World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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A chemical and toxicological study of the tuber extracts of *Rhoicissus revoilii* Planch. (Vitaceae): A Comorian plant used as a pesticide

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World Journal of Advanced Research and Reviews, 2025, 26(03), 1764-1777

Publication history: Received on 07 May 2025; revised on 14 June 2025; accepted on 16 June 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.3.2359

Abstract

This study aimed to assess the acute toxicity of *Rhoicissus revoilii* Planch tuber extracts. This plant from the Vitaceae family is used to treat various pathologies and as a pesticide in the Comoros. From dried tuber powder previously treated with hexane, a methanolic extract (ME) was obtained with a yield of 20.33%. Phytochemical screening of the powder revealed the presence of flavonoids, tannins, polyphenols, coumarins, triterpenes and steroids. The toxicity of ME was assessed in mice and cold-blooded animals. In mice, symptoms of intoxication by the intraperitoneal (i.p.) and oral routes mainly included decreased motor activity, muzzle itching, diarrhoea, and increased respiratory rate. The LD_{50} (24 h) of ME in mice was estimated to be between 21.29 and 22.3 mg/kg body weight (b.w.) by the i.p. route. By the oral route, it was estimated to be between 273 and 275 mg/kg b.w. When administered by these two routes, ME caused dose-dependent histopathological lesions in the liver, lungs and kidneys. The LC_{50}s were estimated to be 7.491 µg/ml, 8.055 µg/ml and 685 µg/ml, respectively, for alvins, legless frog tadpoles and mosquito larvae at stage three of development.

Keywords: *Rhoicissus revoilii*; Vitaceae; Tubers; Toxicity; Pesticide

1. Introduction

Rodents represent a significant threat worldwide, causing substantial economic losses and considerable inconvenience to humans. These small mammals, including rats and mice, cause significant damage to various sectors, including agriculture, public health, the food industry and construction [1, 2]. On farms, rodents damage crops by nibbling them, leading to a significant drop in food production and considerable financial losses. Not only do they damage property, they also carry a number of serious infectious diseases, such as leptospirosis, salmonellosis and plague. They often transmit these diseases through their excrement, urine or the parasites they harbour [3] posing a real threat to public health.

To deal with this threat, various methods of rodent control have emerged, ranging from traps to chemical products [4]. However, these approaches often pose problems regarding effectiveness, cost, and environmental impact. It is therefore

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crucial to explore new natural and sustainable alternatives for controlling these pests. Certain plants, traditionally used for their repellent properties, could provide a more ecological and less intrusive solution.

Rhoicissus revoilii Planch. (Vitaceae), a plant widely grown in the Comoros, is recognised not only for its potential as a natural pesticide (mousicide, rat poison), but also for its therapeutic properties in the Comoros and in East Africa such as Kenya and Tanzania [5, 6, 7, 8].

The main objective of this study was to assess the toxic effects of methanolic extract of *Rhoicissus revoilii* tubers on various animals.

2. Material and methods

2.1. Materials

2.1.1. The plant

(Source: the authors)

Figure 1 *Rhoicissus revoilii*: a) Whole plant; b) Twigs; c) Leaves and fruits; d) Tubers

Rhoicissus revoilii Planch., one of the 12 species of the *Rhoicissus* genus, is a woody climber with tendrils that can reach heights of between one and ten metres. Its trifoliate leaves are dark green shiny and have entire margins. The leaflets are elliptical and asymmetrical and may be hairless or hairy. Its edible fruits are reddish to black in colour, rounded, and two-lobed [8].

Tubers are generally fleshy. They have a rough, brownish to beige skin and white or yellowish flesh.

This plant is distributed in East Africa (Ethiopia, Sudan, Democratic Republic of Congo, Zambia, Zimbabwe, Mozambique, South Africa), as well as in Ghana, the Comoros, Saudi Arabia and Yemen [8].

In the Comoros, it is known by the vernacular names Trambamajji, Pumboubissa and Mhamoussi.

The plant (Figure 1) was harvested in May in Chaoueni, in the southeast of Anjouan Island, 60 km from Mutsamudu, at the following geographical coordinates: 12°35'61“ S; 44°50'13“ E.

Identification was carried out at the Malagasy Institute of Applied Research (IMRA) by Rakotonirina Benja.

2.1.2. Animals used in experiments

The mice

White *Mus musculus* OF1 mice were used for the experiments. Five-week-old male and female mice weighing an average of 25 ± 2 g were selected.

Frog tadpoles

Legless frog tadpoles *(Ptychadena mascareniensis)* were used in the experiments. They were captured in bodies of water around the university campus three days before the test so that they could acclimatise to laboratory conditions.

Carp alvins

The carp alvins *(Cyprinus carpio)* were supplied by an approved fish farmer. They ranged in size from 3 to 5 cm and weighed an average of 3 g. They were placed in an aquarium in the laboratory for three days to acclimatise.

Mosquito larvae

The larvae of the mosquito (*Culex quinquefasciatus*), in stage 3 of their development, were captured on the day of the test in the stagnant water around the university campus.

2.2. Methods

2.2.1. Preparation of study material

The tubers were dug up, washed, sliced into 4 mm-thick pieces and dried for 72 h in a well-ventilated area. The cossettes obtained were then reduced to a powder and stored in airtight jars at room temperature.

2.2.2. Preparation of the extract

The exhaustion extraction method was used to extract the plant's toxic principles.

The powder was stripped with hexane (at a ratio of 1:10 w/v) until complete discoloration was achieved. To do this, the powder was suspended in the solvent and the mixture was subjected to magnetic stirring at room temperature for 3 h [9, 10].

The dried pomace was then exhausted with methanol following the same extraction procedure. After filtration, the supernatant from each extraction was evaporated at low pressure and 40°C using a rotary evaporator. The dry residue, dissolved in 10 ml of distilled water, constituted the methanolic extract (ME).

2.2.3. Phytochemical screening

The reactions used to detect chemical groups in tuber powder and ME were those developed by Marini-Bettolo *et al*. [11] and Fong *et al*. [12].

2.2.4. Toxicological study

Study of the effects of ME on mice

Two routes of administration were used to assess toxicity in mice: intraperitoneal (i.p.) and oral. For each 25 g b.w., 0.3 ml of extract was administered, after which the animals were observed for 24 h. Mice given a physiological solution (0.9% NaCl) served as negative controls.

Both routes of administration were used in acute toxicity tests. These tests included determining symptoms of intoxication, acute toxicity indices, and anatomo-pathological lesions.

Acute toxicity indices (LD_0, LD_{50} and LD_{100}) for ME in mice were determined using the methods of Reed and Muench [13] and Boyd [14].

2.2.5. Anatomopathological study

Any mice that developed symptoms of intoxication were sacrificed. Their organs were then removed and fixed in a 10% formaldehyde solution before being embedded in paraffin. Histological sections of 3 µm were taken and stained using the standard haematoxylin-eosin method [15]. The slides were prepared using a technique developed by Hould [16] and Diebold *et al.* [17]. They were observed under a light microscope (10x and 40x magnification).

Study of the effects on cold-blooded animals

The tests carried out on cold-blooded aquatic animals (carp alvins, frog tadpoles and mosquito larvae) followed the protocols described by Razanatseheno *et al*. [18]. The LC_{50} (24 h) was determined by testing different concentrations. The results were analysed using ANOVA with GraphPad Prism 7 software. Statistical values were expressed with 95% confidence intervals.

3. Results

3.1. Extraction and purification process

The tuber powder extraction process is shown in Figure 2.

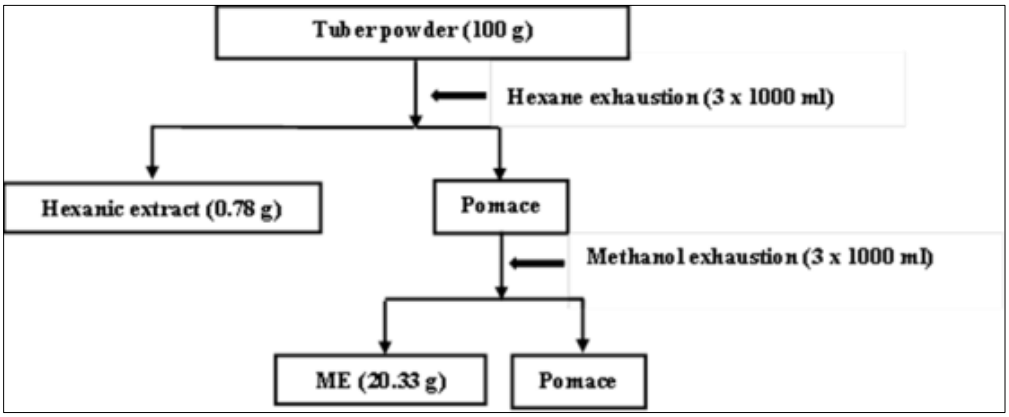

Figure 2 Summary diagram of the preparation of the ME extract

3.2. Phytochemical screening results

The results of the phytochemical screening of the tuber powder and ME are shown in Table 1.

Table 1 Phytochemical screening of *Rhoicissus revoilii* tuber powder and ME

Chemical group	Detection reaction	Tuber powder	ME
Alcaloïds	Mayer test	-	-
	Wagner test	-	-
	Dragendorff test	-	-
Flavonoids	Flavonols	+	+
	Flavones	+	+
	Flavonones	+	+
Tannins	1% gelatin test	+	+
	10% salted gelatin test	+	+
Polyphenols	10% $FeCl_3$ test	+	+
Leucoanthocyanins	Bate-Smith	-	-

Coumarins	NaOH (hot)	+	+
Triterpenes	Liebermann-Burchard test	+	-
Steroids		+	+
Iridoids	HCl (hot)	-	-
Saponins	Foam test	-	-

+: positive; -: negative

Table 1 showed that flavonoids, tannins, polyphenols, coumarins and steroids were present in the tuber powder and in the ME, while triterpenes were only detected in the tuber powder.

3.3. Effects of extracts on mice

3.3.1. Symptoms of intoxication and influence of administration routes

The symptoms of intoxication observed included itching around the muzzle, reduced motor activity, diarrhoea, paralysis of the limbs and increased respiratory rate. These symptoms were the same for both routes of administration, with the exception of abdominal contortions and polyuria (i.p. route) and convulsions (oral route). The results depended on the route of administration. At LD_{100}, mice died after 20 min by the i.p. route and after 47 min by the oral route.

3.3.2. Determination of LD_{50} (24 h)

The acute toxicity indices (LD_0, LD_{50} and LD_{100}) of ME in mice via i.p. and oral administration are presented in Table 2. Seven increasing doses ranging from 12.6 mg/kg to 48 mg/kg were used for the i.p. route, and six dose increments ranging from 75 mg/kg to 600 mg/kg were used for the oral route.

Table 2 Acute intraperitoneal and oral toxicity of ME in mice

Lethal dose (LD)	i.p. route (mg/kg)	Oral route (mg/kg)
LD_0	12.6	75
LD_{50}	21.29-22.3	273-275

3.3.3. Anatomopathological lesions in mice

After dissecting the animals, the organs were observed macroscopically and then examined under the microscope to detect any lesions or anatomical changes. The effects of ME on organs (brain, lungs, heart, liver, small intestine, kidneys) were studied for the two routes of administration at different doses. A control group of untreated mice was included for comparison.

Macroscopic observations

No macroscopic changes were observed when the harvested organs were examined.

Microscopic observations

Lesions observed after 24 h by i.p. route

The main lesions caused by 2 doses of ME (12.6 and 15.6 mg/kg b.w.) on each organ are summarised in Table 3 and illustrated in Figure 3.

Table 3 Lesions caused by 2 doses of ME in mice by i.p. route (24 h)

Organs	**Untreated mice**	**Observed lesions**	
		LD_0 (12.6 mg/kg)	**Sub-lethal dose (15.6 mg/kg)**
Brain	Normal aspect	Normal structure	Normal aspect
Lungs	Normal aspect	- Dilated inter-alveolar blood vessels - Neutrophils in the parenchyma - Neutrophil infiltration (oedema)	- Dilated inter-alveolar blood vessels - Presence of alveolar macrophages loaded with haemosiderin - Presence of red blood cells in the alveoli - Haemorrhagic sheets
Heart	Normal aspect	Normal aspect	Normal aspect
Liver	Normal aspect	No steatosis or inflammation	- Inflammatory infiltrate - Dilated capillaries - Presence of neutrophils - Ruptured sinusoids
Small intestine	Normal aspect	Normal aspect	Normal aspect
Kidneys	Normal aspect	- Dilated glomerular capillaries - Presence of neutrophils	- Inflammation of the interstitium and tubules - Glomerular infiltrate - Mesangial proliferation

Lesions were particularly marked in the lungs (haemorrhagia), kidneys and liver (inflammation), while the brain and small intestine were histologically normal.

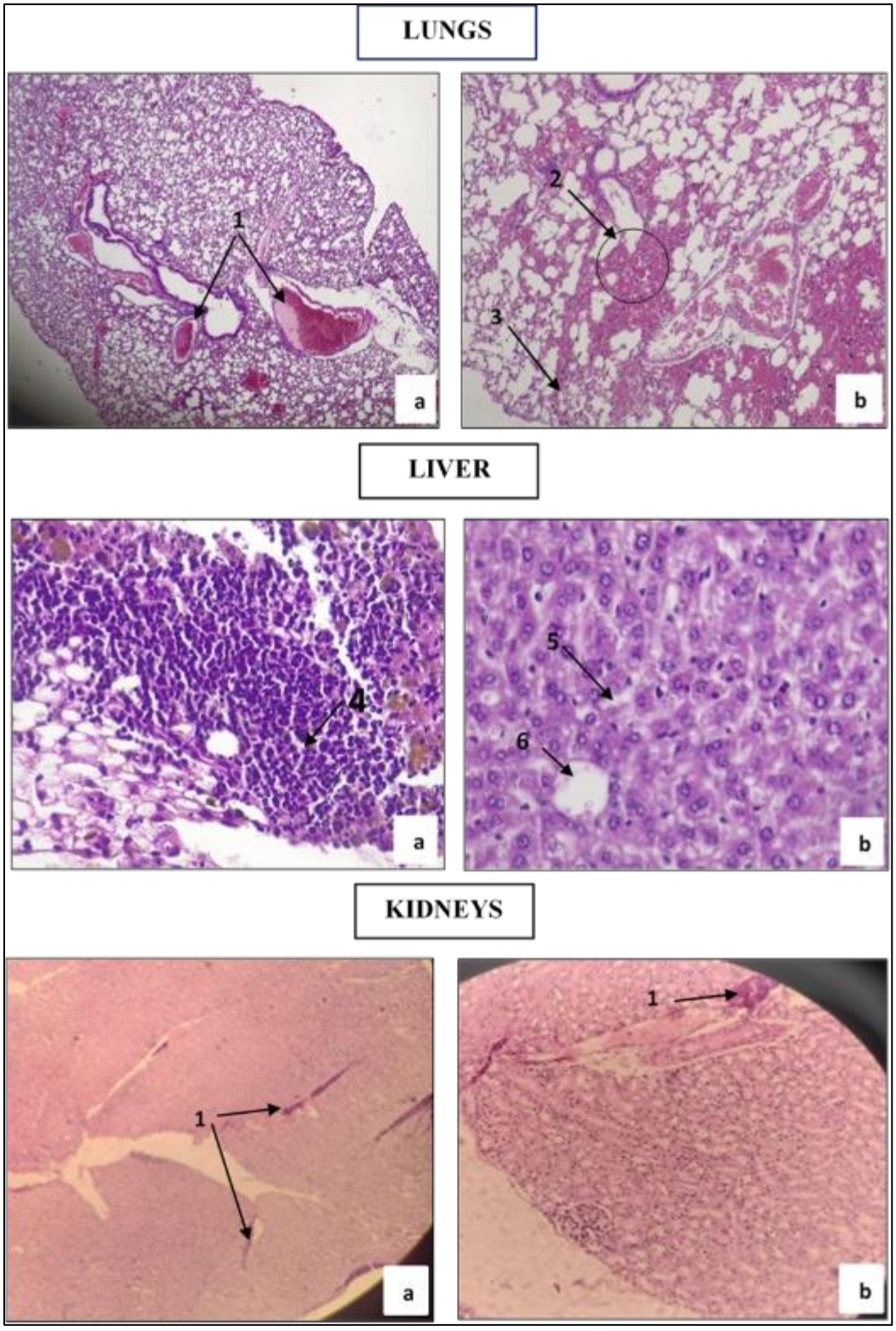

Figure 3 Lesions caused by ME at a dose of 15.6 mg/kg (b.w.) by i.p. route in the lungs, liver and kidneys (a: 10x magnification; b: 40x magnification)

1: Dilated vessels; 2: Haemorrhagic foci; 3: Destroyed alveoli; 4: Neutrophils; 5: Dilated sinusoidal capillary; 6: Dilated centrilobular vein; Lesions observed after 24 h by oral route

Two doses of ME corresponding to LD_0 (75 mg/kg) and LD_{100} (600 mg/kg) were administered orally over a 24 h period. Table 4 below shows the histopathological lesions observed following these treatments.

Table 4 Lesions caused by different doses of ME on mice taken orally (24 h)

Organs	Untreated mice	Observed lesions	
		LD_0 (75 mg/kg)	**LD_{100} (600 mg/kg)**
Brain	Normal aspect	Normal structure	Histologically normal
Lungs	Normal aspect	- Dilated inter-alveolar blood vessels - neutrophils in the parenchyma - neutrophilic infiltrate (oedema)	- Dilated blood vessels - Alveolar oedema (lymphocytic) - Dense lymphocytic cellular infiltrate - Congested capillaries and venules - Proliferation of lymphocytes and macrophages - Dilated inter-alveolar blood vessels - Neutrophils in the parenchyma - Haemorrhagic layer
Heart	Normal aspect	Normal aspect	Normal aspect
Liver	Normal aspect	Normal aspect	- Intracellular lipid accumulation - Hepatocyte necrosis
Small intestine	Normal aspect	Normal aspect	Normal aspect
Kidneys	Normal aspect	Normal aspect	- Interstitial inflammatory infiltrate - Lymphocytic infiltration between tubular epithelial cells - Interstitial oedema - Dilated glomerular capillaries - Neutrophils

These results show that a dose effect was noted. The lesions observed were more severe at 600 mg/kg than at 75 mg/kg. In the lungs, haemorrhagia and proliferation of lymphocytes, macrophages and polymorphs were observed at the highest dose. In the liver, hepatocyte necrosis was also observed at the same dose. In the kidneys, inflammatory infiltrates associated with interstitial oedema were found. At the lowest dose (LD_0), the changes were limited to dilatation of the vessels in the lungs. There were no significant effects on other organs, notably the brain, heart and small intestine.

Figure 4 Illustrates the different tissue lesions induced by oral administration of ME at 600 mg/kg

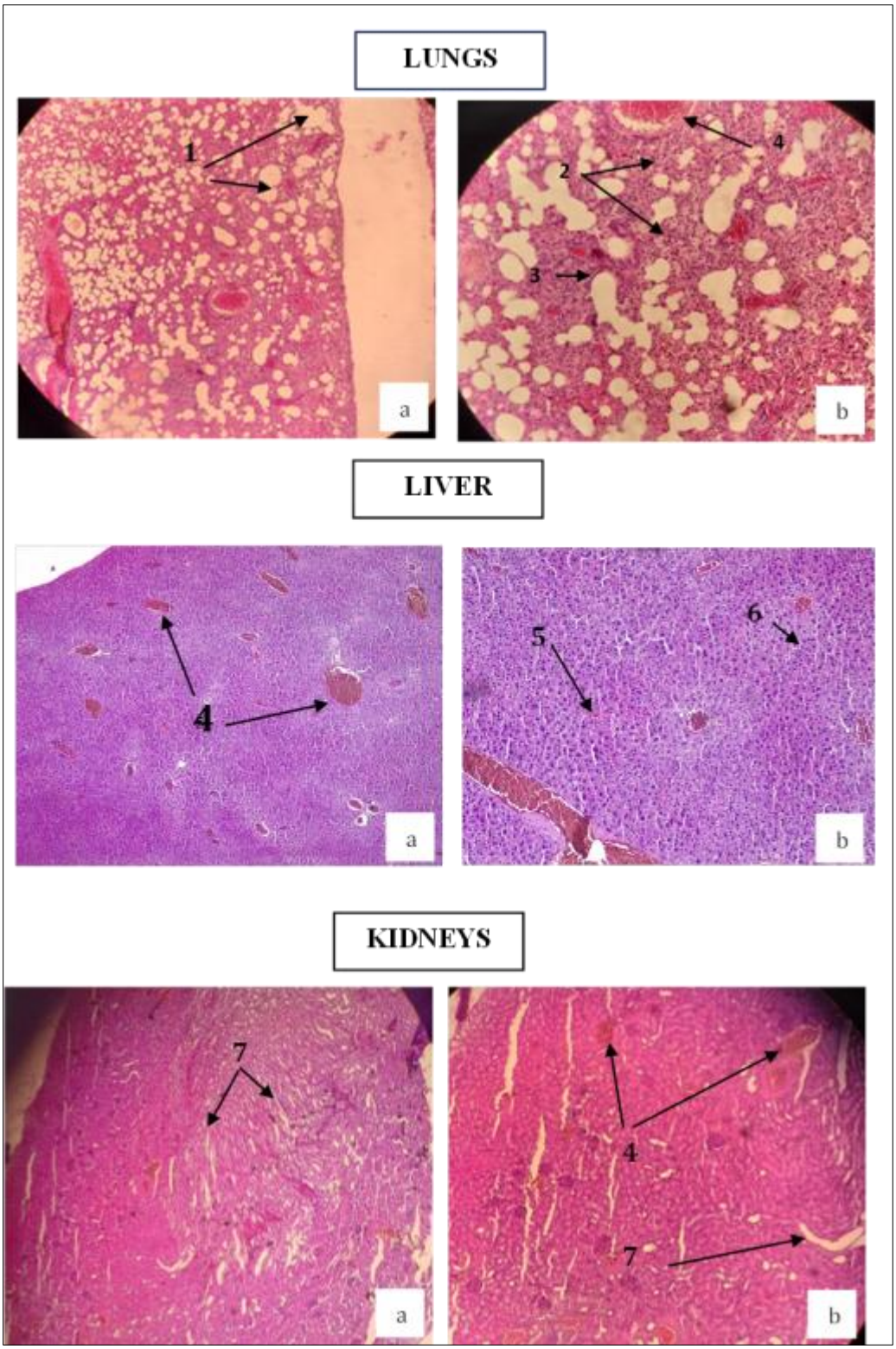

Figure 4 Lesions caused by ME at a dose of 600 mg/kg (b.w.) orally in the lungs, liver and kidneys (a: 10x magnification; b: 40x magnification)

1: Dilated alveolus; 2: Inflammatory infiltrate; 3: Thickened bronchiolar wall; 4: Dilated blood vessel; 5: Dilated sinusoidal capillaries; 6: Hepatocytic cords; 7: Tubular dilatation

3.4. Effects of methanolic extract on cold-blooded animals

3.4.1. Effects on carp alvins

Six increasing concentrations of ME ranging from 5 to 9.21 µg/ml with a geometric reason of r =1.12, were tested on six batches of five alvins. The LC_{50} of ME in fish alvins was estimated at 7.491 µg/ml (Figure 5).

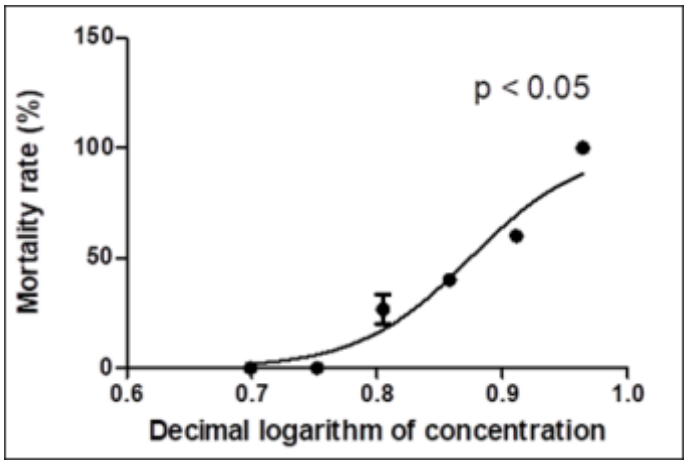

Figure 5 Dose effect and mortality caused by ME on carp alvins

3.4.2. Effects on frog tadpoles

Seven concentrations of ME in geometric progression with reason r of 1.12 ranging from 5 to 10.4 µg/ml were tested on seven batches of five tadpoles. The LC_{50} of ME on the tadpoles was estimated to be 8.055 µg/ml (Figure 6).

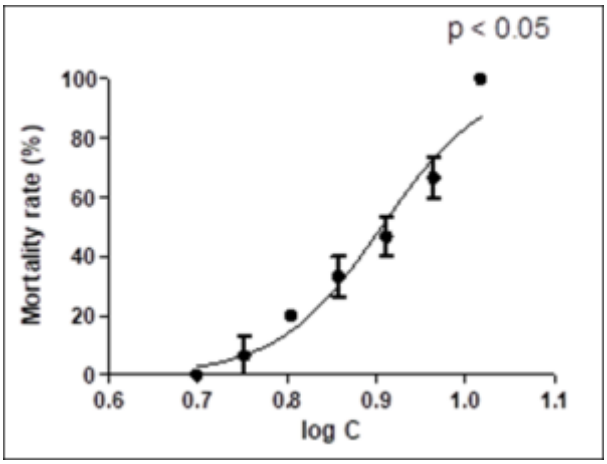

Figure 6 Dose effect and mortality caused by ME on frog tadpoles

3.4.3. Effects of ME on mosquito larvae

Six different concentrations of ME in geometric progression of reason r of 1.4 ranging from 0.25 to 1.81 mg/ml were tested on 6 batches of 25 larvae. The LC_{50} of ME on the larvae was estimated at 0.685 mg/ml (Figure 7).

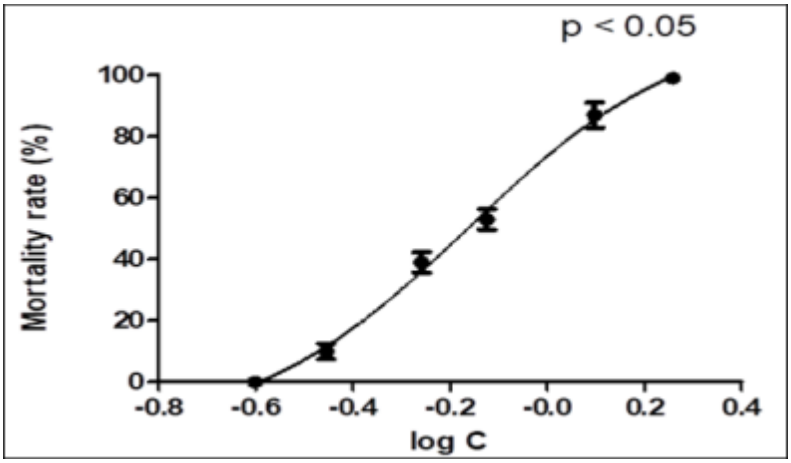

Figure 7 Dose-effect and mortality caused by ME on mosquito larvae

4. Discussion

The extraction yield of ME was 20.33%. This yield was higher than that of the methanolic extract of *Rhoicissus revoilii* tubers from Kenya (4%) [19].

Phytochemical screening of ME revealed the presence of tannins, flavonoids, polyphenols, coumarins and steroids. However, in previous studies on *Rhoicissus revoilii*, the presence of flavonoids, alkaloids, saponins, steroids and anthraquinones has been demonstrated [20, 8]. With the exception of flavonoids, certain compounds such as alkaloids and saponins were not present in the plant. This showed that the diversity of environmental conditions in the countries where plants are grown can result in different chemical profiles for the same plant species [21].

Test results indicated that the secondary metabolites present in ME were toxic to mice.

According to the Hodge and Sterner [22] scale, ME could be classified as very toxic in mice (LD_{50} between 21.29 and 22.3 mg/kg b.w.) by the i.p. route, while ME was moderately toxic by the oral route (LD_{50} between 273 and 275 mg/kg b.w.).

The toxicity of ME on mice could be due to the synergistic action of the various secondary metabolites it contains, or to the individual action of some of these compounds, such as coumarins [23].

Compared with other plants already studied at LABASM, in mice, ME was more toxic than the ethanolic extract of *Rhodocodon madagascariensis* bulbs (Liliaceae) (LD_{50} = 170 mg/kg b.w.) but less toxic than the methanolic extract of *Dioscorea antaly* tubers (Dioscoreaceae) (LD_{50} between 2.88 g/kg and 2.93 g/kg b.w.) [24].

By the oral route, ME showed high toxicity (LD_{50} between 273 and 275 mg/kg b.w.) in mice compared with methanolic extracts of *Dioscorea antaly* tubers (LD_{50} between 4.95 and 5.06 g/kg b.w.) and *Rhodocodon madagascariensis* bulbs (LD_{50} between 1.09 and 1.43 g/kg b.w.) [24].

The symptoms of intoxication observed were similar for the two routes of administration tested (i.p. and oral), with the exception of abdominal contortions and polyuria (i.p.) and convulsions (oral). The majority of symptoms suggested damage to the nervous system, although an increase in respiratory rate was also recorded. Histological observations suggested that this increase in respiratory rate could be linked to lesions affecting the respiratory organs.

The anatomopathological study revealed that the lungs, kidneys and liver were the organs most affected by ME. These three organs were primarily responsible for eliminating xenobiotics from the body. Lesions were more marked at the highest doses (15.6 mg/kg i.p. and 600 mg/kg orally). These results suggested that, even at LD_0, tissue damage could occur, although less markedly.

Histopathological examination of the kidneys treated by i.p. route revealed interstitial and tubular inflammation, associated with glomerular infiltration indicating nephritis [25]. Histological observations of the liver treated orally

revealed an accumulation of intracellular lipid vacuoles, indicating focal microvacuolar steatosis [26]. This disturbance in hepatic metabolism was accompanied by hepatocyte necrosis and could be linked to a dose-dependent toxic effect.

Pathological analysis of the lungs of orally-treated mice showed vascular dilatation, alveolar oedema and a dense lymphocytic cellular infiltrate, indicating inflammatory pneumonitis.

The activity of ME was not selective. In addition to its toxicity to warm-blooded animals, it also acted on cold-blooded animals. ME was toxic to carp alvins (LC_{50} = 7.491 µg/ml), frog tadpoles (LC_{50} = 8.055 µg/ml) and mosquito larvae (685 µg/ml). Saponins are known for their toxic effects on cold-blooded animals [27]. However, due to their absence in ME, the synergy between the active ingredients could contribute to its toxicity on these organisms.

Compared with other extracts already studied at LABASM whose toxicity was assessed under the same conditions, ME was less toxic in alvins than the methanolic extract of *Albizia viridis* and *Albizia androyensis* whose LC_{50}s were respectively 3.56 µg/ml and 4.50 µg/ml [10] but more toxic than extracts of *Albizia aurispara* and *Albizia tulearensis*, whose LD_{50}s were 60 µg/ml and 15.04 µg/ml respectively [10].

For frog tadpoles, ME was less toxic than methanolic extract of *Albizia tulearensis* (LC_{50} = 2.28 µg/ml) but more toxic than methanolic extract of *Pittosporum ochrosiaefolium* (LC_{50} = 13.85 µg/ml) [28].

With regard to toxicity to mosquito larvae, ME showed a lower toxic activity than crude extracts of *Euphorbia primulifolia* var latex, with an LC_{50} of 0.48 mg/ml [29] and *Vitex trifolia* (Verbenaceae) with an LC_{50} of 41.41 mg/ml [30]. However, it had a higher larvicidal activity than the methanolic extract of *Pavonia zeylanica* (Malvaceae) for which the LC_{50} was estimated at 2214.7 mg/ml [30].

5. Conclusion

In conclusion, the methanolic extract (ME) of *Rhoicissus revoilii* Planch. showed marked toxicity in mice, with lesions mainly in the lungs, kidneys and liver, and also high toxicity in aquatic animals. This toxicity showed that this plant could be a natural alternative to chemical pesticides. However, due to its proven toxicity, precautions must be taken when using it for medicinal purposes. Tests on mosquito larvae have also proved promising. However, ME was also toxic to frog tadpoles and carp alvins, so its use must also be controlled to minimise negative impacts on the aquatic ecosystem.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Pasteur Institute of Madagascar (IPM) for providing laboratory animals, and the Central Pathological Anatomy Laboratory of the University Hospital Centre (CHU) Joseph Ravoahangy-Andrianavalona for contributing to the work on histopathological studies.

Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of ethical approval

All the tests on animals were approved and in line with the standard established by Ethics Committee of the IPM.

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