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

R Allouni1*, H Guergour1,2, N Mahdeb1, M Omrane3, A Bouzidi1

1Departement of Biochemistry, Faculty of natural sciences and life, University Ferhat Abbas, Setif19000, Algeria
2Departement of Biology, Faculty of natural sciences and life, University Elbachir El Ibrahimi, Bordj Bou Arriridj 34000, Algeria
3Hospitalo-Universitary Centre, CHU of Setif, Algeria

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ABSTRACT

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

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

INTRODUCTION

Medicinal plants were one of the only sources of cure for many diseases, including more than 80 per cent of the population of the countries in the process of development and almost exclusively used in traditional medicine for their primary health needs. In addition, around 25% of the prescriptions worldwide are herbal2. Algeria has a vegetable flora rich and diversified, among the medicinal plants that constitute the vegetal cover, is the genus Ruta belongs to the family Rurtaceae, this last is widely distributed especially in the mountainous regions. Many species of this type are used in traditional medicine because they contain several molecules endowed with therapeutic activities; among the most well-known species are *Ruta montana*, commonly called Fidjel; Plant with yellow flowers, strong odor, ornamental, aromatic, known for its richness in secondary metabolites. Phytochemical studies indicate the presence of various compounds: essential oils (aliphatic ketones)9, coumarins (rutarin, furanocoumarins: psoralen, bergapten, xantotoxin) Furoquinoines and acridone derivatives)24, flavonoids (rutside) and tannins2,6. This plant widely used as a medicinal plant for its abortifacient and anti-fertility properties15, antispasmodic, analgesic, antiinflammatory, emmenagogue and antiparasitic2. However, the use of plants as a remedy does not mean that they are always beneficial to human health, and following the observations of Masri et al., 2015 of a case of acute poisoning by *Ruta montana* where the toxicological analysis of the urine shows a strong presence of alkaloids, starting from theirs works, we try to evaluate the lethal dose 50 and the acute toxicity of the total alkaloids from the aerial parts of *Ruta montana* in male mice.

MATERIALS AND METHODS

Plant collection and identification

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

Extraction of totals alkaloids

50 g of air dried powdered of aerial parts was defatted with 200 ml of petroleum ether under reflux, and then the powder were witted with 40 ml of NH4OH (0.5N) for 24 h, and were extracted to exhaustion with CHCl3 using a soxhlet apparatus for 5 h. The organic extract (containing free alkaloids with lipophilic impurities) is then shaken three times with 150 ml aqueous sulphuric acid (0.5N). The acid extracts (alkaloids salts) are treated three times with 50 ml NH4OH(0.5N) to pH 9 to liberate the free alkaloids which are separated by extraction with 150 ml CH2Cl2, and then dried with Na2SO4 and concentrated to dryness under reduced pressure to obtain crude alkaloids3. The yield of this extract was approximately 0.27 ± 0.02% (w/w).

*Author for Correspondence: ryme87@yahoo.fr*
Experimental animals
Male Swiss albino mice of weights 22-32g, they were purchased from the Pasteur Institute in Algiers, were used to evaluate the lethal dose50, for acute studies. The mice were housed in hanging transparent plastic cages (55 × 33 × 19 cm) in the animal room. The animals were fed with a standard pellet and tap water ad libitum. The animals are acclimated to the conditions of the animal room for two weeks prior to the experiments.

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

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

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

Blood analysis
The hematological components including white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) were determined using the automated hematology analyzer (Medonic).

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

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

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

RESULTS
Lethal Dose 50
The intra-peritoneal administration of total alkaloids extract of aerial parts of *Ruta montana* characterized by severe clinical symptoms, including decrease in locomotors activity with paralysis of the hind legs, cyanosis and tachycardia. Though there were deaths 15th minute to 24 hours; however, the animals which survived exhibited a normal behavior, similar to the animals of the control group. The toxicity was observed to be a dose-dependent phenomenon. The LD50 value of the extracts was calculated to be 389.04 mg/kg (See Tab. 1, Fig. 2).

\[ \chi^2 \]

\[ n= 4, \text{ so theoretical } \chi^2 = 9.49 \text{ therefore experimental } \chi^2 < \text{ theoretical } \chi^2. \]

LD16= 226.46 mg/kg, LD50= 389.04 mg/kg, LDs4= 512.86 mg/kg

Acute toxicity
In acute toxicity studies, the intra-peritoneal administration in Swiss albino mice of single dose of 129.68 mg/kg body weight of alkaloid extract showed no mortality or abnormal behavior. As compared to control group, no biological significant effects of total alkaloids extract were noted on body weight gain (See Tab. 2) In addition, no significant pathological changes in the colors and textures of vital organs, including the liver, kidney, brain, heart, lung, spleen and testes were observed via macroscopic examination, but statistically there were significant decrease in the relative weight of the lungs of the mice sacrificed after 24h and 5 days with also significant decrease in the relative weight of liver of the mice sacrificed after 5 days as showed in Table 3. The haematological parameters of the mice treated with total alkaloid extract are presented in Table 4. There was a statistically no significant change in the values of
Table 2: Body weights of male mice treated with 129.68 mg/kg of total alkaloids extract from aerial parts of *Ruta montana*. Values are mean ± SEM.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Groups</th>
<th>1st Day</th>
<th>5th Day</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>30 ± 2.062</td>
<td>32.45 ± 1.054</td>
<td>2.1 ± 0.352</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.28 ± 2.036</td>
<td>32.45 ± 1.054</td>
<td>2.1 ± 0.352</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30 ± 0.597</td>
<td>32 ± 1.658</td>
<td>1.9 ± 0.213</td>
</tr>
</tbody>
</table>

DISCUSSION

*Ruta montana* has been used in traditional medicine against stomach ailment, respiratory and liver diseases\(^{16}\), in digestive disorders and helminthiasis, it’s traditionally known for its abortive and aphrodisiac effects\(^{9,14}\), emmenagogue\(^{13}\), as disinfectant; antipyretic and pest-destroying\(^{10}\). In fact, their therapeutic properties are due to the presence of thousands of secondary metabolites in particular essential oils and alkaloids\(^2\). However, no study dealing with the safety assessment of this popular food and medicinal plant is reported. Therefore, the acute intraperitonial toxicity of the total alkaloids extract of the aerial parts of *Ruta montana* was carried out in order to evaluate its safety.

Sigma chi squared = 0.1687, theoretical value of chi squared for P = 0.05 correspond to n[Number of degrees of freedom = K (Number of doses) -2].
In this study we determined the lethal dose 50 and we tested the acute toxicity of the total alkaloids extract from aerial parts of *Ruta montana* in male mice. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis or toxicologic classification of substances. Under these conditions oral or parenteral administration of a single dose of *Ruta montana* was 389.04 mg/kg and a LD50 was obtained with an acute toxicity test. When oral administration of *Ruta montana* was repeated, the LD50 value was 129.68 mg/kg, therefore, the acute toxicity study, the alkaloid extract from aerial parts of *Ruta montana* was found to be safe in mice. In this study, no significant changes were observed in the relative organ weight of lungs (after 24 h and 5 days) and liver (after 5 days) were significantly decreased, therefore the decrease in the liver-body weight ratio in groups receiving extract may be attributed to atrophy. Hematological and biochemical parameters are the main diagnostic criteria in clinical practice. The values of some hematological and biochemical parameters indicate the adverse effects of drugs on organs or systems. The haematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in humans and animals. In this study, no significant differences were found in some hematological parameters at the end of the treatment as compared with control group. Thus, the results suggested that the total alkaloid administrated by intra-peritoneal route at 129.68 mg/kg has no influence on the system of blood in male mice. Alkaline phosphatases are often used to assess the integrity of plasma membrane and endoplasmic reticulum. ALAT is a sensitive and specific marker of hepatocellular affect is exclusively present in the cytoplasm of hepatocytes and released into the circulation after cellular damage. ASAT is less sensitive and less specific than ALAT for the liver; it is also found in other organs, especially in the skeletal muscles, heart muscle and brain while PAL, this enzyme is found especially in the liver and bones. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood. Therefore, no changes in ASAT and ALP activities but there are a considerable change in ALAT on first day and

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lungs</th>
<th>Heart</th>
<th>Brain</th>
<th>Spleen</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0464±0.0019</td>
<td>0.0122±0.0008</td>
<td>0.0074±0.0002</td>
<td>0.0054±0.0001</td>
<td>0.0129±0.0001</td>
<td>0.0054±0.0001</td>
<td>0.0074±0.0002</td>
</tr>
<tr>
<td>1st day</td>
<td>0.0493±0.0037</td>
<td>0.0135±0.0011</td>
<td>0.0066±0.0001*</td>
<td>0.0048±0.001</td>
<td>0.0137±0.0001</td>
<td>0.0051±0.0004</td>
<td>0.0068±0.0004</td>
</tr>
<tr>
<td>5th day</td>
<td>0.0255±0.0069*</td>
<td>0.0129±0.0005</td>
<td>0.0061±0.0001***</td>
<td>0.0116±0.000</td>
<td>0.0135±0.0049</td>
<td>0.0067±0.0004</td>
<td>0.0064±0.0004</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Teste</th>
<th>RBC</th>
<th>MCV</th>
<th>RDW</th>
<th>HCT</th>
<th>PLT</th>
<th>MPV</th>
<th>WBC</th>
<th>HGB</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^6/mm³</td>
<td>µm³</td>
<td>%</td>
<td>%</td>
<td>10³/mm³</td>
<td>µm³</td>
<td>3/mm³</td>
<td>g/dL</td>
<td>pg</td>
<td>pg</td>
</tr>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.66 ± 0.5187</td>
<td>42.75 ± 0.6756</td>
<td>20.83 ± 1.791</td>
<td>32.75 ± 2.293</td>
<td>471 ± 1.571</td>
<td>8.733 ± 1.038</td>
<td>8.1 ± 0.3177</td>
<td>11.22 ± 0.9864</td>
<td>14.98 ± 2.371</td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>7.863 ± 0.6623</td>
<td>42.25 ± 0.6457</td>
<td>19.52 ± 3.487</td>
<td>33.45 ± 5.985</td>
<td>443.2 ± 0.4743</td>
<td>7.217 ± 0.8083</td>
<td>12.18 ± 0.6814</td>
<td>15.68 ± 0.6058</td>
<td>37.42 ± 2.259</td>
<td></td>
</tr>
<tr>
<td>5th day</td>
<td>7.225 ± 0.3034</td>
<td>42.73 ± 0.7775</td>
<td>19.85 ± 0.4264</td>
<td>30.90 ± 3.642</td>
<td>582.3 ± 3.4175</td>
<td>6.583 ± 0.2777</td>
<td>11.10 ± 0.4740</td>
<td>10.73 ± 1.393</td>
<td>14.90 ± 34.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>
reverses on the 5th day, as known ALT is considered the most liver-specific enzyme because it is present in higher quantities in hepatocyte cytosol. Therefore, its presence in the serum is considered a marker of hepatocellular necrosis.

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

However, the results of acute study showed no adverse effects on the usual markers of liver and kidney toxicity (ASAT, ALAT, ALP, Urea and Creatinine). It may be concluded that the alkaloid extracts of Ruta montana aerial parts did not induce significant damage to these organs. The liver and kidney are sensitive organs, and their functions are known to be affected by a number of factors, such as drugs, including plant phytochemicals, ultimately leading to renal failure and liver toxicity.

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

![Graph showing biochemical parameters of liver function](image1)

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

![Graph showing biochemical parameters of kidney function](image2)

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

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

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

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

CONCLUSION

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

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