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Acute Oral Toxicity Profile of the Aqueous Extract of Six Plants Used in Congolese Traditional Medicine

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ABSTRACT

Several plants are traditionally used in the treatment of various pathologies but to date, there is no documented evidence corroborating its safety. This study thus aimed to determine the toxicity profile of the aqueous extracts of six plants widely used in traditional medicine in the Democratic Republic of Congo by determining its effects after acute oral administration in guinea pigs at a single dose (1-3 g/kg body weight). These plants include *Cussonia corbisieri* De Wild (Araliaceae), *Desmodium repandum* (Valh) DC (Fabaceae), *Dialiopsis africana* Radlk (Sapindaceae), *Pericopsis angolensis* (Baker) Meeuwen (Fabaceae), *Solanum lycopersicum* L. (Solanaceae) and *Vernonia shirensis* Oliv. & Hiern (Asteraceae). Some clinical parameters indicating intoxication in the incidence of general behavioral adverse effects namely alopecia, convulsion, paralysis, weight loss, death and measurement of the activity of *alanine aminotransferase*, *aspartate aminotransferase*, creatinine, and urea were monitored. The mortality rate also increases with increasing dosage by Dragstedt and Lang's method. The results recorded showed symptoms of toxicity such as convulsion, paralysis, coma, as well as deaths. Biochemical disturbances were also observed by a slight increase in ALT, AST, and urea. The LD_{50} values obtained with the six plant species range from 3000 to 5000 mg/kg. These data allowed classifying all these plants in low toxic plants class. Overall, the findings of this study indicate that the use of these plants in traditional medicine at a low dose (1000 mg/kg) would be without acute toxicity. This constitutes a hope for per os recipes obtained by maceration and used in the treatment of various pathologies.

INTRODUCTION

In Africa, the use of traditional recipes that make extensive use of medicinal plants is the basis of primary health care [1-4] and represents, through the richness of its flora, an important therapeutic arsenal to the traditional healers [5-8]. Unfortunately, the therapeutic uses of many plants are known but the toxicity of most of them is little studied. However, more than one-third of plants used in Traditional Medicine contain toxic substances, most of which are not yet identified [9,10]. Fortunately, some recipes used for millennia are harmless as shown by few investigations done in the world [11-13]. But the so-called harmless plants show a delayed or cumulative toxicity which appears in use. In Africa, human poisoning from plants accounts for more than 6,5 % of poisonings and are responsible for significant morbidity and mortality [14,15]. Unfortunately, several therapeutic accidents can sometimes be tragic [16].

This study is part of a broad program to improve and enhance African traditional medicine by assessing the toxicity of some plants widely used in traditional medicine in Southern Katanga. Thus, *Cussonia corbisieri* De Wild, *Desmodium repandum* (Valh) DC, *Dialiopsis africana* Radlk, *Pericopsis angolensis* (Baker) Meeuwen, *Solanum lycopersicum* L. and *Vernonia shirensis* Oliv. & Hiern plants abundantly cited by the traditional healers of Lubumbashi for their various medicinal properties were thus retained for an evaluation of their acute toxicity. These six plants are cited in the management of several ailments including hemorrhoids, diarrhea, malaria, liver diseases, various infections, diabetes, sexual impotence and intestinal worms [2,5,9]. They were also selected on the basis of the preliminary results of their biological activities and the absence of studies on their safety.

Faced with this broad application of these plants in the traditional therapeutics to which a good fringe of the population resorts, the study of their oral toxicological parameters by using their aqueous extract (the traditional use) is necessary.

MATERIALS AND METHODS

Harvesting and identification of plants

The plant material includes the roots of *Desmodium repandum* harvested at the Kasamba village on GPS data: 11° 48' 1'' South and 27° 27' 40.4" Est-West, at an altitude of 1274 meters, the leaves of *Dialiopsis africana* harvested in the forest zones of the Kasungami

village on GPS data: 11 ° 45 '56.6' 'South and 27° 28' 17.8" Est-West at altitude of 1258 meters; *Vernonia shirensis* harvested at Kasamba village on following GPS data ; south: 11° 48' 26.0 " and East-West: 27° 26' 58.1 ", at an altitude of 1276 meters; The stem bark of *Pericopsis angolensis* was harvested at Kasamba village on GPS data: 11° 49' 23.5" South and 27° 27' 22.6" East-West, at an altitude of 1223 meters, bark stem of *Cussonia corbisieri* was harvested at Kasamba village on GPS data: 11° 36' 93.2" South and 27° 28' 99.9" East, at 1293 meters above sea level and the stems of *Solanum lycopersicum* was harvested in Lubumbashi town on following GPS data ; South: 11° 38' 19.6" and East-West: 27° 29' 07.3" at an altitude of 1278 meters. After collection, the harvested organs were weighed, washed with water, dried and stored away from the sun at room temperature for about 2 weeks. The harvest was carried out with the support of healers. Authentication was made at the Herbarium of the Institut National pour l'Etude et les Recherches Agronomiques (INERA-KIPOPO, DR Congo). Voucher of different plants have been made and are awaiting registration.

Obtaining the aqueous crude extracts

The plant's extracts were prepared by maceration following the traditional method of preparing the recipes [17]. Five hundred grams of the dry plant material was pulverized and macerated in a liter and half of distilled water for 24 hours. This operation was repeated three times. The mixture was drained with cotton wool and then filtered on Wattman paper N^{o} 1. The solvent was evaporated using a rotavapor (BIBBYRE 200B, Germany) at 40°C temperature. The extracts obtained were weighed and stored in a freezer at -20° C in sterile, hermetically sealed vials until further use [18].

Acute oral toxicity study an animal model

The acute toxicity test of the various plant extracts was carried out on guinea pigs. This experiment was conducted in accordance with the principles for laboratory animal use and care as found in Canadian Committee [19].

A total of seventy-six (76) male guinea pigs weighing 460 ± 30 g were purchased from the University of Lubumbashi's faculty of Agronomic Sciences where they were bred under conventional conditions for research purposes.

These animals were housed in cages at a room temperature of 20 ± 2° C (guinea pig's favorable temperature is between 18-24°C [19]) regularly controlled for 2 weeks before manipulation for a period of acclimatization to the laboratory conditions. They were fed with rodent pellets (Alilapin®; MIDEMA/DR Congo), with water ad libitum and vitamin C supplement by gavage at 20 mg/kg/ day. The animals were divided into three groups of four guinea pigs each in order to reduce the variability of animal's responses to the plants' aqueous extracts and were, as closely as possible, matched for weight and size per group. For each plant extract, twelve animals divided into three groups were used as described below [20]: the animals of experimental groups, received respectively for each plant 1000, 2000 and 3000 mg of extract per kg of weight. Distilled water was used as a vehicle for the plants extract doses and it was used for the control group (3 mL/kg weight). The administration was carried out following the OECD guidelines for the study of acute toxicity [17,21,22]. Animals of all groups were fasted for about 14 hours before experiment [23].

Recording clinical signs and examination

After administration of plant extracts, animals were continuously monitored for 14 days for recording all deviations in general behavior associated with the administration of plant material such as diet, locomotion, external appearance, abdominal state and respiration and the number of deaths [23]. After administration of the substances according to the batches, the guinea pigs were observed immediately, after 30 minutes, and then 1 hour thereafter 2 hours with the resumption of feeding. The observation was then resumed 6 hours later and finally after 12 hours for the first day. Subsequently, they were followed every 6 hours for 3 days, and finally, every 12 hours for the last 10 days [24]. Cases of dead animals were also noted, which subsequently allows calculation of the LD_{50} according to the Dragstedt and Lang method [25].

Evaluation of biochemical parameters

In this study, direct observation of clinical signs of intoxication (eating disorders, alopecia, dyskinesia, asphyxia, bedsore, etc.), biochemical analyses were performed at the Analytical Laboratory of the university clinic of the University of Lubumbashi [24,26,27,28,29,30]. Alanine Transaminase (ALT), Aspartate Transaminase (AST) were performed on an analyzer using a kinetic rate method for the enzymes, modified rate.

Jaffe method for creatinine and urea were analyzed for this purpose as they are the most remarkable parameters among those appearing early in poisoning [31]. These parameters allow measuring both the liver and the kidneys function to suppress the toxic substances of the blood [32,33].

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical comparisons between the data for the control and treatment groups were performed using one-way analysis of variance (ANOVA) test. Results were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Observations of clinical disorders

The results on the clinical symptoms observed after administration of the aqueous extracts are summarized in Table I.

Table I: Clinical disorders data observed

Plant species	Dose (mg/kg)	Weight Before (g) – After (g)	Clinical signs observed
Cussonia corbisieri	1000	460.6±6.5 – 440.8±3.8	Eating disorders, paralysis.
	2000	464.9±7 – 421.6±4	Eating disorders, hypomobility, priapism, alopecia, death.
	3000	468.5±4 – 419.8±5	
Desmodium repandum	1000	450.8±9 – 360.7±4	Agitation, aggressiveness, eating disorder, dyspnea, irritation, alopecia, upper limb paralysis, death.
	2000	462.9±6 – 373.7±8	Agitation, aggressiveness, dyspnea, irritation, alopecia, upper limb paralysis, priapism, eating disorders, dyskinesia, death.
	3000	487.5±6 – 366.5±5.5	
Dialiopsis africana	1000	432.5±4 – 430.6±2	Priapism, alopecia, restlessness, tearful eyes, immobility, trembling, death.
	2000	432.5±5 – 355.9±4.5	Priapism, alopecia, restlessness, watery eyes, immobility, general tremor, bradycardia, dyskinesia, posterior limb paralysis, death.
	3000	432.5±4 – 295.5±9	In addition to the signs observed at 2000 mg/kg, we notice convulsion and dyspnea.
Pericopsis angolensis	1000	450.3±20 – 420.5±20	Alopecia, dyskinesia, pressure sores, hypoactivity, upper limb paralysis, weight loss, eating disorders.
	2000	452.8±9–373.6±9	Alopecia, dyskinesia, pressure sores, hypoactivity, upper limb paralysis, weight loss, eating disorders, death.
	3000	485.6±5 – 360.8±14	Alopecia, dyspnea, dyskinesia, pressure sores, hypoactivity, weight loss, eating disorders, death.
Solanum lycopersicum	1000	430.5±9 – 389.8±7	Hypoactivity, death.
	2000	432.6±6.5 – 401.4±4.5	Alopecia, abdominal bloating, hypoactivity, bloody lesions, death.
	3000	436.8±5 – 408.4±6	
Vernonia shirensis	1000	451.5±4 – 352.5±6	Polypnea, tachycardia, hypoactivity, weight loss, limb paralysis, increased food requirements, and death.
	2000	455.5±4 – 389.3±2.5	Dyspnea, tachycardia, hypoactivity, weight loss, stomach bloating, alopecia, bloody lesions, increased food requirements, paralysis of the hind limbs and death.
	3000	460.5±7 – 364.3±8	Dyspnea, tachycardia, hypoactivity, weight loss, belly bloating, alopecia, bloody lesions, paralysis of the hind limbs and death.
Control group	3 ml distilled water/kg	400±6 – 670.2±4	Nothing to report

The results above show that several signs of intoxication were recorded during the 14 days of observation. These signs are manifested with all the plants studied and with a similarity of effects at equal doses.

Dyspnea has been observed with *P. angolensis*, *C. corbisieri*, *D. repandum*, and *V. shirensis*. It is due to a hypotensive effect of the extracts of the plants concerned. This hypotensive effect may be due to the presence of flavonoids and comparable to that of acetylcholine as reported by other studies [34]. Indeed, some flavonoids [35] such as chalcone and quercetin have been shown to have a hypotensive effect [36].

A convulsion and a hypoactivity observed in this study may be due to the decrease in elements necessary for animal activity as also found by Mukinda and Syce [37] during the acute and chronic toxicity of the aqueous extract of *Artemisia afra* Jacq. Ex. Willd. in rodents.

Very pronounced dose-dependent alopecia was observed with all plants. Alopecia is the most common symptom of plant poisoning in animals as demonstrated by Doumbia *et al*. [38], Djyh *et al*. [39], Hobou *et al*. [16], Adamu *et al*. [40], Nair and Staden [41], and Dar *et al*. among others [42]. Alopecia may be due to a various number of causes including a defective production of the hair follicle and/or an overproduction of estrogen-progestogens [36]. Antihypertensive substances are also reported to have this effect [36]. Alopecia may, therefore, be related to the presence of flavonoids in the studied plants. Several *in vivo* acute toxicity studies have also reported these signs of intoxication [24,43,44], which are relatively proportional to the dose administered for each plant.

The results of this study also show that the weight of the animals was significantly decreased ($p < 0.05$) in proportion to the dose. Several studies show that weight change is generally a toxicity index after exposure to a toxic substance [23,45,46]. This reduction in weight can be explained by a reduction in food consumption, but also by the possibility of dose/absorption interactions and by the reduction in the amount of food absorbed. Other studies have also demonstrated a reduction in the weight of the rats after oral administration of recipes of some plants which can be toxic such as *Chiococca alba* (L.) Hitchc (Rubiaceae) extract [47] and that of *Stryphnodendron adstringens* (Mart) Coville (Fabaceae) [48].

Mortality rate and Determination of lethal doses 50

The deaths recorded for each group of animals allow calculating the mortality rate which led to the 50 lethal doses for each plant as proposed by Dragstedt and Lang [25] and taken over by Fané [49], Diallo [50] and Nene bi [51].

Table II: Mortality Rate and LD_{50} Values (n = 4)

Plant species	Dose (mg/kg)	Number of dead/used	Mortality rate (%)	LD_{50} (mg/kg)
Cussonia corbisieri	1000	0/4	0	3520
	2000	2/4	25	
	3000	3/4	41.66	
Desmodium repandum	1000	1/4	25	3000
	2000	2/4	37.5	
	3000	3/4	50	
Dialiopsis africana	1000	1/4	25	4125
	2000	2/4	37.5	
	3000	2/4	41.66	
Pericopsis angolensis	1000	0/4	0	5000
	2000	2/4	25	
	3000	2/4	33.33	
Solanum lycopersicum	1000	1/4	25	3000
	2000	2/4	37.5	
	3000	3/4	50	
Vernonia shirensis	1000	1/4	25	3000
	2000	2/4	37.5	
	3000	3/4	50	
Control group	3 ml distilled water/ kg	0/4	-	-

This table II shows that oral administration of aqueous extracts of the six plants at 1000, 2000 and 3000 mg/kg resulted in guinea pig deaths in some groups in a dose-dependent manner. No deaths were recorded in the control group. Dose-dependent mortality has already been observed in other toxicity studies as reported by Mukinda and Eagles [30] by evaluating the acute and subchronic toxicity of aqueous extracts of *Polygala fruticosa* P.J.Bergius (Polygalaceae), by Hobou *et al*. [16] after evaluating the toxicity of *Stachytarpheta indica* (L.)Vahl (Verbenaceae) in mice and by Doumbia *et al*. [38] who demonstrated this does effect by studying the acute toxicity of *Mareya micrantha* (Benth.) Müll.Arg. (Euphorbiaceae) in mice.

These mortality rates were calculated over 14 days with a first death recorded with *Vernonia shirensis* 1 hour after gavage of 2000 mg/kg and 3 hours after gavage of *Desmodium repandum* at 1000 mg/kg. The LD_{50} values were 3000 mg/kg for *Desmodium repandum, Solanum lycopersicum*, *Vernonia shirensis*, 3250 for *Cussonia corbisieri*, 4125 and 5000 mg/kg respectively for *Dialiopsis africana* and *Pericopsis angolensis*.

The LD_{50} obtained, ranged from 3000 mg/kg to 5000 mg/kg, allow to consider the plants studied as low toxicity in guinea pigs under the conditions of this study according to the Diezi [52] classification as taken up by Nene bi *et al*. [51].

Analyzed biochemical parameters

In the surviving guinea pigs up to the 14th day, the blood was taken for biochemical analyzes and the results are shown in the synoptic table III below.

TABLE III: Results of analysis of biochemical parameters

Plant species	Doses (mg/kg)	ALT (IU/l)	AST (IU/l)	Creatinine (mg/dl)	Urea (mg/dl)
Cussonia corbisieri	1000	78.78±5.9	91.07±13.87	0.59±0.04	39.07±19.20
	2000	148.96±21.03	133.8±71.99	0.55±0.06	34.84±4.77
	3000	132.07±26.05	110.61±22.26	0.63±0.03	48.38±7.66
Desmodium repandum	1000	94.23±18.73	132.4±23.22	0.74±0.007	31.82±6.64
	2000	194.8±18.13	63±12.88	0.72±0.09	53.36 ±5.67
	3000	96.05±21.8	99.78±24.74	0.66±0.022	22.17±6.38
Dialiopsis africana	1000	54.73±3.14	49.04±2.47	0.84±0.12	44.69±3.93
	2000	58.56±17.42	54.92±34.18	0.788±0.01	51.47±12.20
	3000	67.06±12.55	92.73±33.56	0.768±0.03	53.63±12.18
Pericopsis angolensis	1000	160.2±7.5	138.6±7.5	0.67±0.08	33.35±7.50
	2000	66.6±10.9	193.9±58.9	0.82±0.08	39.40±2.71
	3000	107.2 ±15.6	130.8±6.2	0.74±0.17	27.11±4.59
Solanum lycopersicum	1000	152.23±11.06	74.80±11.99	0.49±0.04	32.38±6.90
	2000	135.65±4.06	92.80±5.92	0.49±0.07	42.91±4.48
	3000	57.8±5.16	52.74±3.34	0.56±0.05	23.23±2.42
Vernonia shirensis	1000	86.42±9.11	116.39±13.21	0.63±0.06	21.41±3.98
	2000	173.79±29.43	98.01±3.32	0.71±0.05	56.16±12.23
	3000	121.18±48	102.18±11.00	0.71±0.07	24.32±3.14
Control group	3 ml distilled water/kg	65.95±5.22	63.94±7.25	0.70±0.09	36.51±10.50
Normal values of the literature (18)	-	27-68	25-59	0.6-2.2	9-31.75

The data from the experimental groups (animals receiving the plant extracts) were compared with those of the control group.

The analysis showed a significant increase ($P < 0.05$) of ALT at all doses (1000, 2000 and 3000 mg/kg) between the undervaluation and control groups. The observed increase would be

due to the toxic action of the extracts attested by the destruction of the liver cells, which contain high amounts of ALT [24,42,53,54]. The same observation of increase made on the ALT was made for AST. On the other hand, no significant difference was observed between the creatinine and urea levels obtained between the experimental groups and the control group and even compared to the normal values of the literature.

It is well known that ALT and AST activity and creatinine and urea levels are good indicators of liver and renal function respectively [24]. The only fluctuation of ALT and AST shows that the extracts of the studied plants would have a toxic effect although little pronounced on the liver function and null on the kidney.

CONCLUSION

This assessment of the acute toxicity of the aqueous extracts of *Pericopsis angolensis, Cussonia corbisieri, Desmodium repandum, Dialiopsis africana, Solanum lycopersicum, Vernonia shirensis* at doses of 1000, 2000 and 3000 mg/kg showed that the plants would have low toxicity dose-dependent. Their use at doses reported in traditional medicine would be weakly dangerous.

This makes these plants a real expectation in the treatment of the various pathologies noted in the literature, namely hemorrhoids, liver pathologies, sexual impotence, female infertility, in the fight against intestinal worms and dysmenorrhea. However, at high doses, these plants have significant liver, hepatic and renal toxicity. Further studies to determine the sub-chronic and chronic effects of these plants are ongoing to complete the safety profile of them.

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