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Original Research Article

Acute and Subchronic Toxicity Study of Methanol Extract of the Aerial Parts of *Ruta montana* L. on Adult Female Wistar Rats

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ABSTRACT

Ruta montana L. locally known as fidjel belongs to the rutaceae family. This plant is used in traditional medicine as an aphrodisiac, abortive and as hypoglycemic. The aim of this research is to evaluate the mean lethal dose (LD50) and subchronic toxicity of methanol extract of *Ruta montana* L. on female rat. To determine the LD_{50}, 30 female rats were divided into 5 groups; each group received intraperitoneally, a single dose (800, 1000, 1200, 1400 and 1600 mg/kg bw) of the extract, toxicity symptoms and mortality were registered for 14 consecutive days. In subchronic toxicity (45 days), 14 female rats were divided into 2 groups; treated group received intraperitoneally 38,7149 mg/kg bw = 1/30 LD_{50} of the extract and control group received physiological water. Body weight, relative organ masses, reproductive hormones, haematological, biochemical and histopathological parameters were evaluated. The LD_{50} value was calculated to be 1161.4486 mg/kg by Litchfield and Wilcoxon method. In the subchronic study, there was a significant reduction in body weight in the 2nd and 7th week ($P<0.05$). Relative mass of the brain and ovaries registered a significant decrease and increase, respectively ($P<0.05$). Follicle stimulating hormone (FSH), luteinizing hormone (LH) and biochemical parameters did not record significant difference. Haematological parameters presented significant increase and decrease in mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW), respectively ($P<0.05$). The histopathology revealed no abnormalities in different studied tissues. The current study revealed that the methanol extract of *Ruta montana* at the tested dose had no toxic effect on subjected organs.

Keywords: *Ruta montana* L., Female rats, LD_{50}, Subchronic toxicity, Reproductive hormone.

Introduction

Medicinal plants are used for centuries as medications for human illnesses including fertility problems,[1] due to the therapeutic value of their constituents,[2] without the actual knowledge of their toxic potential. Among the problems of the medication by plants; are the imprecise prescribed doses, frequently resulted in overdose.[3]
Medicinal plants are used by more than 80% of people in the word (according to the WHO) for their health problems, where the traditional use, the natural source and the economic side are some reasons for this worldwide utilization.[4] Among these plants we find *Ruta montana* L. *Ruta montana* L. (Rutaceae) is a plant of 20-40 cm,[5] originating in Europe, particularly the mediterranean area, but it is widely distributed in all the moderate and tropical areas.[6]
Ruta montana L. locally known as fidjel,[7] contains many compounds belonging to different chemical classes such as alkaloids, coumarins, flavonoids, essential oils, saponins, triterpenes, phenols and lignans.[8] The richness of this plant by these compounds probably a reason for its large use in traditional medicine.[9] This plant is not used almost any more in Europe; on the other hand it remains a plant appreciated by the tradipraticians in particular in the mediterranean basin and in South America;[10] where it is used as an emmenagogue, an aphrodisiac, abortive,[10-13] also as an antihelmintic,[10] hypoglycemic,[14] antirhematic,[11,15] antipyretic,[16] in the treatment of hepatic diseases,[17] hypertension, [18] and vitiligo.[19]
To our knowledge, no scientific investigations concerning the toxicological studies of *Ruta montana* L. on female rats (albinos wistar) such as the determination of the LD_{50} value and the subchronic study. The purpose of this study is to determine the LD_{50} value and the subchronic toxicity study of the methanol extract of the aerial parts of *R. montana* L. on adult albino wistar female rats.

Materials and Methods

Plant material
The fresh aerial parts of *R. montana* L. was collected in June 2020 from Beni Aziz, a place at North East of Setif (Algeria). Taxonomic identification was done by Dr. Nouiwa W. (Department of Plant Biology and Ecology, Faculty of Nature and Life Sciences, Sétif 1 University, Algeria) (Figure 1). The specimen with voucher number USRB 6-20 was deposited in the department of plant biology and ecology of the same Faculty.
The aerial parts were dried at room temperature in shade for fifteen days; it was manually purified from stems and all impurities and stored in paper bags in the refrigerator at - 4 °C until use.

Plant extraction
Leaves, flowers and fruits of *R. montana* L. was powdered using an electric grinder, the powder (100 g) was macerated with 500 mL of methanol (90%) at room temperature for a week, where the mixture was daily agitated for one hour. The filtrate was evaporated at 30ºC at low pressure.[20]

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Figure 1: (A) The *Ruta montana* L. plant, (B) the fruit of the *Ruta montana* L.

Animals
Adult female rats (albinos wistar) obtained from the Pasteur institute (Algiers- Algeria) were used to evaluate the acute and subchronic toxicity. The ethical approval for the study was obtained from the scientific council of the faculty of Nature and Life Sciences, Sétif 1 university, Algeria).The rats were housed in metal grid cages (55x33x19 cm) and were fed with standard diet and tap water *ad libitium*, however the litter is renewed twice per week. The animals were acclimated to the conditions of the animal room of the faculty of Nature and Life Sciences, Setif 1 University for two weeks prior to the experiments.

Acute toxicity study (Determination of LD_{50})
Adult female rats weighing between 110-155 g were used in this study; the rats were divided into 5 groups each containing 6 rats. The animals were fasted overnight with free access to water prior the treatment and they were weighed just before the injection. Each group received intraperitoneally a single dose (800, 1000, 1200, 1400 and 1600 mg/kg bw) of the methanol extract of *R. montana* dissolved in methanol and solubilized in physiological water, where each rat received a volume of 1 mL/ 200 g bw. The treated rats were observed every hour during the first 24 hours and daily for 14 days to note mortality, signs of toxicity and all changes in their behavior.
The LD_{50} and its confidence interval were calculated using the Litchfield and Wilcoxon method. [21]

Subchronic toxicity study
Adult female Wistar rats weighing between 150-190 g were divided into 2 groups each containing 7 rats; the treated group received daily for 45 days the dose of 38, 7149 mg/kg bw = 1/30 LD_{50} of *R. montana* methanol extract by intra-peritoneal route, the control group received only physiological water with few drops of methanol. After 45 days of treatment, rats were fasted overnight with free access to tap water. On the morning of the 46th day, They were anaesthetized by inhalation of chloroform and blood samples were taken from the orbital vein using haematocrit tubes for the measurement of haematological parameters (EDTA tubes), biochemical parameters and the reproductive hormones (FSH and LH) (Heparin tubes). The organs (kidneys, liver, lungs, spleen, heart, brain and ovaries) were observed macroscopically *in situ*, and then removed, placed in physiological water, degreased, dried and weighed to calculate the relative mass, the kidneys, liver and ovaries were fixed in 10% formalin for histopathological study.[22]

Blood analysis
The haematological parameters such as HGB (haemoglobin), RBC (red blood cells), HCT (haematocrit), MCH (mean corpuscular haemoglobin), RDW (red cell distribution width), MCHC (mean corpuscular haemoglobin concentration), MCV (mean corpuscular volume), WBC (white blood cells) and PLT (platelet) were analyzed with an automatic hematologic analyzer Sysmex coulter XN 350 (Kits- Sulfolyser, fluorocell, cellpack, lysercell, Germany). The analysis of the biochemical parameters including UREA (urea), CREAT (creatinine), GPT (glutamate-pyruvate transaminase), GOT (glutamate-oxaloacetate transaminase) (Kits- biosystems sa costa brava 30 Barcelona, Spain), ALP (alkaline phosphatase) (Kits- Spinreact S.A.U, Spain) and bilirubin (Kits- Biolabo, France) were evaluated with Biosystem apparatus. For the hormonology; LH (luteinizing hormone) and FSH (follicle stimulating hormone) dosages were carried by automatic analyzer Vidas (Kits- Biomerieux, France).

Histopathological analysis
The kidneys, liver and ovaries fixed in 10 % formalin were dehydrated, embedded in paraffin, and after routine histological procedures, 4 µm sections were mounted on slides and stained with hematoxylin and eosin (H&E) for reading with an optical microscope.[22]

Statistical analysis
The obtained results were statistically analyzed by Sigma Stat 3.5 software using the one-way ANOVA test followed by the Tukey test, where differences were considered significant at $p < 0.05$. The results were expressed as the mean value ± standard deviation.

Results and Discussion

The most of new treatments were originally derived from ancient herbal traditions,[2] in the world, about 25% of modern drugs developed from plants.[23] Plants contain a diversity of metabolites, some of which may be useful or very toxic to humans, [24] mentioning that pharmaceutical drugs are therapeutic at one dose and toxic at another.[23] In order to ensure safety, toxicity tests are useful in obtaining information on the different biologic activities of a substance and understanding its mechanisms of action. In addition, the evolution of medicaments and the rise of their therapeutic potentiels are essentially based on the toxicological evaluation studies.[24]
Ruta montana locally known as Fidjel,[7] belongs to the rutaceae family.[25] This plant contains above the phytochemicals compounds such as alkaloids, flavonoids, essential oils, coumarins and phenols, [19,26] which make it widely used in traditional medicine; especially as an emmenagogue, anaphrodisiac and abortive, but also it is known for its toxic properties where The most frequent intoxication cases by it are observed following abortion essay during which the plant is administered either in the form of drinkable decoction, or in the form of vaginal enemas.[14,19] The extraction by maceration of the aerial parts of *R. montana* L.in methanol gave a crude extract in the form of a sticking paste of a green color with an extraction yield of 12.4258 %. The yield of total phytochemical content of a plant is significantly affected by the characteristics of extracting solvents, among this, the solvent polarity, which is an important parameter. So that the higher the polarity is the better the solubility of compounds.[27] Previous investigations show that the methanol extracts more phytoconstituents than another solvent. In this study methanol 90% gave a yield more than obtained by,[28] this difference is probably due to the environmental conditions where it grew, or the harvest period.[29]
The LD_{50} of a given substance is the quantity that induces mortality of 50% of a tested group of animals, habitually mice or rats, treated by a specific route. It is presented as the amount of administered substance (mg) per the body weight of the tested animal (100g of small animals and kg for greater subjects).[30] The simple tool, that's frequently used to get general information on the toxicity of a chemical substance; is the determination of the LD_{50}.[31] The intra-peritoneal administration of the methanol extract of *R. montana* L., caused different symptoms including: Erect hairs, difficulty of walk (paralysis), regrouping in the corner of the cage, tachycardia, difficulty in breathing followed by cyanosis. Mortality was recorded from the first hour of treatment (after almost 15 minutes) up to the 24th hour. For the survived animals, the symptoms were regressed gradually, and after 24 hours, normal behavior was observed compared to that of the controls. The toxicity was observed to be a dose-dependent phenomenon (see Table 1, Figure 2).

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Table 1: Dose-effect of the crude methanol extract of the aerial parts of *Ruta montana* in female rats treated intraperitoneally.

Dose	No. of deaths /	Log dose	Observed effect		Expected effect		% difference	$\chi 2$
mg/kg	group		%	Probit	%	Probit		
800	0/6	2.9030	0.5	2.42	1.4	2.8	0.9	0.006
1000	1/6	3	16.7	4.03	18.5	4.1	1.8	0.002
1200	4/6	3.0791	66.6	5.43	58.1	5.2	8.5	0.03
1400	5/6	3.1461	83.3	5.97	86.5	6.1	3.2	0.0085
1600	6/6	3.2041	98.6	7.20	95.8	6.73	2.8	0.018

Table 2: Relative organ masses of female rats controls and treated under the conditions of subchronic toxicity with 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana* L.

	Liver	kidney	Heart	Spleen	Brain	Lung	Ovaries
Treated Group	$0.0309 \pm 2.01e^{-3}$	$0.00669 \pm 4.41e^{-4}$	$0.00332 \pm 3.09e^{-4}$	$0.00294 \pm 7.52e^{-4}$	$0.00825* \pm 4.05e^{-4}$	$0.007 \pm 1.04e^{-3}$	$0.000819* \pm 1.69e^{-4}$
Control Group	$0.0314 \pm 3.54e^{-3}$	$0.00672 \pm 5.09e^{-4}$	$0.00323 \pm 2.07e^{-4}$	$0.00285 \pm 5.02e^{-4}$	$0.00988 \pm 5.95e^{-4}$	$0.00735 \pm 8.33e^{-4}$	$0.000602 \pm 4.86e^{-5}$

The values are presented as mean ± SD. * significantly different for (P<0.05).

Table 3: The hematologic parameters values of female rats controls and treated under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta Montana*.

	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	IDRa	PLT	PDW	MPV	WBC	P- LCR
	10^{12}/l	g/dl	%	Fl	pg	g/dl	%	fl	10^9/l	fl	Fl	10^9/l	%
Control group	7.33 ± 0.19	13 ± 0.231	40.41 ± 0.51	55.14 ± 1.78	17.74 ± 0.54	32.19 ± 0.35	15.63 ± 0.76	35.3 ± 0.66	711 ± 90.30	8.74 ± 0.17	5.54 ± 0.13	4.66 ± 1.86	2.8 ± 0.54
Treated group	7.00 ± 0.42	12.84 ± 0.75	39.87 ± 2.77	56.96 ± 1.88	18.36* ± 0.55	32.24 ± 0.48	14.54* ± 0.44	35.57 ± 1.31	618 ± 67.19	8.83 ± 0.26	5.58 ± 0.22	4.84 ± 2.27	3.6 ± 1.30

The values are presented as mean ± SD. * significantly different for (P < 0.05).

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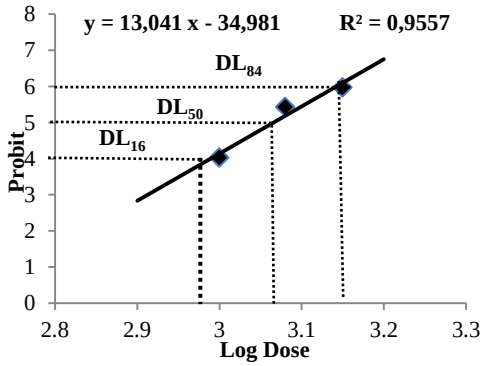

Figure 2: Straight line for determining the LD_{50} in female rats treated intraperitoneally with the methanol extract of *Ruta montana* L. by the method of Litchfield and Wilcoxon (1949).

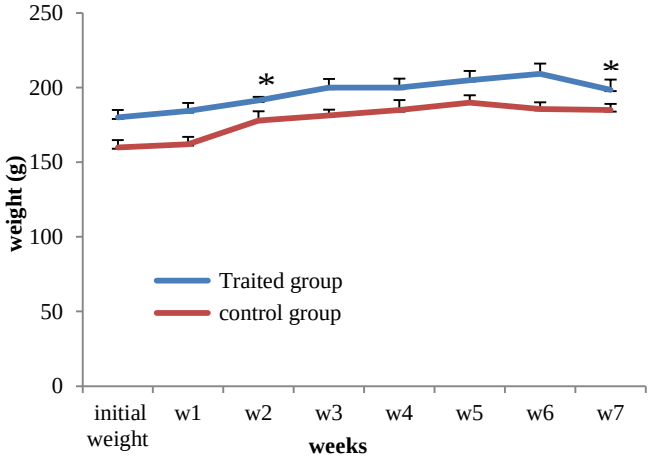

Figure 3: The body weight (g) evolution of female rats controls and treated under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana*. The values are presented as mean ± SD. * significantly different for ($P<0.05$).

χ2 of the right: Σ cont. χ2.N / K = 0.0645.30 / 5 = 0.387. (N / K: average number of animals per dose, N: total number of animals, K: total number of doses).

χ2 theoretical: for the probability threshold p = 0.05 and for the number of degrees of freedom n = 5-2 (total number of doses-2) is 7.82. Therefore χ2 experimental < χ2 theoretical.

The LD_{50} value of the extract was calculated to be 1161.4486 mg/kg, DL_{16} = 977.2372 mg/kg and DL_{84} = 1380.3842 mg/kg. In this experiment the value of the LD_{50} of the extract was between 500 and 5000 mg/kg and according to, [31] our extract is considered as moderately toxic substance, and as slightly toxic substance according to.[32] Advanced subchronic toxicity studies in animal model can contribute in the prevision of the toxic effect of the plant extracts, from which, a possible interpolation of the response may be correlated with humans. Also, it can give an idea about the target organ.[33] The female albinos wistar rats treated with 38.7149 mg/kg = 1/30 LD_{50} of the methanol extract of *Ruta montana* plant under the subchronic conditions toxicity didn't show any sign of toxicity during 45 days of observation. Also the main aspect of toxicity investigation is formed by the rate of mortality.[24] In our work there was no mortality in subchronic treatment. The apparent marker of the harmful effects of chemical substances is the body weight changes.[34] The body weight evolution of the treated female rats recorded a significant reduction in the 2nd and the 7th week of 7.63 % and 7.34 % respectively in comparison with the control group, however the weeks leftovers knew a normal evolution. The control group presented a normal evolution in its weight, see figure 3. This reduction, was probably due to the administered extract; which may provoke an anorexia and disorders in the metabolism of carbohydrates, fat or proteins as proposed by,[35] or may be explained by the interaction between tannins (including ellagitannins) present in the methanol extract of the aerial parts of *R. montana*, [28,36] and proteins. This interaction inhibits the digestion of endogenous protein, resulting in weight reduction.[37]

Another important indicator of pathological and physiological states in animals is the organ weight. [27] Macroscopically, no abnormalities were found in the internal organs in all the rats, but statistically a significant reduction in the relative mass of the brain of 16.5% and a significant rise in the relative mass of the ovaries of 36.05% in treated rats in comparison with the control group see table 2. As reported by,[38] that the number of ovary corpora lutea or edema can affect the ovary weight. In our study, treated rats ovaries revealed the absence of edema or other histological abnormalities, so the increased relative mass of ovaries may be due to the number of corpora lutea in the ovary. The kidneys and liver are important organs in the waste excretion and in metabolism, respectively. To assess the toxicity of any compounds a biochemical analysis is carried out because it is the main tool to verify the state of these two essential organs.[4] Cases of hepatic and renal toxicity were registered after administration of phytotherapeutic products.[39] The evaluation of the kidney function is generally based on the determination of the level of creatinine and urea in the blood.

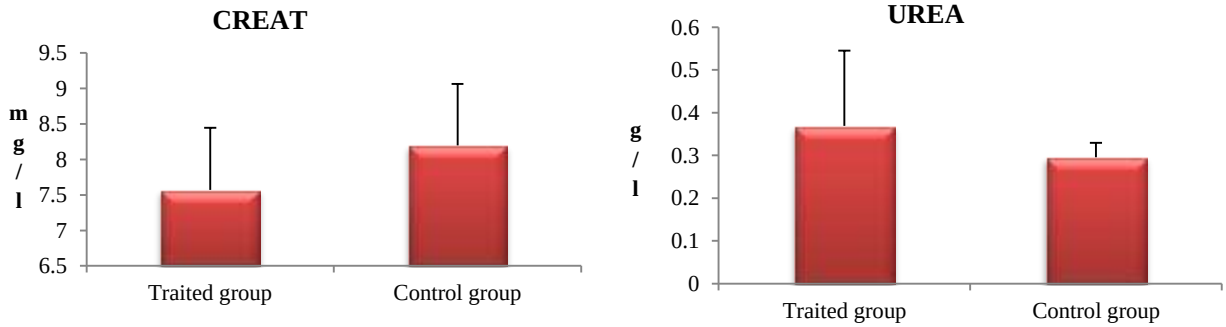

Figure 4: Effect of *Ruta montana* methanol extract on creatinine and urea in female rats in subchronic toxicity. The values are presented as mean ± SD.

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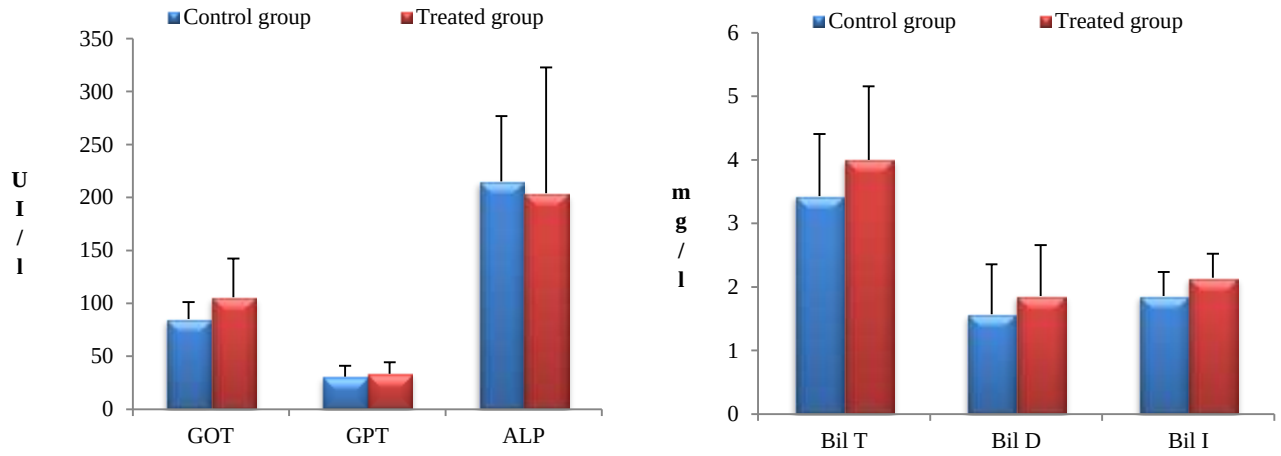

Figure 5: Effect of *Ruta montana* methanol extract on some biochemical parameters of the evaluation of the hepatic function in female rats in subchronic toxicity. The values are presented as mean ± SD.

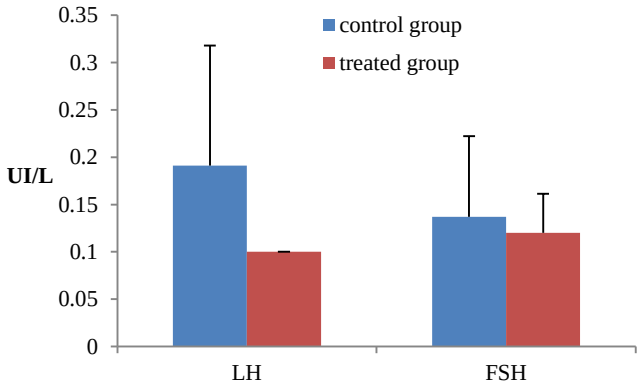

Figure 6: Serum rate of FSH and LH of female rats controls and treated under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana*. The values are presented as mean ± SD.

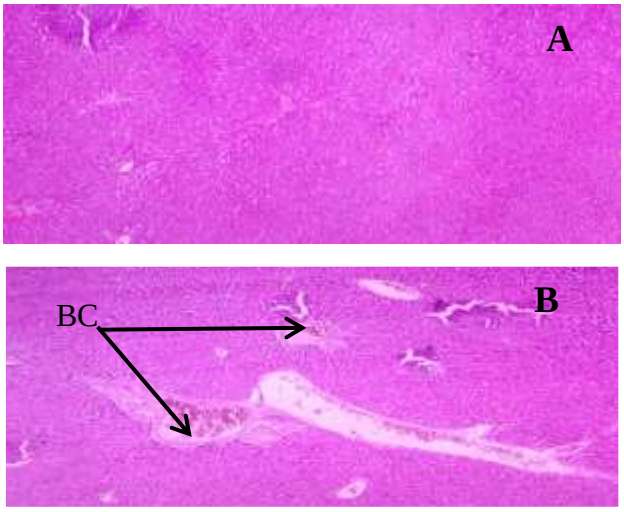

Figure 7: The histological sections of liver (x40) of female rats controls (A) and treated under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana* (B). BC: blood congestion. (H & E stain).

These parameters, in cases of chronic or acute renal toxicity, are frequently increased to quadruple compared to the normal value of control animals.[39] The biochemical parameters of the evaluation of the renal function were presented in figure 4, no significant difference was found in the concentrations of urea and creatinine, so this extract didn't affect the kidney function and this was confirmed by the absence of abnormalities in the histological study except the presence of some blood congestions (Figure 7). These confirm the absence of renal toxicity. As show figure 5 biochemical parameters of the estimation of the liver function like ALP for the bile duct alterations, the GOT and GPT for the integrity of hepatocytes and bilirubin for the liver function,[37] did not show significant changes following administration of *R. montana* methanol extract. Moreover the absence of histopathological changes in the liver (except the presence of some blood congestions) (Figure 8) confirms that the extract has no hepatotoxic potential. LH and FSH are glycoprotein hormones released from anterior pituitary gland.[42] They are important regulators of ovarian follicular growth and differentiation,[43] where the former controls the estradiol production in ovarian theca cells and the latter regulates the development and the maturation of the follicles in the ovaries, so the increased FSH concentration may elevate the number of the follicles and the weight of ovaries.[44] Reduction in the LH and FSH values in treated animals may be probably due to the inhibitory effect of the extract on the hypothalamus or the anterior pituitary.[45] The regulation of these two hormones liberation is primary realized by gonadotropin releasing hormone (GnRH) released from the hypothalamus,[45] and also by steroid hormones such as estrogens.[44] The values of FSH and LH (reproductive hormones) dosages did not show significant difference after subchronic treatment (see figure 6), so the *Ruta montana* methanol extract had no effect on the levels of FSH and LH. The histology of ovaries indicated a normal ovarian parenchyma (Figure 9), but regrettably, the determination of corpus luteum number was not possible. Based on the obtained results we suggest that the extract did not affect the hypothalamus-pituitary-ovary axis at this dose. The hematopoietic system is an essential indicator of pathological and physiological status in humans and animals also is a very sensitive target of poisonous compounds.[40] Haematological parameters are the pertinent indicators for the risk assessment.[23] The MCHC and the MCV are indices of RBC, which are used in anemia determination in most animals,[40] where the first one with MCH express the haemoglobin concentration while the second one reveals the RBCs size. The HGB and the packed cell volume (PCV) are other blood indices related with the RBCs total population. The haematocrit or the PCV designates the RBCs proportion in the blood and used in anemia diagnosis. The abnormal reduction or increase in the platelets rate causes hemorrhage or clotting respectively.[23] According to,[41] modifications of the haematological system possess a great prediction importance for human toxicity when the data are extrapolated from animal studies.

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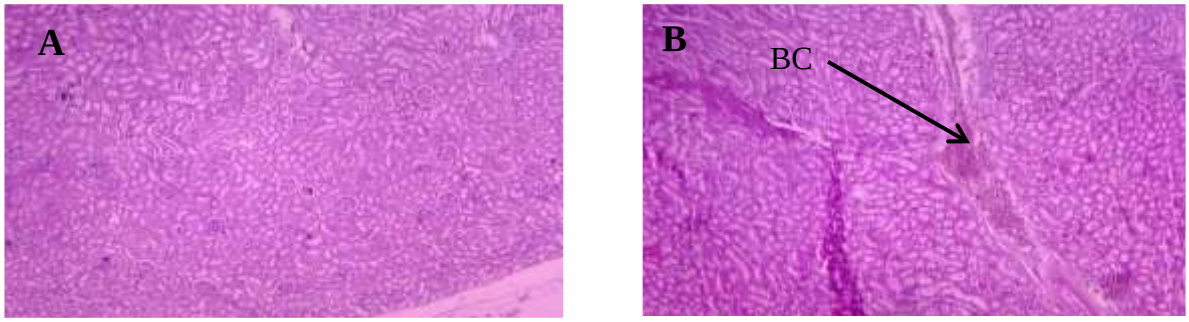

Figure 8: The histological sections of kidney (x40) of female rats controls (A) and treated under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana* (B). BC: blood congestion. (H & E stain).

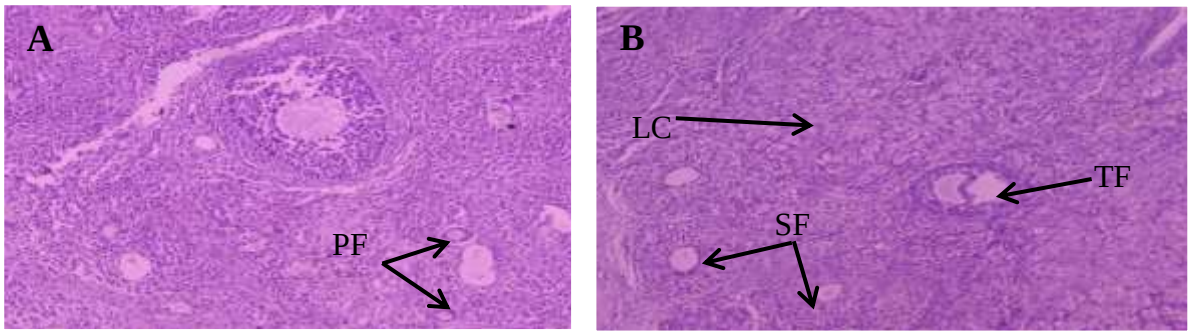

Figure 9: The histological sections of ovaries of female rats controls (A) (x40) and treated (x100) under the subchronic toxicity conditions by the dose of 38.7149 mg/kg = 1/30 LD_{50} of the crude methanol extract of the aerial parts of *Ruta montana* (B). PF: Primary follicle; SF: Secondary follicle; TF: tertiary follicle; CL: Corpus luteum. (H & E stain).

The values of the different haematological parameters are mentioned in table 3. There was a significant increase in the value of MCH of 3.46 % and a significant decrease in the percentage of RDW of 6.95 % in treated rats in comparison with the control group. However the other parameters didn't mark significant difference. According to our results, the *Ruta montana* methanol extract, at this studied dose, probably will not provoke any abnormalities such as bone marrow suppression, anemia or hemorrhage in humans.

Conclusion

According to the results, it seems that the *R. montana* methanol extract according to its LD_{50} in female rats is considered as moderately toxic. The subchronic study of this extract at the dose of 38.7149 mg/kg = 1/30 LD_{50} in female rats had no marked effects on haematological parameters, ovogenesis and liver and kidney functions.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Almasad MM, Qazan WS, Daradka H. Reproductive toxic effects of Artemisia herba alba ingestion in female Spague-dawley rats. Pak J Biol Sci. 2007; 10(18):3158-3161.
2. Qazan WS. Effects of short and long term treatment of *Ballota undulate* on female albino rats fertility and pregnancy. Pak J Biol Sci. 2008; 11(4):638-642.
3. Usman MM, Sule MS, Gwarzo MY. Toxicological studies of aqueous root extract of Euphorbia lateriflora: (Schum and Thonn) in rats. J Med Plants Stud. 2014; 2(2):58-62.
4. Kpemissi M, Metowogo K, Melilaa M, Veerapurc VP, Negru M, Taulescu M, Potarniche AV, Suhas DS, Puneeth TA, Vijayakumar S, Gadegbeku KE, Aklikokou K. Acute and subchronic oral toxicity assessments of Combretum micranthum (Combretaceae) in Wistar rats. Toxicol Rep. 2020; 7:162-168.
5. Bonnier G and Douin R. The great flora in color by Gaston Bonnier. Bellin: Paris; 1990; 205p.
6. Harsha SN and Latha BV. *In vitro* antioxidant and in vitro anti-inflammatory activity of *Ruta graveolens* methanol extract. Asian J Pharm Clin Res. 2011; 5(1):32-35.
7. Bouzidi MA, Faraoun F, Meliani H, Boubekeur I, Kralifa M, Zattal Z. Characterization of the soil occupied by Ruta genus species in Tessala mountain (north-west of Algeria). Lab. Technol. 2012; 7(28):79-84.
8. Zellagui A, Belkassam A, Belaidi A, Gherraf N. Environmental impact on the chemical composition and yield of essential oils of Algerian *Ruta montana* (Clus.) L and their antioxidant and antibacterial activities. Adv Environ Biol. 2012; 6(10):2684-2688.
9. Kara Ali W, Ihoual S, Abidli N. 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. 2016; 8(12):215-223.
10. Masri W, Belwaer I, Khlifi F, Nouioui A, Ben salah D, Amira D, Hedhili A. Acute poisoning by *Ruta montana*: A case report. Phytothérapie. 2015; 13(1):36-38.

11. Bellakhdar J, Claisse R, Fleurentin J, Younos C. Reportory of standard herbal drugs in the Moroccan pharmacopoeia. J Ethnopharmacol. 1991; 35(2):123-143.
12. Kambouche N, Merah B, Bellahouel S, Bouayed J, Dicko A, Derdour A, Younos C, Soulimani R. Chemical composition and antioxidant potential of *Ruta montana* L. Essential oil from Algeria. J Med Food. 2008; 11(3):593-595.
13. Pollio A, De Natale A, Appetiti E, Aliotta G, Touwaide A. Continuity and change in the mediterranean medical tradition: Ruta spp. (rutaceae) in Hippocratic medicine and present practices. J Ethnopharmacol. 2008; 116(3):469-482.
14. Benkhnigue O, Ben Akka F, Salhi S, Fadli M, Douira A, Zidane L. Catalog of medicinal plants used in the treatment of diabetes in the region of Al Haouz-Rhamna (Morocco). J Anim Plant Sci. 2014; 23(1):3539-3568.
15. Ouarghidi A, Gary JM, Abbad A. Botanical identification and ethno-medicinal uses of some underground part of medicinal plants collected and traded in Marrakech region. J Med Plants Res. 2013; 7(29):2165-2169.
16. Bnouham M, Mekhfi H, Legssyer A, Ziyyat A. Medicinal plants used in the treatment of diabetes in Morocco. Int J Diabetes Metab. 2002; 10(1):33-50.
17. Lakhdar L. Evaluation of the antibacterial activity of Moroccan essential oils on aggregatibacter actinomycetemcomitans: in vitro study. Mohamed V University of Rabat. 2015; 18p.
18. Eddouks M, Maghrani M, Lemhadri A, Ouahidi ML, Jouad H. Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). J Ethnopharmacol. 2002; 82(2-3):97-103.
19. Daoudi A, Hrouk H, Belaidi R, Slimani I, Ibijbijen J, Nassiri L. Valorization of *Ruta montana* and *Ruta chalepensis*: Ethnobotanical study, phytochemical screening and Antibacterial activity. J Mater Environ Sci. 2016; 7(3):926-935.
20. Sofowora A. Medicinal plants and traditional medicine of Africa. Karthala; 2010; 25p.
21. Litchfield JRJT, Wilcoxon f. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther. 1949; 96 (2):99-113.
22. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological Stains: A literature review and case study. Glob J Health Sci. 2015; 8(3):72-79.
23. Alelign T, Chalchisa D, Fekadu N, Solomon D, Sisay T, Debella A, Petros B. Evaluation of acute and sub-acute toxicity of selected traditional antiurolithiatic medicinal plant extracts in wistar albino rats. Toxicol Rep. 2020; 7:1356-1365.
24. Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in wistar rats. Toxicol Rep. 2019; 6:161-175.
25. Takhtajan A. Flowering Plants. (2nd ed). Springer: Russia; 2009; xli p.
26. Hammiche V, Merad R, Azzouz M. Poisonous plants for medicinal use around the Mediterranean. Springer: Paris; 2013; 212p.
27. Ogbuehi IH, Ebong OO, Obianime AW. Oral acute toxicity (LD_{50}) study of different solvent extracts of *Abrus precatorius* Linn leaves in wistar rats. Eur J Exp Biol. 2015; 5(1):18-25.
28. Allouni R. Study of the morphological, phytochemical and pharmacotoxicological aspects of the *Ruta montana* plant. Farhet Abbas, Setif University.2018; 60p.
29. Chollet S, Papet Y, Mura P, Brunet B. Determination of atropine and scopolamine contents in wild and ornamental varieties of Datura. Ann Anal Toxicol. 2010; 22 (4):173-179.
30. Gadanya AM, Sule MS, Atiku MK. Acute toxicity study of "gadagi" tea on rats. Bayero J Pure Appl Sci. 2011; 4 (2):147-149.
31. Zbinden G and Flury-Roversi M. Significance of the LD_{50} test for the toxicological evaluation of chemical substances. Arch Toxicol. 1981; 47(2):77-99.
32. Loomis TA and Hayes WA. Loomis's Essentials of Toxicology. (4th ed). Academic Press: London; 1996; 25p.
33. Sharif HB, Mukhtar MD, Mustapha Y, Gabi B, Lawal AO. Acute and Subchronic Toxicity Profile of *Euphorbia pulcherrima* methanol extract on wistar Albino rats. Adv Pharm. 2015; 2015:1-9.
34. Tédong L, Dzeufiet PDD, Dimo T, Asongalem AE, Sokeng DS, Flejou JF, Callard P, Kamtchouing P. Acute and subchronic toxicity of Anacardium occidentale linn (anacardiaceae) leaves hexane extract in mice. Afr J Trad CAM. 2007; 4 (2):140-147.
35. Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of *Acorus calamus* L. rhizome in experimental animal models. J Trad Compl Med. 2016; 6(4):431-436.
36. Benziane MM. Phytochemical screening of the *Ruta montana* plant. Extraction of essential oil and rutin. Antioxidant activity of the plant. Es-Senia University of Oran. 2007; 48p.
37. Patel C, Dadhaniya P, Hingorani L, Soni MG. Safety assessment of pomegranate fruit extract: Acute and subchronic toxicity studies. Food Chem Toxicol. 2008; 46 (8):2728-2735.
38. Rockett JC, Narotsky MG, Thompson KE, Thillainadarajah I, Blystone CR, Goetz AK, Rena H, Best DS, Murrell RN, Nichols HP, Schmid JE, Wolf DC, Dix D J. Effect of conazole fungicides on reproductive development in the female rat. Reprod Toxicol. 2006; 22(4):647-658.
39. Arsad SS, Esa NM, Hamzah H, Othman F. Evaluation of acute, subacute and subchronic oral toxicity of *Rhaphidophora decursiva* (Roxb.) Schott extract in male Sprague Dawley rats. J Med Plant Res. 2013; 7(41):3030-3040.
40. Amna FO, Nooraain H, Noriham A, Azizah AH, Husna NR. Acute and oral subacute toxicity study of ethanolic extract of *Cosmos caudatus* leaf in sprague dawley rats. Int J Biosci Biochem Bioinforma. 2013; 3(4):301-305.
41. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. 2000; 32(1):56-67.
42. Jashni HK, Jahromi HK, Ranjbary AG, Jahromi ZK, Kherameh ZK. Effects of aqueous extract from *Asparagus officinalis* L. roots on hypothalamic-pituitary-gonadal axis hormone levels and the number of ovarian follicles in adult rats. Int J Reprod BioMed. 2016; 14(2):75-80.
43. Yaghmaei P, Parivar K, Jalalvand F. Effect of Imatinib on the oogenesis and pituitary –ovary hormonal axis in female Wistar rat. Int J Fertil Steril. 2009; 3(1):11-16.
44. Monsees TK and Opuwari CS. Effect of rooibos (*Aspalathus linearis*) on the female rat reproductive tract and liver and kidney functions *in vivo*. S Afr J Bot. 2017; 110:208-215.
45. Lilaram and Raichur AN. Effect of ethanolic seed extract of *Caesalpinia bonducella* on female reproductive system of albino rat: a focus on antifertility efficacy. Asian Pac J Trop Dis. 2012; 2:S957-S962.