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Toxicity Study of *Pittosporum ochrosiaefolium* Bojer (Pittosporaceae) a Medicinal Plant of Madagascar

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Abstract: The present work aimed to assess the leaf toxicity of *Pittosporum ochrosiaefolium* Bojer, a well-known medicinal plant endemic to Madagascar. Leaf methanolic extract (LME), obtained after successive extractions by hexan and methanol, was tested *in vivo* on warm and cold-blooded animals and *in vitro* on isolated atria of guinea-pig. LME was toxic to mice with a LD_{50} of about 46.69 mg/kg of body weight by intraperitoneal route. It induced mainly nervous disorders (body fasciculation, clonic convulsions), respiratory troubles (reduction of respiration frequency and cyanosis) and diarrheas. By intraperitoneal route, LME (46.69 mg/kg) caused histopathological lesions in lungs, liver, kidneys, small and large intestines but had no effects on brain, heart and stomach. Vascular congestion, inflammatory infiltrates, edema and necrosis were frequently observed. LME had a positive inotropic effect but no significant chronotropic one on isolated atria. It did not alter renal and hepatic functions at 21.24 mg/kg. It was highly toxic to the frog *Ptychadena mascareniensis* (LC_{50} of 13.51 µg/mL) and the fish *Cyprinus carpio* (LC_{50} of 8.2 µg/mL). It was also toxic to mosquito larvae *Culex quinquefasciatus* and *Aedes albopictus* with LC_{50} of 720 ppm and 910 ppm respectively. Different chemical compound groups were found in LME but only saponins proved to be toxic. Under certain conditions, *P. ochrosiaefolium* might be exploited as source of pesticides or therapeutic molecules.

Keywords: *Pittosporum ochrosiaefolium*, Leaf Methanolic Extract, Saponins, Toxicity, Histopathological Lesions, Pharmacological Activity

1. Introduction

This study was undertaken to investigate the toxic effect of leaf methanolic extract (LME) of *Pittosporum ochrosiaefolium* (Pittosporaceae), an endemic species to Madagascar used in traditional medicine. It was the continuation of our preliminary investigations on the toxicity of Malagasy *Pittosporum* species [1, 2].

The *Pittosporum* genus, originating from East Asia, is one of the 9 genera belonging to the Pittosporaceae family. It comprises about 160 species growing wild in tropical and subtropical regions [3]. It is often cultivated as ornamental plants in the Mediterranean region [4].

Pittosporum has other uses. Its timber is used in marquetry. Some species are employed as a fish poison [5-7].

Different species are widely used as medicinal plants for the treatment of various diseases. *P. floribundum* parts are used against skin diseases and itches [7]. In high doses, its bark acts as narcotic used as an antidote to snake poison, general weakness and also as a stimulant [8]. Australian Aborigines use *P. phylliraeoides* to treat a variety of conditions. An infusion of the leaves, seeds, fruit pulp or

wood is utilized to treat bruises, muscle aches, sprains and cramps. *P. phylliraeoides* infusions are drunk to treat cough and cold and to induce lactation [9]. A decoction of fruit is used both externally and by ingestion to treat eczema and pruritus [10]. In south of India, *P. tetraspermum* is an expectorant and possesses febrifuge and narcotic properties. It is used to cure chronic bronchitis, leprosy and skin diseases. The paste of the root bark is applied to inflammatory and rheumatoid swelling [11]. In Azores archipelago, infusion of *P. undulatum* fruits in alcohol or vinegar is employed for its anti-inflammatory activity [12]. In South Africa and Kenya, *P. manii* is used for the treatment of fever, malaria, inflammation, stomachache and as an antidote for insect bites [13, 14]. Some *Pittosporum* species displayed an antidote activity for snake poisoning [7].

Several active compounds were isolated from *Pittosporum*. Triterpene saponins were found in *P. senacia* (senaciapittosides A and B) [15], *P. viridiflorum* (Pittoviridoside) [16], *P. verticillatum* [17], *P. manii* [18]. Sesquiterpene glycosides were isolated from *P. undulatum* (undulatumosides A and B) [19] and *P. viridiflorum* [20]. Iridoid glycosides (6α-hydroxygeniposide) were obtained from *P. glabratum* [21], carotenoids (tobiraxanthins) from *P. tobira* [22]. Essential oil was extracted from *P. undulatum* [23], *P. neelgherrense* and *P. viridilum* [24].

In addition, a number of *Pittosporum* species were the subject of pharmacological studies. Methanolic extract from the bark of *P. manii* showed activity against *Plasmodium falciparum* strains [18]. Antimicrobial and antifungal activities of several species such as *P. tobira* [25], *P. floribundum* [7]. *P. neelgherrense* [26], *P. undulatum* [23], *P. viridulum* [24], *P. tetraspermum* [27] were demonstrated. Larvicidal activity of *P. tobira* was reported [28]. Anti-inflammatory activity of *P. tetraspermum* [11] and *P. undulatum* [19] was revealed. The methanol and aqueous extracts of stem bark from *P. dasycaulon* showed antioxidant activities [29]. Cytotoxicity properties of *P. verticillatum* [17], *P. venulosum* [30] and *P. tobira* [22] were reported.

In Madagascar, *Pittosporum* is represented by 11 species, 9 of which are endemic. Different species are widely used as medicinal plants. They have anti-inflammatory, antimicrobial and antispasmodic activities [31, 32].

P. ochrosiaefolium Bojer *var madagascariense* Danguy Cufod is a shrub or tree up to 10 m high growing in rainforest, in hot and humid areas of the eastern part of Madagascar.

The leaf infusion is employed for the treatment of gonnorhea. The bark decoction is a vermifuge in moderate doses. The stem and leaf infusion serve to calm bellyache and chewed leaf is an antidote for poisonous spider bites [31, 32]. It is also used against cough and rash. In North East of Madagascar, bark decoction is recommended to fight against tiredness, back pain and urinating difficulty [33]. In Ranomafana, according to traditional healers, *P. ochrosiaefolium* is used to heal wounds.

The present work mainly focused on the assessment and characterization of the LME toxic effects on animals and the chemical nature of the compound(s) responsible of its toxicity. Its effects on disease vector insects were also investigated.

2. Experimental

2.1. Plant Materials

Leaves were harvested in Ranomafana (Region of Vatovavy-Fitovinany, 400 km South East of Antananarivo), in February 2013. Voucher specimens of *P. ochrosiaefolium* were deposited in the herbarium of Plant Biology and Ecology Department of the Faculty of Sciences of the University of Antananarivo.

2.2. Animals

OF-1 strain Albino mice (*Mus musculus*), weighing 25±2 g, came from the Pasteur Institute of Madagascar breeding farm.

Male or female Guinea pigs weighing 300±50 g were used. They were purchased from local farmers and kept at least a week in the IMRA animal house before use. Those rodents were housed under standard environmental conditions and fed with standard rodent diet and water *ad libitum*.

Fishes (*Cyprinus carpio*), Royal strain, 2-4 cm size, were provided by a fish farmer.

Apode frog tadpoles (*Ptychadena mascareniensis*) were harvested from the ponds in the vicinity of the Antananarivo University site.

Fishes and tadpoles were allowed to acclimatize to the aquarium conditions for three days after their arrival in laboratory.

The mosquito larvae *(Culex quinquefasciatus, Aedes albopictus*) were furnished by Entomology department of Pasteur Institute of Madagascar (IPM).

One day old chicks (*Gallus gallus domesticus*), Hubbard classic strain, were provided by poultry farmer.

2.3. Leaf Methanolic Extract Preparation

The dried leaves of *P. ochrosiaefolium* were ground into powder. Leaf powder (100 g) was extracted with hexan until complete discoloration. The colorless powder was extracted with 3x1000 mL of methanol for 24 h under stirring. The filtrate was evaporated under reduced pressure to yield dry extract powders. The water solution of this leaf powder was named LME.

2.4. Extraction of the Crude Saponins

The extraction of the crude saponins was performed as following. LME residue was dissolved in methanol. A mixture of acetone-ether (v/v) was added dropwise to the resulting solution placed in ice-bath until no more crude saponins precipitated. Precipitate was dissolved in distilled water and constituted the crude saponin fraction. The supernatant was evaporated to dryness. Dry residue dissolved in distilled water constituted the non saponosidic

fraction. The toxicity activity of both fractions was assessed on mice.

2.5. Phytochemical Screening

The detection reactions of chemical groups on LME were carried out according to the methods of Fong *et al*, 1997 [34] and Marini-Bettolo *et al*, 1981 [35].

2.6. Acute Toxicity Tests

Toxic effects on mice and chicks were evaluated by intraperitoneal route and oral route. By intraperitoneal route, LME was injected at a volume of 0.25 mL per 25 g of body weight while by oral route, it was given by gavage by means of a curved distal end needle at the rate of 0.25 mL per 25 g of body weight.

Subcutaneous route was employed to assess LME toxicity on mice (0.25 mL per 25±2g of body weight).

2.6.1. Behavioral Observations of Intoxicated Mice

All changes in intoxicated mice behavior were observed during 24 h after LME administration, continuously for the first 4 h and then every hour. Changes in general behavior and other physiological activities or death were noted [36, 37].

2.6.2. LD_{50} Assessment on Mice

The toxicity study was carried out using 40 female mice. The animals were randomly distributed into 8 groups of 5 animals: one control group and seven treated groups. Animals were provided with food and water *ad libitum*.

Seven different doses (from 42.48 to 60.24 mg/kg body weight) of LME were intraperitoneally injected. The control group received physiological serum.

The LD_{50} (24 h) of the extract was determined by calculation and graphical methods of Reed and Muench, 1938 [38] and Boyd *et al*, 1968 [39].

2.6.3. LC_{50} Assessment on Cold Blooded Animals

The LC_{50} (24 h) of LME was determined on fishes, frog tadpoles and mosquito larvae. Eleven groups of five animals (fishes or frog tadpoles) were placed each in 500 mL crystallizer containing 250 mL of spring water. Ten groups were treated with 10 different concentrations of LME and the last one served as control.

For the mosquitoes, 25 larvae were placed in 25 mL of distilled water. Different concentrations of LME were added in the medium. One group without extract is used as control. The tests were performed in triplicate.

2.7. Anatomopathological Study

Three groups of 10 mice were used. Animals of group 1 were injected with 46.49 mg/kg, a dose corresponding to LD_{50}, then sacrificed 3, 9, 24, 48 h after treatment. Animals of group 2 were treated orally with a single daily dose of 42.48 mg/kg (LD_0 dose) for 10 days. At the end of experiences, the animals were sacrificed. Animals of group 3 served as control.

Brain, lungs, heart, stomach, liver, kidneys, large and small intestines were harvested afterwards and preserved in formol 10%.

The preparation of the organ sections for histopathological examinations was carried out using a classical method including the following steps: body fixation, inclusion, organ sections, preparation by microtomy, glass slide mounting, staining and microscope examination [40].

2.8. Study of LME Cardiac Effect

The effect of LME on the chronotropy and inotropy of the guinea pig isolated atria was assessed utilizing the method described in previous paper [41].

Increasing concentrations of LME from 12.5 to 50 µg/mL were tested on the same animals. The responses of atria were recorded at 1, 3 and 5 min after each injection. The number of beats per min and the amplitude of contraction were measured and percentage of stimulation or inhibition was calculated according to the parameter controls.

2.9. Study of LME Effect on the Renal and Hepatic Functions

This study consisted in the daily administration of a sub-lethal dose of the extract during 30 days and the assessment of the biochemical parameters to evaluate hepatic and renal function after treatment in mice. After exposure to a few possible toxic substances, there will be changes in body weight gain which would reflect toxicity [42].

The animals were weighed and divided into two groups of five animals each. They were treated daily at a fixed time in the morning by gavage of 10 mL/kg of distilled water for the control animals and 10 mL/kg of the extract prepared in distilled water for the treated animals. The tested LME dose was 21.24 mg/kg body-weight. This dose corresponded to the 1/2 of LD_0 by intraperitoneal route. The animals were then weighed every day, from the start until the end of the treatment, to note any weight variation. On the 31st day, the animals were anesthetized by chloroform inhalation. Their blood was collected on the retro-orbital.

Mean plasma levels of ALAT, ASAT and creatinine from control animals were statistically compared with those of the extract treated animals.

2.10. Statistical Analysis

The results of LC_{50} assessment were treated using the method of statistical analysis ANOVA (Graphpad prism 5 software). Those of the pharmacological study were expressed as mean ± standard error mean (SEM). Significant differences were determined using a Student's t test and the differences were considered significant if $p<0.05$ [43].

3. Results and Analysis

3.1. LME Extraction Yields

Extraction of the dried leaves of *P. ochrosiaefolium* gave a green colored extract with a yield of 20.59%.

3.2. Major Chemical Groups in LME

The results of phytochemical screening of LME revealed the presence of saponins, triterpenes, unsaturated sterols, alkaloids, flavonoids, iridoïds, tannins and polyphenols (Table 1).

3.3. Acute Toxicity of LME on Mice

3.3.1. Behavior Studies

The signs developed by intoxicated animals were various. The signs of the nervous system disorders were developed by hypoactivity, exophthalmos, fasciculation, paralysis of the hind legs and clonic convulsions. The respiratory systems attacks were manifested by reduction of respiration frequency, cyanosis etc. Other disorders as diarrhea were noted.

3.3.2. Influence of Administration Route

The influence of the administration route on LME effect was shown in Table 2. The intraperitoneal route was by far the most efficient. At doses about 52 times higher than the intraperitoneal LD_{100} (60.24 mg/kg), no mortality was observed by subcutaneous and oral routes.

3.3.3. LD_{50} Values

The LD_{50} of *P. ochrosiaefolium* value was assessed between 46.24 mg/kg and 47.15 mg/kg.

Table 1. *Phytochemical screening of leaf preparations of P. ochrosiaefolium.*

Chemical groups	Tests	Results	
		powder	LME
Alkaloids	Mayer	+	+
	Wagner	+	+
	Dragendorff	+	+
Flavonoids	Willstätter	+	+
Iridoïds	Hot HCl	-	-
Leucoanthocyanins	Bate-Smith	-	-
Saponins	Froth test	+	+
Steroids	Liebermann-Burchard	-	-
Triterpenes		+	+
Unsaturated sterols		+	+
Cardenolides		-	-
Cyanogenic glycoside	Grignard	-	-
Insaturated lactones	Kedde	-	-
Coumarin		-	-
Tannins and polyphenols	Gelatin 1%	-	-
	Gelatin-salt 10%	+	+
	$FeCl_3$	+	+
Quinones	Borntrager	-	-

Table 2. *Effect of LME on mice according to administration route.*

DOSE (mg/kg)	Mortality rate (%)		
	Intraperitoneal	Subcutaneous	Oral
60.24	100	0	0
463.68	100	0	0
3000	100	0	0
3500	100	100	30
4000	100	100	100

3.4. Anatomopathological Studies

The macroscopic analysis of the organs did not show any significant changes in aspect, size, color or texture when compared with the control group.

Brain, heart and stomach appeared normal. Three hours after LME injection, vascular exudative inflammation appeared as vascular congestion in lungs, liver, kidneys and large intestine. Inflammatory infiltrates of variable density consisting of neutrophil polymorphonuclears were visible. No histologic lesion was observed in small intestine. Hemorrhagic foci with the presence or not of hemosiderin pigments appeared in purifier organs (lungs, liver and kidneys) after 9 h of intoxication. Edematous and necrotic foci were also noted in liver. Hemorrhagic areas or edematous foci and residual inflammatory cells were found at 24 h after injection. A starting tissue repair in inflammation target organs with capillary hyperplasia (small and large intestines) appeared after 48 h.

As examples of organ injuries, lesions in lungs, liver, kidney, small and large intestines, which were more visible after at 9 h of poisoning were shown in Fig. 1.

Lesions by oral intoxication were generally the same as by intraperitoneal route after 3 h of intoxication. However, some lesions were not observed.

3.5. Effects of LME on Three Major Physiological Functions

3.5.1. Effects of LME on the Cardiac Contraction in Guinea-Pig

On the one hand, compared to the magnitude of the contraction control, the perfusion of LME at a concentration of 25 µg/mL significantly increased auricular force of contraction at 3rd and 5th minute ($p<0.05$) of recording (Fig. 2). At 50 µg/mL, it produced a positive inotropic effect time-dependent. On the other hand, up to 50 µg/mL, no significant effect was recorded on the auricular frequency of contraction ($p>0.05$).

3.5.2. Effects of LME on the Renal and Hepatic Functions in Mice

(i). Effects of LME on the Body Weight

The mean weekly body weight gain of control and daily treated mice with LME during 30 days was presented in Table 3.

The body weight of all treated animals increased. The weight gain was even higher in treated animals.

(ii). Effects of LME on the Biochemical Parameters

The impact of a sub-chronic intoxication by LME (21.24 mg/kg) on hepatic and renal function was assessed by any rate change of respectively transaminases (ASAT and ALAT) and creatinine. No significant change of the rate of the 3 biochemical parameters ($p>0.05$) was observed under the experiment conditions (Table 4).

3.6. Effects of LME on Chicks and Cold Blooded Animals

At 60.24 mg/kg, a dose corresponding to LD_{100} on mice,

LME had no effect on chicks by intraperitoneal and oral routes.

LME had toxic effects on *Ptychadena mascareniensis* tadpoles, *Cyprinus carpio* alvins and *Culex quinquefasciatus* and *Aedes albopictus* larvae (Table 5).

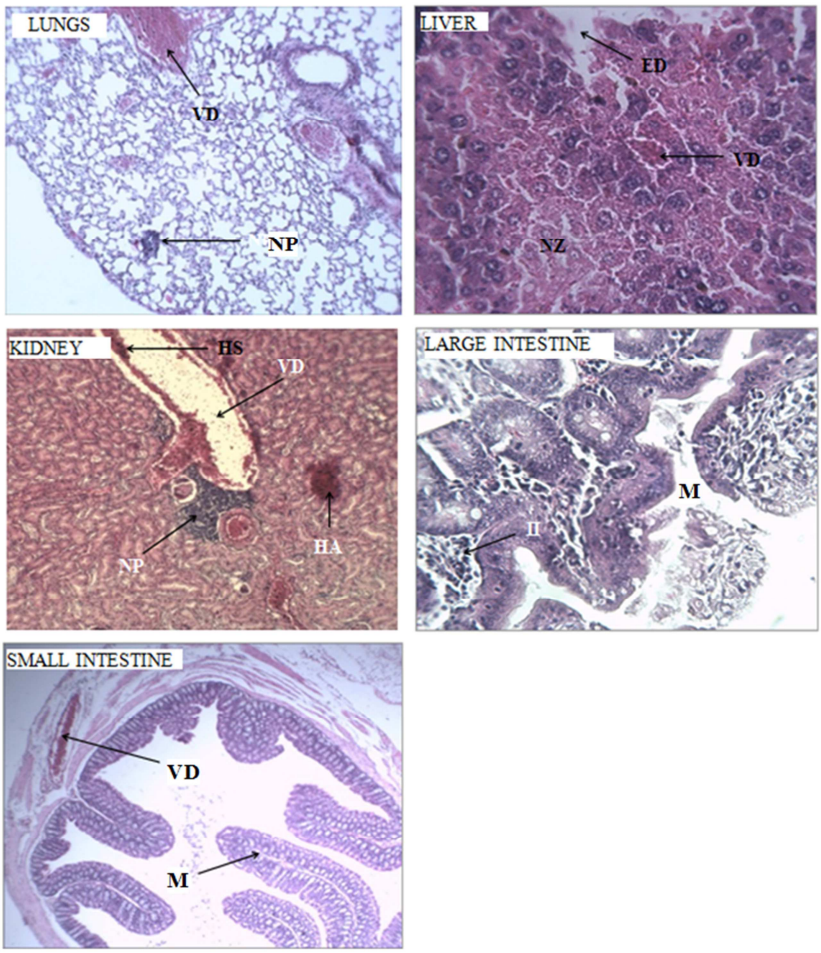

Figure 1. *Main lesions induced by LME on lungs, kidney, small intestine (G x10), liver and large intestine by intraperitoneal route after 9 h of exposure (magnification factor x 40).*

ED: edema; HA: hemorrhagic area; VD: vasodilatation; NP: neutrophil polymorphonuclears; NZ: necrotic zone; HS: hemosiderin pigments; II: inflammatory infiltrate; M: mucus

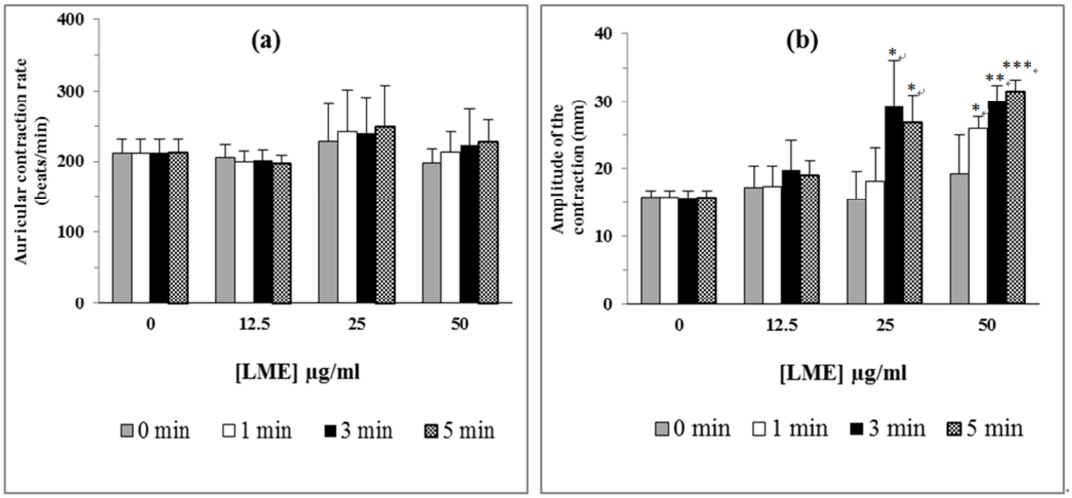

Figure 2. *Effect of different concentrations of LME and contact time on the number of beats per minute (a) and the amplitude of contraction (b) and of the isolated guinea pig atria (n=3); *: p<0.05; **: p<0.02; ***: p<0.01.*

Table 3. *Evolution of body weight of mice treated orally with LME (21.24 mg/kg) during 30 days.*

Treatments	Evolution of body weight (g)		Increase (%)
	Initial	Final	
Distilled water (Control)	25.25±2.03	29.94±1.92	18.57
LME (21.24 mg/kg)	26.06±1.46	34.23±2.02	31.35

Table 4. *Effects of LME on two major physiological functions on mice.*

	Physiological function		
	Hepatic		Renal
	ALAT (UI/L)	ASAT (UI/L)	Creatinine (mg/L)
Control	34.00 ± 10.19	476.33 ± 66.08	2.95± 0.48
LME	35.33 ± 7.93	350.50 ± 91.86	3.78 ± 0.37

Table 5. *Effects of LME on different cold blooded animals.*

Animal class	Species	LC_{50}
Amphibians	*Ptychadena mascareniensis*	13.51 µg/mL
Fishes	*Cyprinus carpio*	8.2 µg/mL
Insects	*Culex quinquefasciatus*	0.72 mg/mL (720 ppm)
	Aedes albopictus	0.91 mg/mL (910 ppm)

3.7. Effects on Mice of Crude Saponins and Non Saponosidic Compounds

The effects on mice of crude saponins and non saponosidic compounds were evaluated at two doses corresponding respectively to LME LD_{50} (46.69 mg/kg) and LD_{100} (60.24 mg/kg).

At 46.69 mg/kg, crude saponins were found to be more toxic than LME. As to non-saponosidic compounds, even at 60.24 mg/kg, they were not lethal to mice but caused slight symptoms which disappeared within few hours.

4. Discussion

4.1. Phytochemistry

Several chemical groups were found in leaf extract of *P. ochrosiaefolium* such as saponins, triterpenes, unsaturated sterols, alkaloids, flavonoids, iridoïds, tannins and polyphenols, but saponins appeared to be the main chemical group if not the only involved in the toxicity of this plant. As mentioned above, saponins were found to be responsible of the toxic activity of a number of *Pittosporum* species. Given the endemicity of *P. ochrosiaefolium* and the diversity of saponins already isolated from *Pittosporum*, *P. ochrosiaefolium* might contain new saponins.

Investigations on other chemical groups, whose pharmacological interests were demonstrated in foreign *Pittosporum* species, are ongoing.

4.2. Acute Toxicity

LME was toxic to warm and cold blooded animals. In mice, by intraperitoneal route it was highly toxic but by oral route it was much less toxic, explaining why there were no reported side-effects.

Compared to LD_{50} of leaf extract of other Malagasy species, assessed under almost the same conditions (animal, administration route), LME was as toxic as *P. verticillatum* (LD_{50} of 46.4 mg/kg) [1] but less toxic than *P. senacia* (LD_{50} of 26.73 mg/kg) [2]. The comparison to the foreign *Pittosporum* species toxicity was not easy because their LD_{50}, were sometimes assessed on other animals and by other administration route and concerned other parts extract of the plant. For information, the LD_{50} on rats by oral route of the methanol and aqueous extracts of *Pittosporum floribundum* bark were 1834.6 mg/kg and 1337.5 mg/kg respectively [44]. The LD_{50} 24 h values of *P. tobira* green seeds extract were respectively of 25 mg/kg and 1275 mg/kg by intraperitoneal and oral routes in mice and rats [22].

In comparison to the toxicity of other plants we studied, LME was more toxic than *Rhodocodon madagascariensis* bulb extract (LD_{50} of 170 mg/kg) and *Albizia bernieri* seed extract (LD_{50} of 55 mg/kg) [41].

The signs developed by intoxicated animals were various but the symptoms of nervous and respiratory systems attacks were the most visible. This diversity was probably due to the presence of more than one toxic secondary metabolite in LME.

Crude saponins proved to be more toxic than LME. The symptoms with non-saponin group might be due to small amounts of non-extracted saponins or other compounds.

4.3. Anatomopathogical Study

The evolution over time of lesions caused by LME by intraperitoneal route was comparable to the normal progress of the inflammatory response in animals. The vasculo-exsudative phase resulted in vasodilatation, edematous or hemorrhagic foci by loss of red blood cells or intravascular fluid and neutrophil polymorphonuclears. The cleaning phase occurred through the presence of plasma cells, histiocytes to clean altered polymorphonuclear leukocytes, edematous or hemorrhagic foci. The scarring process (fibrosis, capillary hyperplasia) began after 24 h.

The hemorrhagic area observed in liver and lungs were certainly due to the tensioactive and hemolytic activities of saponins present in large amount in LME.

4.4. Effects on Major Physiological Functions

The result showed that LME possessed a concentration and time-dependent positive inotropic effect. Three main categories of substances are known to have a positive inotropic effect, the digitalis, the phosphodiesterase inhibitors and the β1-adrenergic agonists. The positive inotropic effect of digitalis is often accompanied by a negative chronotropic effect [45]. They contain in their chemical structure some steroid nucleus which is responsible of their pharmacological activity. According to the phytochemical screening, this plant did not contain any steroid compounds. On the other hand, the positive inotropic effect of the β1-adrenergic agonists was accompanied by a positive chronotropic effect.

LME did not act on the chronotropy but only on inotropy, indicating that it could not contain any molecules capable to stimulate the beta-1 adrenergic receptors. The pharmacological profile of the cardiac effect of this tested extract was closer than that of the phosphodiesterase inhibitors. Effectively, the PDE inhibitors have only a positive inotropic effect [46]. So, its positive inotropic effect could be due to a PDE type III inhibition mechanistic.

The body weight of the animals did not show any significant change when compared to control group.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the extracts.

The function of renal filtration was not significantly affected. The creatinine plasma level was statistically not different for the group of treated animals and those of the control group.

There were no changes in the ALAT and ASAT levels, which revealed that LME did not affect the liver function/ or metabolism.

Therefore, it can be inferred that LME at 21.24 mg/kg did not affect the normal hepatic and renal functions on 30 days treatment.

4.5. Effects of LME on Other Animals

LME toxicity was not selective since cold-blooded animals were also found to be susceptible to its action. The fish *Cyprinus carpio* was more sensitive to LME than the frog tadpole *Ptychadena mascareniensis*. Compared to the toxicity of the seed extracts of Malagasy *Albizia* assessed in the same conditions [41], LME (LC_{50} of 13 µg/mL) was more toxic to *Ptychadena mascareniensis* than the seed methanolic extract of *Albizia aurisparsa* (LC_{50} of 60 µg/mL) but less toxic than *Albizia androyensis* (LC_{50} of 3.56 µg/mL). LME was more toxic to *Cyprinus carpio* than *Albizia aurisparsa* (LC_{50} of 15 µg/mL) but less toxic than *Albizia tulearensis* (LC_{50} of 2.28 µg/mL).

The high toxic effects of LME on cold-blooded animals were probably due to saponins. The toxicity of these compounds to cold blooded animals is well-known. It accounts for the use of many plants containing saponins as poison fishing in several countries. Many species of *Pittosporum* were also reported to be used as fish-poisoning [5, 7].

Concerning the toxicity to mosquito larvae, LME was compared to leaf methanolic extracts of other plants [47]. LME (LC_{50} of 720 ppm) was much more efficient against *Culex quinquefasciatus* than *Pavonia zeylanica* (Malvaceae) and *Acacia ferruginea* (Leguminosae) whose LC_{50} were 2214.7 ppm and 5362.6 ppm respectively. On the contrary, it was less efficient than *Vitex trifolia* (Verbenaceae) (LC_{50} of 41.41 ppm). To *Aedes albopictus,* LME (LC_{50} of 910 ppm) was less toxic than *Annona squamosa* (Annonaceae) LC_{50} of 20.26 ppm).

5. Conclusion

Our results showed that *P. ochrosiaefolium* contained saponosides toxic to mice, fishes, tadpoles and mosquitoes. These scientific data are necessary before exploiting any pharmacological property of this plant. They also demonstrated the weak toxicity of LME by oral route which justifies the use of *P. ochrosiaefolium* in traditional medicine. However, any use by injection is strongly not recommended.

Keeping in mind the various pharmacological properties concerning the saponosides isolated from foreign *Pittosporum* species and saponosides in general, investigations are ongoing for isolating *P. ochrosiaefolium* saponins and exploring their properties.

The toxicity of LME to cold blooded animals was interesting but its rational use in pest control must take account its non-selective effects.

Our results contributed to a better knowledge of medicinal and poisonous plants from Madagascar.

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