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ACUTE TOXICITY STUDY OF TOTAL ALKALOID OF *MITRAGYNA CILIATA (RUBIACEAE)* IN FEMALE RATS

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ABSTRACT

Mitragyna ciliata (MYTA) is a plant species whose stem barks are traditionally used in the treatment of malaria. The toxicity of total alkaloids extracted from this plant was studied in female rats. The extract was administered orally in single doses of 2000 mg / kg and 5000 mg / kg bw. The animals were observed continuously for 24 h and daily for 14 days. During this period, the clinical signs were recorded, and the body weight of the animals noted once a week. The blood of the rats was also drawn weekly for the determination of the biochemical and hematological parameters. The results obtained revealed an absence of toxic effect of the alkaloids of this plant, with a lethal dose 50 (LD50) greater than 5000 mg / kg bw. It emerges from these results that the total alkaloids of MYTA are therefore classified in category 5 of the globally harmonized classification system for chemical substances, a category characterizing substances of low toxicity.

KEYWORDS: acute toxicity, total alkaloid, *Mitragyna ciliata*, clinical signs.

INTRODUCTION

Man, through an apprenticeship that undoubtedly involves many failures and a few successes, has experimented on himself with remedies drawn from the plant world and sometimes from the animal world. Some of these remedies have become classics of modern pharmacopoeia. We will cite the main remedy against pain, morphine, extracted from the poppy (*Papaver somniferum, Papaveraceae*) and that against malaria, quinine extracted from cinchona (*Cinchona spp, Rubiaceae*). These successes have authorized research in the twentieth century on natural substances which have led, from medicinal or poisonous plants to the isolation of classes of anticancer drugs, the bi-indole alkaloids or a new class of antimalarial drugs, derivatives of artemisinin (Jean Bruneton et al., 1993). Their chemical structures have also served as a model for the synthesis of less toxic and sometimes more powerful derivatives (Sévenet et al., 1994). This operation to improve the performance of products by the synthesis called medical chemistry requires permanent collaboration between chemistry laboratories and pharmacology laboratories. Pharmacological exploration of plants requires confirmation of the medical interest of the preparation tested. This involves biological models that mimic human disease as much as possible, or mechanisms for the establishment or functioning of this disease (Sauvain, 2002).

It is in this context that we are interested in *Mitragyna ciliata (Rubiaceae),* a plant whose stem barks are traditionally used to treat malaria. This species has been the subject of several studies both in terms of its biological activities and its chemical composition. The methanolic extract obtained from the stem bark of this plant has shown interesting biological activities. An antiplasmodial effect on a chloroquine-resistant strain of Plasmodium falciparum FcM29 (Adjétey et al., 2007), an inhibitory effect of the Na^+ / K^+ ATPase dependent pump (Bidié et al., 2008), a cardiotonic effect on the isolated heart of the rabbit and a hypotensive effect on the carotid arterial pressure of the rabbit (Bidié et al., 2010). In addition, the methanolic extract of Mitragyna ciliata has been shown to be moderately toxic (Bidié et al., 2010). The stem bark of this plant contains several chemical compounds, including alkaloids. The objective of this work is to assess the possible toxic effects of total alkaloids extracted from the stem bark of Mitragyna ciliata in female rats of the wistar strain.

MATERIAL AND METHODS

Plant material

The bark of Mitragyna ciliata was harvested in the Toukouzou swamps in the Grand Lahou department (Ivory Coast). The identity of the plant has been confirmed at the National Floristic Center of Félix HOUPHOUËT-BOIGNY University in Abidjan (Ivory

Coast). The rinds were cut up, dried at room temperature and finely ground.

Extraction of total alkaloids

For the preparation of the total extract of the bark of M. ciliata, 50 g of the vegetable powder were dissolved in 1.5 L of 100% methanol. The methanolic mixture was stirred for 24 h at room temperature using a Heidolph brand magnetic stirrer (Lab-Mix 35). After 24 h, the mixture was left to stand for 15 min then; three successive filtrations were carried out as follows: once on white cotton cloth, then once on hydrophilic cotton and finally once on 3mm Wathman paper. The filtrate obtained was evaporated at reduced pressure at a temperature of 40 ° C using a rotary evaporator of the BUCHI brand. The brown-colored powder obtained constituted the total methanolic extract. 300 mg of this extract were then dissolved in 10 mL of a methanol / water mixture (v/v). The pH of the solution was adjusted to 2 by adding hydrochloric acid (HCl). Then a series of liquid-liquid extraction of the filtrate (4 times) was carried out by successive addition (4 times) of 5 mL of dichloromethane. Then, sodium carbonate (Na2CO3) was added to the aqueous phase to bring the pH to 10. The mixture was again subjected to liquid-liquid extraction of the filtrate (3 times) in the presence of 5 mL of dichloromethane. The different organic phases were combined and 25 g of anhydrous magnesium sulfate was added to the solution to remove traces of water. Filtration and concentration of the organic phase to dryness were finally carried out using a rotavapor at a temperature below 40 ° C in order to avoid denaturation of the alkaloids (Koffi et al., 2009; Konkon et al., 2006).

Characterization of total alkaloids

The characterization of the total alkaloids was carried out using specific reagents: Dragendorff and Bouchardât reagents which, in the presence of alkaloids, give orange and reddish-brown precipitates respectively (Bézanger-Beauquesne, 1958). Thus, 0.2 g of the alkaloid extract was dissolved in 2 mL of 60% alcohol and divided into two test tubes. Two drops of each reagent were added to each of these tubes.

Acute toxicity test

Our study involved white rats, female (*Rattus norvegicus*), of the Wistar strain, with an average weight of 138.40 g and about 3 months old. The breeding of these animals took place in the animal house of the Ecole Normale Supérieure of the University Félix HOUPHOUËT-BOIGNY. The ambient temperature in the room was 24 ± 2 ° C. Water and food were provided to them ad libitum. The method used is the limit test corresponding to a dose level of at least 2000 mg / kg body weight (exceptionally 5000 mg / kg). The animals were fasted for 12 h. Nine rats (9) female rats were divided into 3 groups. Batch 1 constituted a control batch. Animals in this batch were orally administered 1 mL / 100 g body weight (bw) of distilled water. Lots 2 and 3 constituted the lots of animals treated. They were administered orally at 2000 and 5000 mg / kg bw, respectively, of M. ciliata total alkaloids extract. The different solutions were administered to the rats using a gastric tube, at a rate of one dose per batch and 1 mL of solution per 100 g of body weight. The time interval between administrations of each dose depends on the duration and severity of the toxic effects observed. The absence of death and / or moribund state of the rats from the previous batch is a condition for the use of the next dose. After administration of the extract, the animals were observed individually at least once for the first 30 minutes, regularly for the first 24 hours and daily for 14 days. During this period, the number of dead rats as well as the behavior and symptomatic disturbances observed with the naked eye were recorded. The toxicity class of M. ciliata total alkaloids was determined based on the highest dose used and clinical signs (OCDE 423).

Evaluation of haematological parameters

The complete blood count is the quantitative and qualitative cytological study of the elements of the blood. The blood samples were taken at the start of the treatment of the rats (Do), one week later (D7) and at the end of the experiment (D14), using a Pasteur pipette through the retro sinus orbital at the level of the orbital vein. Blood was collected in pediatric tubes containing EDTA K2 type dry anticoagulant and analyzed on the SYSMEX XP-300 machine.

Evaluation of some serum biochemical parameters

Some serum parameters related to lipid metabolism (cholesterol and triglycerides), carbohydrate metabolism (glucose), hepatic functions (transaminases) and renal function (creatinine) were measured in animals which survived the different doses of total alkaloids administered. To do this, the blood collected from the rats was centrifuged at 2000 rpm for 10 min. Plasma or serum was collected in wells and analysis of serum parameters was performed using the Cobas c311 analyzer from Roche Hitachi.

Statistical analysis

Data were entered into an Excel sheet (Microsoft Office 2007, USA) and analyzed with Statview software version 5.0 (SAS Institute., Inc., USA). Quantitative data has been presented as the mean ± standard deviation (SD) in graphs and tables.

RESULTS

Extraction and characterization of total alkaloids

Extraction of the total alkaloids in acidic and alkaline medium from 300 mg of total methanolic extract of *M. ciliata* made it possible to recover 100 mg of brown solid product with a yield of 33.33%. This product gives a positive test with the Dragendorff and Bouchardât reagents, characteristic of alkaloids **(Table 1).**

Table 1: Results of the characterization test for the total alkaloids of M. ciliata.

Sr. No	Reagent	Total alkaloids	
		Acid medium	Alkaline medium
1	Dragendorff reagent	+ + +	+
2	Bouchardât reagent	+ + +	+

+++: Strongly positive; +: Weakly positive

Acute toxicity

Effect of M. ciliata Total Alkaloids on the General Condition of Rats

Clinical signs were observed during the 30 min following administration of the product to the animals. The rats having received the same doses of the product presented all the same clinical signs with recovery times of 5 to 10 min and 20 to 30 min respectively for the doses of 2000 mg / Kg of bw and 5000 mg / Kg of bw. No mortality was recorded **(Table 2).**

Table 2: Clinical signs recorded in each animal according to the doses administered.

Sr. No	Clinical signs	Group 1 (distilled water mg / Kg of bw)			Group 2 (2000 mg / kg bw)			Group 3 (5000 mg / kg bw)		
		R1	R2	R3	R1	R2	R3	R1	R2	R3
1	Reduced activity	-	-	-	-	-	-	+	+	+
2	Difficulty breathing	-	-	-	-	-	-	+	+	+
3	Convulsion	-	-	-	-	-	-	-	-	-
4	Diarrhea	-	-	-	-	-	-	-	-	-
5	Coma	-	-	-	-	-	-	-	-	-
6	Blood in the urine	-	-	-	-	-	-	-	-	-

+: positif ; - : negatif ; R : rat

Effect of M. ciliata Total Alkaloids on Rat Body Weight

The variation in the weight of the animals is presented in Table 3. The results indicate a reduction in the weight of the rats of the order of - 4.48% and - 6.34%, in the animals of batches 2 and 3, treated respectively with 2000 mg / kg bw and 5000 mg / kg bw of the alkaloid extract of M. ciliata. The control batch, there is a weight gain of + 1.45 in the same period (D14). However, these variations are not significant.

Table 3: Variation in the body weight of the rats as a function of the doses.

Sr. No	Animals groups	Body weight of animals in g		
		J0	J7	J14
1	Control	138,0 ± 8,11	139,4 ± 9,47 **(1,01%)**	140 ± 10,50 **(1,45%)**
2	Group 1 (2000 mg/Kg)	138,40 ± 10,30	135,5 ± 10,75 **(-2,09%)**	132,2 ± 5,83 **(-4,48%)**
3	Group 2 (5000 mg/Kg)	138,80 ± 10,27	137,0 ± 7,23 **(-1,30%)**	130,0 ± 14,34 **(-6,34%)**

Effect of M. ciliata total alkaloids on biochemical parameters

Each biochemical parameter is represented in the table by the mean of the values, accompanied by the standard deviation and the variation from J0 (%) indicated in parenthesis. The analysis of the serum of the rats on D0 and D14 shows that there is a non-significant decrease in the activity of alanine amino transferase (ALAT), and of the level of creatinine, triglycerides, cholesterol and glucose in treated rats compared to control rats. On the other hand, the activity of aspartate amino transferase (ASAT) increased in the order of + 5.94% and + 8.54% respectively at doses of 2000 mg / Kg of Pc and 5000 mg / Kg of Pc at the end of D14. This increase is, however, not significant. The results are shown in Table 4.

Table 4: Variation of biochemical parameters in female rats.

Sr. No.	Parameters	Control		Group 2 (2000 mg/Kg de Pc)		Group 3 (5000 mg/Kg de Pc)	
		J0	J14	J0	J14	J0	J14
1	Créatinine (mg/L)	6,9±0,21 (0 %)	5,3±0,31 (-23,18 %)	6,3 ±0,26 (0 %)	4,8±0,49 (-23,81 %)	5,9±0,30 (0 %)	4,1±0,83 (-30,51 %)
2	ALAT (UI/L)	42,8±7,16 (0 %)	73,3±15,07 (-71,26 %)	35,3±6,29 (0 %)	64,5±11,68 (-82,72 %)	41,2±4,29 (0 %)	73±25,63 (-77,79 %)
3	ASAT	149,9±28,03	117,0±12,03	117,9±33,25	**124,9±16,05**	99,5±18,10	**108,0±9,63**

	(UI/L)	(0 %	(-21,95 %)	(0 %)	**(+5,94%)**	(0 %)	**(+8,54)**
4	**Triglycéride (g/L)**	0,72±0,08 (0 %)	0,88±0,22 (+22,22 %)	0,76±0,20 (0 %)	0,98±0,19 (28,95 %)	0,94±0,24 (0 %)	**0,64±0,36 (-31,91 %)**
5	**Cholestérol (g/L)**	0,78±0,05 (0 %)	0,87±0,23 (+11,53 %)	0,76±0,13 (0 %)	0,76±0,28 (0 %)	0,70±0,30 (0 %)	0,69±0,12 (-1,42%)
6	**Glucose (g/L)**	0,92±0,01 (0 %)	0,93±0,01 (+1,09 %)	0,92±0,02 (0 %)	**0,85±0,04 (-7,60%)**	0,92±0,02 (0 %)	**0,87±0,03 (-5,43 %)**

Effect of M. ciliata total alkaloids on haematological parameters

The blood count results reveal a slight increase in the level of white blood cells on D14 compared to D0 in the animals of the control group and those of the group treated with the extract at a dose of 2000 mg / kg of Pc. Unlike the two previous batches, there is a slight decrease in leukocytes in batch 3 on D14. In the red blood cells, the results indicate an increase in the rate in the batch of control animals, on the other hand, in the treated animals; a slight decrease is noted from D0 to D14. As for hemoglobin levels, a slight increase is observed in all batches (batch 1, 2 and 3). At the level of haematimetric constants (HCT, MCV, MCH, MCHC), only the hematocrit level which decreases in the animals of the control group and the group treated with 2000 mg / kg of bw. Finally, concerning platelets, an increase in the rate is observed in all batches from D0 to D14.

Table 5: Variation of haematological parameters in female rats.

Sr. No.	Parameters	Control		group 2 (2000 mg/Kg de Pc)		Group 3 (5000 mg/Kg de Pc)	
		J0	J14	J0	J14	J0	J14
1	**WBC (10^3/L)**	6,13 ±0,91 (0 %)	9,13 ±1,99 (32,10 %)	6,90 ±2, 31 (0 %)	**7 ,20 ±5,7 (4,34 %)**	10,87 ±3,10 (0 %)	**8,40 ±0,21 (-22,92 %)**
2	**RBC (10^6/µl)**	5,80 ±1,06 (0 %)	6,60 ±1,30 (13,79 %)	6,00 ±1,27 (0 %)	**5,92 ±1,91 (-1,36 %)**	6,99 ±0,36 (0 %)	**6,88 ±0,46 (-1,57 %)**
3	**HGB (g/dl)**	11,40 ±2,21 (0 %)	12,30 ±2,14 (7,89 %)	11,47 ±2,31 (0 %)	11,54 ±1,86 (4,61 %)	13,80 ±1,15 (0 %)	14,43 ±1,40 (4,56 %)
4	**HCT (%)**	35,30 ±6,80 (0 %)	33,90 ±9,07 (3,96 %)	35,17 ±8,30 (0 %)	**33,60 ±12,08 (-4,46 %)**	40,40 ±3,01 (0 %)	**42,30 ±4,14 (-4,70 %)**
5	**MCV (fl)**	59,33 ±3,10 (0 %)	59,4 ±2,93 (0,12 %)	58,60 ±2,36 (0 %)	59,20 ±1,93 (1,02 %)	57,80 ±1,68 (0 %)	**61,30 ±4,08 (6,05 %)**
	MCH (pg)	19,50 ±0,55 (0 %)	19,50 ±0,36 (0 %)	19,23 ±1,10 (0 %)	19,80±1,27 (2,96 %)	19,70 ±0,70 (0 %)	**20,93 ±1,91 (6,24 %)**
	MCHC (g/dl)	32,20 ±0,76 (0 %)	33,40 ±1,15 (3,72 %)	32,83 ±1,58 (0 %)	34,10 ±0,98 (3,87 %)	34,13 ±0,40 (0 %)	34,17 ±1,33 (0,12 %)
	PLT (10^3/µl)	797,30±12,7 (0 %)	921,00 ±24 (15,51 %)	877,3±24,6 (0 %)	906,30(±28,9) (3,30 %)	974,0±16,7 (0 %)	998,3±14,2 (2,49 %)

DISCUSSION

Extraction with methanol and then with dichloromethane in an acidic and basic medium of the bark of Mitragyna ciliata made it possible to obtain a total extract with a yield of 33%. The phytochemical analysis of the product made it possible to demonstrate the richness of the bark of this plant in alkaloids (Bidié et al., 2010). MYTA total alkaloids administered orally at doses of 2000 mg / Kg and 5000 mg / Kg (OECD 423) did not cause any deaths throughout the study. Lethal dose 50 is greater than 5000 mg / kg bw. This value of the LD50 makes it possible to classify the total alkaloids of MYTA in category 5 of the globally harmonized classification system for chemical substances, a category characterizing substances of low toxicity (OECD, 2001). The variation in weight is a very important parameter in the monitoring of animals subjected to a toxicity test. During the 14 days following treatment of the animals, a non-significant decrease in body mass was observed in the rats given the doses of M. ciliata alkaloid extract. The M. ciliatad alkaloids extract could therefore lead to loss of appetite in animals, even if this change in weight is not significant.

The biochemical tests carried out have made it possible to demonstrate the effect of MYTA alkaloids on renal functions through the determination of urea and creatinine. The results showed no significant difference in urea and creatinine levels in the rats given the M. ciliata alkaloid extract compared to those in control rats. These results are in agreement with those of Coulibaly (Coulibaly et al. 2004) who worked with the total extract of this plant.

Transaminases are enzymes with significant metabolic activity inside cells. They are involved in certain energy reactions. The increase in their serum level reflects cell damage, particularly in the liver (Kew, 2000) and in certain heart cells. The results obtained in this study show that the total alkaloids of MYTA caused an

insignificant variation in the levels of ASAT and ALAT. This is in agreement with the previous work of Bidié et al. (2010) on the cardiovascular system of the rabbit. In terms of lipid and carbohydrate metabolism, the results show that the administration of the total alkaloids of MYTA at a dose of 5000 mg / kg bw to animals does not influence lipid and carbohydrate metabolism.

Like the other parameters, CBC analysis shows no significant variation. This means that the extract has no cytotoxic effect on the figurative elements of the blood.

CONCLUSION

This study showed that the stem barks of Mitragyna ciliata are rich in total alkaloids, with an extraction yield of 33.33%. These studies also reveal that the extracts are low in toxicity with a lethal dose 50 (LD50) greater than 5000 mg / kg bw. The extracts do not exhibit any toxic effect on the key organs of the heart, liver and kidneys and do not interfere with carbohydrate and lipid metabolism. On the other hand, the consumption of extracts at the doses indicated leads to weight loss in the consumer.

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