine levels. The decrease in creatinine may reflect both skeletal muscle mass and physical activity level (Baxmann et al., 2008). This may also be explained by the fact that rats rapidly degrade and eliminate alkaloids and their metabolites (Hardman et al., 1998). An increase in the degradation of protein molecules may help to explain the rise in urea (Lullmann et al., 1998). Analysis of renal histological sections revealed some glomerular atrophy and a low level of renal blood congestion. The kidney continued to function normally despite the damage.

When subacute toxicity at a dose of 100 mg/kg was applied to rats, histological sections of their ovaries revealed that there were more mature follicles without oocytes than oocyte-containing follicles. These findings could be explained by how EBMRM affects meiosis, which occurs during oogenesis. These results are in agreement with those obtained by Ghadjati *et al.* (Ghadjati et al., 2022).

Conclusion

The *Ruta montana* L. plant, which is a member of the Rutaceae family, is reportedly harmful to both humans and animals, according to the bibliographic research. It was able to identify the crude methanolic extract of *Ruta montana* L. as a moderately toxic product due to the acute toxicity circumstances observed in female Albino Wistar rats. Several hematological and biochemical parameters were disturbed, including liver and kidney function, in rats given the crude methanolic extract of *Ruta montana* L. at a dose of 100 mg/kg (≈ 1/4 LD_{50}). Histological examination of the liver, kidney, and ovary indicated some structural abnormalities.

References

Almança CCJ, Saldanhab SV, Sousaa DR, Trivilin LO, Nunesa LC, Porfírio LC, Marinhoc B. G. 2011.Toxicological evaluation of acute and sub-chronic ingestion of hydroalcoholic extract of Solanum cernuum Vell in mice. Journal of Ethnopharmacology, 138 (2): 508– 512. doi: 10.1016/j.jep.2011.09.045 .

Aragon G, Younossi ZM. 2010. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. Cleveland Clinic Journal of Medicine, 77 (3): 195-204. doi: 10.3949/ccjm.77a.09064 .

Atsamo AD, Nguelefacka TB, Dattéb JY, Kamanyia A. 2011. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of Erythrina senegalensis DC (Fabaceae) in rodents. Journal of Ethnopharmacology, 134(3): 697–702. doi: 10.1016/j.jep.2011.01.023.

Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP. 2008. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clinical Journal of the American Society of Nephrology, 3(2):348-354. doi: 10.2215/CJN.02870707 .

Bellakhdar J. 2020. La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires. Casablanca : Le Fennec. ISBN 978-9920-755-22-1.

Brown PN, Murch S J, Shipley P. 2012. Phytochemical diversity of cranberry (Vaccinium macrocarponAiton) cultivars by anthocyanin determination and metabolomic profiling with chemometric analysis. Journal of Agricultural and Food Chemistry, 60(1):261- 271. doi: 10.1021/jf2033335.

Chandra P, Sachan N, Kishore K, Ghosh AK. 2012. Acute, sub-chronic oral toxicity studies and evaluation of antiulcer activity of Sooktyn in experimental animals, Journal of Advanced Pharmaceutical Technology & Research, 3(2):117-123. doi: 10.4103/2231-4040.97290.

Da Silva AFS, De Andrade JP, Bevilaqua LRM, De Souza MM, Izquierdo I, Henriques AT. Zuanazzi JAS. 2006. Anxiolytic, antidepressant and anticonvulsant-like effects of the alkaloid montanine isolated from Hippeastrumvittatum. Pharmacology, Biochemistry and Behavior, 85: 148-154. doi:10.1016/j.pbb.2006.07.027.

Diallo A. 2005. Etude de la phytochimie et des activités biologiques de Syzygium guineense Willd (Myrtaceae). PhD thesis in Pharmacy. University of Bamako, Bamako, Mali.

Frank CL. 1991. Toxicologie, données générales procédures d'évaluation, organes cibles, évaluation du risque. Paris : Masson. ISBN : 2-225-82520-3.

Ghedjati N, Mahdeb N, Bouzidi A. 2022. Acute and Subchronic Toxicity Study of Methanol Extract of the Aerial Parts *of Ruta montana* L. on Adult Female Wistar Rats. Tropical Journal of Natural Product Research, 6(8):1203-1209. doi.org/10.26538/tjnpr/v1i4.5.

Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. 2009. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian Journal of Medical Research, 129(5): 569-578. PMID: 19675387.

Goulle JP, Gilbert P, Christian L. 2004. Botanique, chimie et toxicologie des solanacées hallucinogènes: Belladone, Datura, jusquiame, Mandragore. Annales de toxicologie analytique, 16(1): 55- 65. doi:10.1051/ata/2004023.

Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG. 1998. Les bases pharmacologiques de l'utilisation des médicaments, London : McGraw-Hill. ISBN : 2-7042-1326-7.

Heywood VH. 1996. Les plantes à Fleurs. Paris : Nathan. ISBN 2092410563

Hor SY, Ahmad M, Farsi E, Yam MF, Hashim MA, Lim CP, Sadikun A, Asmawi MZ. 2012. Safety assessment of methanol extract of red dragon fruit (Hylocereus polyrhizus): Acute and subchronic toxicity studies. Regulatory Toxicology and Pharmacology, 63:106–114. doi: 10.1016/j.yrtph.2012.03.006.

Kara Ali W, Ihoual S, Abidli N. 2016. Antioxidant and MDR reversal activity in resistant human ovarian cancer cells of methanolic extract from *Ruta montana* located in the North of Algeria. Der Pharma Chemica, 8(12): 215-223. http://derpharmachemica.com/archive.html.

Kalt W, Ryan DAJ, Duy JC, Prior RL, Ehlenfeldt M K, Vander Kloet SP. 2001. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (Vaccinium section Cyanococcus spp.). Journal of Agricultural and Food Chemistry, 49(10): 4761–4767. doi: 10.1021/jf010653e.

Kifayatullah M, Mohd SM, Pinaki S, Moklesur MRS, Arindam D, Sreemoy KD. 2015. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of Pericampylus glaucus (Lam.) Merr. in BALB/c mice. Journal of Acute Disease, 4(4): 309–315. doi: 10.1016/j.joad.2015.06.010.

Kulkarni YA, Veeranjaneyulu A. 2012. Toxicological evaluation of the methanol extract of Gmelina arborea Roxb Bark in mice and rats. Toxicology International, 19(2):125-31. doi: 10.4103/0971-6580.97203.

Kumar G, Sharmila B, Vanitha PP, Sundararajan M, Rajasekara PM. 2004. Hepatoprotective activity of Trianthema portulacastrum L. against paracetamol and thioacetamide intoxication in albino rats. Journal of ethnopharmacology, 92(1): 37-40. doi: 10.1016/j.jep.2003.12.009.

Kurt R.1971. Chromatographie sur couches minces. Paris : Gauthier-Villars.

Lahsissene H, Kahouadj A, Tijane M, Hseini S. 2009. Catalogue des plantes médicinales utilisées dans la région de Zaër (Maroc occidental). Revue de Botanique Lejeunia, N°186. https://popups.uliege.be/0457-4184/index.php?id=710.

Landray MJ, Toescu V, Kendall MJ. 2002. The cardioprotective role of beta-blockers in patients with diabetes mellitus. Journal *of* Clinical Pharmacy *and* Therapeutics, 27(4): 233-242. doi: 10.1046/j.1365-2710.2002.00419.x.

Lazare T, Jacques DY, Michel OA. 2011. Alcoolisation chronique des rats (Rattus norvegicus) de souche Wistar à une eau-de-vie traditionnelle produit en Côte d'Ivoire (Koutoukou). Journal of Applied Biosciences, 41: 2772-2779. ISSN 1997–5902

Litchfield JT, Wilcoxon FA. 1949. A simplified method of evaluating dose effect experiments. Journal of Pharmacology and Experimental Therapeutics, 96(2): 99-113. PMID: 18152921.

Lullmann H, Mohr K, Ziegler A.1998. Atlas de poche de pharmacologie. Paris : Flammarion Médecine - Science. ISBN-10 : 2257121198.

Magdy MEG . 2013. Acute and repeated-doses (28 Days). Toxicity of thymol formulation in male albino rats. Australian Journal of Basic and Applied Sciences, 7(10): 594-601, 2013. ISSN 1991-8178.

Masri W, Belwaer I, Khlifi F, Nouioui A, Ben salah D, Amira D, Hedhili A. 2015. Acute poisoning by *Ruta montana*: A case report, Phytothérapie, 13(1): 36-38. doi.org/10.1007/s10298-014-0903-1.

Nussey GJ, Vuren JHJ, Du Preez HH.1995. Effects of copper on the haematology and osmoregulation of the Mozambicus tilapia, Oreochromis mossambicus (Cichlidae). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 111 (3): 359-367. doi:10.1016/0742-8413(95)00063-1.

Oduola T, Adeniyi F, Ogunyemi E, Bello IS, Idowu T, Subair H. 2007. Toxicity studies on an unripe Carica papaya aqueous extract: biochemical and hematological effects in Wistar albino rats. Journal of. Medicinal Plants Research, 1(1): 001-004. doi.org/10.5897/JMPR.9001254.

Özcan M, Chaichat JC. 2005. Effect of different locations on the chemical composition of essential oils of Laurel (*Laurus nobilis* L.) leaves growing wild in Turkey. Journal of Medicinal Food, 8(3): 408–411. doi: 10.1089/jmf.2005.8.408.

Ozenda P. 2000. Les Végétaux : Organisation et diversité biologiquE. Paris : Dunod. ISBN : 2-10-004684-5.

Pelletier SW. 1983. Alkaloïds, Chemical, Biological Perspectives. London: Pergamon. ISBN: 9780080527000.

Pollio A, De Natale A, Appetiti E, Aliotta G, Touwaide A. 2008. Continuity and change in the mediterranean medical tradition: Ruta spp. (rutaceae) in Hippocratic medicine and present practices, Journal of Ethnopharmacology. 116(3): 469-482. doi.org/10.1016/j.jep.2007.12.013.

Quezel P, Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques et méridionales. Paris : CNRS.

Selamoglu Z. 2018.The Natural Products and Healthy Life. Journal of Traditional Medicine Clinical Naturopathy, 7(2): 1-2. doi: 10.4172/2573-4555.1000e146.

Touati D, Atta-ur-Rahman, Ulubelen A. 2000. Alkaloids from *Ruta montana* .Phytochemistry , 53(2):277-279. doi: 10.1016/s0031-9422(99)00486-0.

Ulubelen A, Ertugrul L, Birman H, Yigit R, Erseven G,. Olgac V. 1994. Antifertility Effects of Some Coumarins Isolated from *Ruta chalepensis* and *Ruta chalepensis* var. Latifolia in Rodents. Phytotherapy Research, 8: 233-236. https://doi.org/10.1002/ptr.2650080409.

Voigt GL. 2000. Anemias Polychythenias. In: Voigt G, Willey (Editors). Hematology Techniques and Concepts for Veterinary Technicians. Iowa: State University Press. pp. 95–101. ISBN: 0813804914, 9780813804910 (Print).

Wagner H, Bladt S. 1996. Plant drug analysis. A thin layer chromatography atlas. Berlin: Springer-Verlag. http://dx.doi.org/10.1007/978-3-642-00574-9.