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ACUTE, SUB-CHRONIC TOXICITIES AND ANTIPYRETIC EFFECT OF EXUDATES OF *STRYCHNOS CAMPTONEURA* GILG & BUSSE (LOGANIACEAE)

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ABSTRACT

Acute, sub-chronic toxicities and antipyretic activity of *S. camptoneura* exudates were determined by using classical experimental models. Acute toxicity test (300 and 2000 mg/kg) did not reveal any modification of general behavior, nor mortality of mice but, one notes a significant increase of body weight. The L. D_{50} of exudates was evaluated to be superior to the dose of 2000 mg/kg. Sub-chronic toxicity at a single dose of 2000 mg/kg after 45 days showed a significant increase of body weight, unchanged hematological parameters (HGB, VGM, platelets and leukocytes); no significant decrease of biochemical parameters (ASAT, ALAT, Bilirubine, creatinine and uric acid) in rat. However, the exudates provoke a significant decrease of glycaemia. At the doses of 300 and 600 mg/kg, exudates reduced significantly hyperthermia induced by the suspension Brewer's yeast (20%). These results could explain the important use of the exudates of this plant in traditional medicine against all types of fever.

KEYWORDS: *Strychnos campteneura*, exudates, acute toxicity, sub-chronic toxicity, antipyretic.

INTRODUCTION

Fever is a state associated with several pathological conditions and, is currently a very challenging problem for the clinician as the available synthetic agents are causing multiple unwanted effects.[1] Several studies are ongoing worldwide to find natural healing agents with better safety profile but, drug discovery involves many steps which must be carefully carried out. In other hand, many countries in process of development, feel enormous sanitary difficulties in the reason of no access in the health centers, drug-stores and the cost of modern drugs. These difficulties are nowadays responsible of the deficit of health care offer. Of or the increasing interest carried currently toward the medicinal plants accessible to all. In Congo-Brazzaville, *S. camptoneura* (Loganiaceae) is widely used against several pathologies.[1,2] Scientific investigations performed with the stem bark proved that the plant presents a wide pharmacological potentialities and many phytochemical compounds endowed antioxidants potentialities.[3,4,5,6] According to the populations, *S. camptoneura* exudates are used against all type of fever. However, if extract plants are often considered like healthy and natural in comparison to modern drugs, some scientific investigations proved that they could contain toxic substances sometimes dragging active serious negative effects until the death.[7,8,9] A recent study revealed that medicinal plants extracts could have undesirable effects as renal and hepatic attacks, diarrheas, constipations, vomiting and rectal bleedings.[10] The plants would be also toxic by ingestion or by contact and, the treatments basis on plant extracts often spread on long periods. Thus, in order to avoid other more serious pathologies in long term there is the necessity of mastery of administered doses. The current study was thus aimed at evaluating of acute, sub-chronic toxicities and antipyretic activity of *S. camptoneura* exudates.

MATERIALS AND METHODS

Plant material

Plant material was constituted by *S. camptoneura* exudates collected at M'voula (Department of Cuvette West, around 765 km from Brazzaville) in June 2015. The plant has been authenticated by National Research in Exacts and Naturals Sciences Institute (IRSEN) and unregistered under the number 2271. The exudates were directly collected in sterile bottles after section of the stem and then carefully kept in an isothermal cooler during transport and at 4°C in the refrigerator before experimentations.

Animals Material:
Male and female mice weighing between 20-25 g were used for acute toxicity and antipyretic activity; Wistar rats weighing between 150-200g for sub-chronic toxicity. Animal comes from Faculty of Health Sciences of Marien Ngouabi University (Brazzaville-Congo). They were fed and maintained under standard lighting conditions (12 hours' light and 12 hours' dark).

Acute toxicity
The acute toxicity was studied according to the OECD (2001) guide line N° 423 for chemical solutions tests. 3 groups of 3 mice each fasted 24 hours before experimentation and treated as follow: group 1 as negative control received distilled water (0.5 ml/100g); groups 2 and 3 received *S. camptoneura* exudates at the doses of 300 and 2000 mg/kg. After unique administration of the products, the animal's behavior was observed during 4 hours in order to value the signs of toxicity. These observations concerned lethality, ptosis, aggressiveness, vomiting, stools state, pilo-erection and reaction of animals to the external stimulus. This experience continued during 14 days in order to insure if there is toxicity after metabolism of exudates constituents and, mice ponderal evolution followed.

Sub-chronic toxicity
Sub-chronic toxicity was evaluated using the OECD (2009) leading line N° 407 for the chemicals tests with repeated dose during 45 days. Two (2) groups of five (5) rats each was constituted and acclimated for 7 days in the laboratory with free access to water and food before any experimentation. The control group was daily treated with distilled water at 0.5 ml/100 g and the tested group with the exudates at the unique dose of 2000 mg/kg bodily weight. The animal's behavior was observed every day and their weight appropriated every week. Some blood withdrawals were achieved by old-fashioned orbiter way after anesthesia with ether before the treatment and 45 days after treatment with the help of the hematocrit micropipettes. The samples of blood appropriated in EDTA tubes was immediately used to determine the rates of hemoglobin (HGB), middle globular volume (MGV), hemoglobin (Hb), platelets and leukocytes by classical methods. [12,13] The one of dry tubes was centrifuged at 2580 towers/min during 10 min and, the collected serums served immediately to evaluate biochemical parameters (ALAT, ASAT, total Bilirubine; creatinine, alkaline phosphates, uric acid and glycemia).[14]

Antipyretic effect
The hyperthermia was induced in mice by oral administration of aqueous suspension of Brewer's yeast (20 %) at the dose of 20 ml/kg in empty stomach after 24 hours.[15] The rectal temperature was appropriated before administration of Brewer's yeast (basal temperature). 24 hours after administration, the rectal temperature is appropriated again and, the mice received different products as follows: group 1 as negative control receives 0.5 ml/100g of distilled water; group 2 as positive control receives paracetamol (25 mg/kg); groups 3 and 4 received exudates at the doses of 300 and 600 mg/kg respectively. The rectal temperature was appropriated then to 1; 2; 3 and 4 hours with the help of a thermometer electronic HaaK.

STATISTICAL ANALYSIS
The results expressed affected on average of the standard mistake are submitted to an analysis of the variance to a factor followed of Student-Ficher test. The limit of significativity is fixed at $p < 0.05$.

RESULTS AND DISCUSSION
This study was initiated to evaluate acute, sub-chronic toxicities and in the second time, antipyretic activity of *S. camptoneura* exudates. Indeed, several studies having revealed potential toxic effects of plant extract.[16, 17] In the reason of abundant use of the exudates in Congolese traditional medicine against all types of fevers, it was necessary to characterize their toxicological effects on biologic systems before approaching antipyretic aspect. According to the leading line of OECD for chemicals tests of which lethal dose (DL_{50}) is unknown, the biggest quantity of product is managed so that the report dose/mouse/human either bigger. In the case of our study, two doses were used (300, 2000 mg/kg). After unique oral administration and 4 hours of observation the test did not reveal any modification of general behavior, nor of mortality that would denote a toxic effect of the exudates to the mouse. In to insure if there will be toxicity after metabolism of exudates constituents the second observation of 14 days after administration folow the leading line of OECD indicates, do not revealed any modification of general behavior or mortality. However, one notes a significant increase weight of animal that received exudates compared to the control group (figure 1). *S. camptoneura* exudates don't provoke anorexia or death, but would stimulate the appetite to animals, the exudates can be considered as low toxicity and presents a higher LD_{50} superior to 2000 mg/kg.[18] Our results is in agree with those obtained with the stem bark of these specie and *Anthocleista schweinfurthii*, two species of Loganiaceae family.[5,19]

After acute toxicity, the dose of 2000 mg/kg that means the highest dose was chosen, in order to search for the effect on biochemical and hematological parameters. The obtain results show that transaminases (ASAT, ALAT), alkalin phosphates level increases no significantly whereas the one of bilirubine, creatinine and, uric acid decreases no significantly. Transaminases (ASAT, ALAT) are, plasmatic enzymes of hepatic origin that permits to explore liver state. Their increase would indicate cells hepatic lesions. Alkalin phosphates (PAL) are everywhere present enzymes in organism but especially in liver, bone, intestine, kidneys and white globules. The attack of these organs provokes alkalin phosphates liberation. Creatinine is a constituent of muscle proteins and a marker of renal function; who's

revealed the degree of renal filtration then, the increase of seric creatinine level reflect a renal dysfunction.[20, 21] In our experimentation, at the studied dose (2000 mg/kg), exudates don't modify significantly any biochemical parameters level. That suggests the non toxic character of these exudates. However, experimental results showed the significant decreases of glycemia level ($p < 0.01$) after 45 days of treatment at 2000 mg/kg. This decrease suggests that *S. camptoneura* exudates could contain bioactive compound that have a possible hypoglycemia effect.

Hematological parameters give some information on hematopoietic functions, determination of all allergies and white blood cell state.[22] Results shows at the dose of 2000 mg/kg after 45 days, a significant increase ($p<0.01$) of the hematological parameters (HGB, VGM, PLT and GB) in comparison of levels before treatment (table 2). A critical analysis suggests that *S. camptoneura* exudates could have a possible stimulation of hematopoietic function that would let think a possible anti-malarial effect. Indeed, a previous investigation revealed that anti-malarial plants had a stimulating effect on hematopoietic and immune system.[23] Experimentation showed a significant increase of leukocytes rate (GB) and of other blood parameters, joining other authors investigations.[24, 25, 26] These results explain the large and frequent traditional use of the exudates against several pathologies and symptoms as hyperthermia or fever.

Hyperthermia is an elevation of the body temperature; in our case, hyperthermia was induced by oral administration of 20 ml/kg of brewer' yeast (20%) suspension. Brewer' yeast provokes an increase of microbial rate in the organism that entails the liberation of prostaglandins, bradikinins and histamines that produce inflammation, pain and the increase of the body heat to the animal and fever.[27,28,29] Antipyretic test results (figure 3) show an elevation of the body temperature of animals 24 hour after administration of brewer' yeast suspension. When the animals are treats with distilled water, in spite of the time of observation, there is not any reduction of the temperature. Whereas at those treated with paracetamol and exudates, one notes a significant reduction ($p < 0.01$) of the temperature since the first hour then it becomes very significant ($p < 0.001$) at the second hour that follows exudates administration. Mice rectal temperature in control group (distilled water) increases so, the hyperthermia is maintained whereas it decreases very significantly ($p < 0.001$) in test groups. The temperatures of different groups that had reached 38.42 ± 0.17 °C; 38.23 ± 0.27 °C; 38.10 ± 0.6 °C and 38.30 ± 0.19 °C after hyperthermia induction pass to 37.56 ± 0.11; 37.20 ± 0.32 and 36.80 ± 0.21°C correspondent to a reduction of 0.67 ± 0.16 °C; 0.90 ± 0.06 °C and 01.50 ± 0.02°C respectively with paracetamol and *S. camptoneura* exudates (300 and 600 mg/kg). This reduction is maintained very significantly until the 4th hour after administration. Exudates it as lowering the body temperature opposes to the installation of fever therefore. While opposing the fever installation, these exudates present a very interesting antipyretic potentialities since at the studies doses; it appears more efficient than paracetamol.

CONCLUSION

In conclusion, this study has permitted to shown that *S. camptoneura* exudates is not toxic at the studied doses and would not provoke pathologies in longer use. It also permitted to confirm exudates potentialities against fevers as claim by farming populations. These interesting results suggest that *Strychnos camptoneura* exudates would contain bioactive compounds against fever and would explain their use. A phytochemical study of these exudates would be interesting in order to reveal different bioactive compounds against fever.

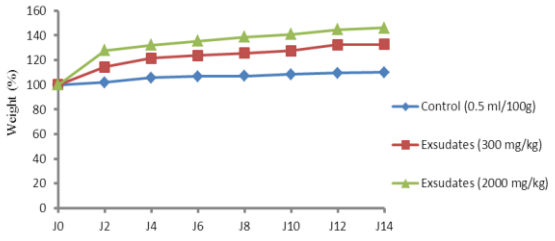

Figure 1: Effect of *S. camptoneura* exsudates on weight evolution (%) in mice, (n = 3), acute toxicity

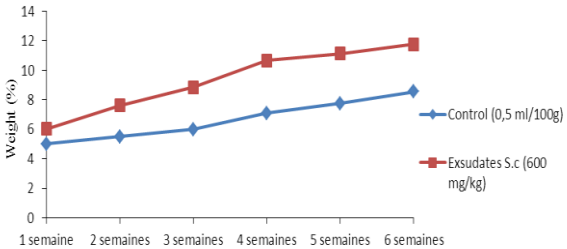

Figure 2: Effect of S. camptoneura exsudates on weight evolution (%) in rats (n=5), sub-chronic toxicity

Table 1: Effect of *S. camptoneura* exudates on biochemical parameters of Wistar rat

Parameters	Gly.	Creat.	ALAT	ASAT	PAL	Bili.	Uric acid
A	1.16±0.10	7.95±0.57	36.78±7.19	188.86±11.04	134.44±11.31	1.278±0.11	21.76±1.430
B	0.76±0.11** (S)	7.43±0.26 (NS)	34.38±5.11 (NS)	125.10±14.37 (NS)	135.7 ± 42.03 (NS)	1.206±0.08 (NS)	21.62±1.31 (NS)

A: Before treatment; B: 45 days after treatment
Gly: Glycemia; Creat: Creatinine; Bili: Bilirubine; PAL: Alkalin phosphates
NS: No significant; S: significant ($p<0.01$ 45 days after)

Table 2: Effect of *S. camptoneura* exudates on hematologic parameters of Wistar rat

Control (0.5ml/100g)	***S. c* (2000mg/kg)**	
Parameters	**45 days**	**45 days**
HGB (dl/L)	13.23±0.34	18.70±0.19**
VGM (μm^3)	55.38±1.23	56.83±0.37**
TMH (Pg)	19.28±0.61	20.00±0.03**
Platelets ($10^3/\mu L$)	440.80±15.86	483.00±7.90*
Leukocytes (C/µL)	05.16±0.86	07.50±0.45**

HGB: Hemoglobin; VGM: mean globular volume; TMH: mean rate in hemoglobin
Control: distilled water. *S. c*.: *Strychnos camptoneura*
The values are the means ± ESM; *p <0.05; **p <0.01 (n = 5)

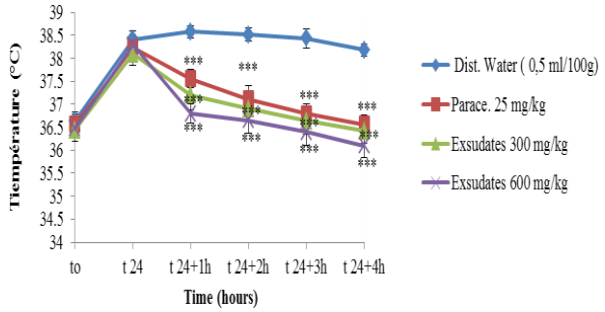

Figure 2: Effect of *S.Camptoneura* exsudaes (300 and 600 mg/kg) and paracetamol (25 mg/kg) on hyperthermia induced by brewer's yeast in wistar rat. Each value represent m ± ESR, ****p<0,001* compared to control group (n=5)

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