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Research Paper

Toxicological study of "*Natural bio juice" a traditional medicine based on Garninia kola (Clusiaceae)*

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ABSTRACT
Objective: The main objective of this research is to evaluate the toxicity of ''Natural bio juice'' a phytomedicine based on G. kola.
Methods: The 28 days sub-chronic toxicity of ''Natural bio juice'' was done. Rats body weight and rat's organ relative weight, haematological and biochemical parameters were determined; and histological sections were performed. The contractile activity on the heart of the toad ''in situ'' was evaluated.
Results: The phytochemical screening of the drug has shown the presence of cardiac glycosides, alkaloids, flavonoids and anthracenes. The administration of ''Natural bio juice'' for 28 days, at 1.7 ml/kg and 10 ml/ kg significantly (p <0.0001) increased the relative weight of the liver and blood sodium level; and decreased significantly (p <0.0001) the platelet count. Histological sections performed on the heart, liver, testes and kidneys showed no histological lesions. The experiment performed of the toad heart in situ have shown a significant decrease (p <0.0001) in heart rate (negative chronotropic effect) and an increase in cardiac amplitude (positive inotropic effect).
Conclusion: ''Natural bio juice'' can be toxic to human liver, blood and heart after a long-term administration. These toxics effects of ''Natural bio juice'' can be attributed to its' contain in cardiac glycosides
***Key words**: Garcinia kola; sub-chronic toxicity; cardiac glycosides, contractile activity.*

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I. INTRODUCTION

The use of plants in traditional medical practice has a long history and remains the mainstay of primary health care in most third world countries [1]. In Africa, the plant-based remedies relieve millions of people, including a large part formed by local communities [2-3].

The main difficulties that follow disease treatment by conventional drugs are their inaccessibility especially because of their high cost [4]. Known by many names in Africa, the "small-Kola" or "bitter kola" is a small kola nut. It belongs to the family of Clusiaceae and its scientific name is *Garcinia kola* [1]. G. kola is popular in southern Nigeria. The plant is used in traditional medicine and as food. Every part of *G. kola* is an important component of traditional herbal medicine in several African countries [5-7]. G. *kola* is a popular ingredient in African ethnomedicine because of its many social and medicinal uses. Scientific studies show that the *G. kola* has sexual stimulant properties, especially in men. Indeed, it contains in theobromine, which is a substance that has the effect of stimulating the central nervous system, *G. kola* is classified in the category of natural aphrodisiacs [8]. *G. kola* is found in the composition of a phytomedicine sold in some markets in Togo. This phytomedicine is called in our study ''*Natural bio juice''*.

Because of the high use of *G. kola* seeds and especially the high use of ''*Natural bio juice'';* because it's said that scientific research is still needed to support the traditional use and the non-toxic effect of *G. kola* [9-12], and as a part of the development of traditional medicines as recommended by WHO, we decided to evaluate the toxicity of ''*Natural bio juice''*.

II. MATERIALS AND METHODS

A Framework

Our study was conducted at the University of Lomé (Togo), in the Animal Physiology Department of the Faculty of Science and in laboratories of Toxicology and pharmacology of the Faculty of Health Sciences.

The traditional medicine we have evaluated and we called in this study ''Natural bio juice''was provided by a traditional therapist living in Lome (Togo). The ''*Natural bio juice"* is indicated in the treatment of many diseases such as: dental disease, blood diseases (diabetes, blood pressure), fibroma, hemorrhoids, painful periods, oligospermia, azoospermia, sexual weakness. The recommended dosage for adults is one small glass in morning or night.

Animals

Male Wistar rats (140 to 270 g) were provided by the department of animal physiology of the University of Lome (Togo) and used for toxicological tests. Toads (*Bufo marinus*) ranged in body weight from 90-160 g (mean 120 g) were captured in night, in damp places and were used for the study of cardiac activity ''*in situ*''. All these animals were housed in environmental normal conditions and fed standard diets of rodents and water.

Phytochemical screening

Plant materials were screened for the presence of alkaloids, saponins, tannins, total phenols, anthraquinones, flavonoids, sterols and cardiac glycosides using the methods previously described by Tona *et al.* [13].

Sub-chronic toxicity study

The repeat dose of oral toxicity study was carried out according to OECD guideline 407 [14]. Three groups (T, D1, D2) of 8 male rats were formed. The control group received distilled water. The groups D1, D2 received the ''*Natural bio juice''* 1.7 mL/kg body weight and 10 mL/kg respectively. The first dose is based on the indicated human dose and the second is the highest dose that rats can take without any perturbation of homeostasis.

The juice was administered daily for 28 days at the same time. The animals were observed at least twice daily for morbidity and mortality. Body weight of the animals was evaluated daily. At the 29th day, after an overnight fast, rats were anaesthetized and blood sample was collected for haematological and biochemical analysis into tubes with or without EDTA respectively. Haemoglobin (Hb), haematocrit (Ht), red blood cells count (RBC), white blood cells count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and platelet count were determined using automatic counter Sysmex (K21, Tokyo, Japan). Biochemical analysis was performed in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min. Standardized diagnostic kits (Labkits) and a Biotrons spectrophotometer were used for spectrophotometrical determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase (ALP), glucose (Glu), total proteins, γGT and urea. Necroscopy of all animals was carried and the organ weights (heart, testis, colon, liver, kidney and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each rat (relative organ weight).

Histological study of organs was done after sacrificing the animals on 29th day. Organs were removed, fixed in 10% formaldehyde, embedded in paraffin and sectioned at 5 mm. Tissue sections were stained with haematoxylin eosin (H and E) for general morphology.

Cardiac activity study in toad

After toads demedullation, a midline ventral incision was made and the pectoral girdle and xiphoid process were removed to expose the heart and major vessels. The apex of the heart with suspended in a fine wire bonded to the transducer coupled to a PowerLab recording system and an application software LabChart all ADI Instruments, listing the frequency and amplitude heart. The software was always calibrated before each experiment. The different doses of the juice and digoxin were administered directly to the heart in vivo. Each test was repeated 5 times [15].

Statistical analysis

The software GraphPad Prism 6.02 (Graph Pad Software, Inc. USA) was used to analyze our results. These were expressed as mean value accompanied by the standard error of the mean (m ± SEM). The number of (n) value was specified in each case. The analysis of variance (ANOVA) was used to compare different groups. The difference between two groups was determined using the Tukey test. The significance level was set at $p < 0.05$

III. RESULTS

Phytochemical screening

The phytochemical screening showed the presence of traces of alkaloids, flavonoids, saponins, tannins and cardiac glucosides. Saponins and tannins are absent.

Sub-chronic toxicity

The ''*Natural bio juice''* resulted in no significant change in rats body weight (Table 1). But there is a significant increased ($p < 0.001$) in liver relative weight at the dose of 1.7 mL/kg and 10 mL/kg body weight (Table 2).

The extract at 1.7 mL/kg and 10 mL/kg body weight increased significantly ($p < 0.001$) the number of platelets after 28 days (Table 3). The table 4 shows the biochemical analysis. *Natural bio juice* decreased significantly ($p < 0.0001$) the blood sodium level.

No significant damage was observed in histological sections of testis, heart, colon, liver, kidney and spleen

The effect of *Natural bio juice on* the toad heart

The ''*Natural bio juice''* at 0.1; 1; 10 and 100 mg/mL caused an increase in toad cardiac amplitude (positive inotropic) and a significantly decrease ($p < 0.0001$) in the heart rate (negative chronotropism) (Table 5).

IV. DISCUSSION

The use of herbal plants as natural remedies, functional foods, and dietary supplements for health care has been increasing in the world. Market estimates suggest that the rate of growth in sales of traditional medicinal products in recent years has been between 5% and 18% per annum [15-16].

In this study we have evaluated the toxicity of ''*Natural bio juice''* is phytomedicine based on *G. kola.* Because of a lack of previous studies on this product, our results were compared to *G. kola* data. The phytochemical screening of *"Natural bio juice"* showed the presence of cardiac glycosides. This result is similar to those of Adesuyi *et al.*, who found a high amount of cardiac glycosides in *G. kola* [1]. Ebana *et al.,* also noted the presence of cardiac glycosides in *G. kola* [17]. We found flavonoids and alkaloids in *"Natural bio juice"*. These results confirm those reported by other authors who found also high levels of flavonoids and alkaloids in *G. kola* [1, 18]. Saponins and tannins were absent, in contrast with Adeyusi *et al.* [1] results who reported a high rate of saponins and low levels of tannins.

In this study, we evaluated the sub-chronic toxicity of *"Natural bio juice"* by using the OECD guidelines 407. ''*Natural bio juice*'' have induced no significant change in body weight of rats. The administration of *''Natural bio juice''* to rats resulted in no significant change in rat's organ relative weight except a significant increase ($p < 0.001$) in liver relative weight at 1.7 mL/kg and 10 mL/kg body weight. The assessment of the weight of organs such as the liver, kidney, spleen, testes, heart, pancreas, brain and language are very important in toxicological studies. The weight of a body or more, the relative weight is an important index used in physiology and toxicology [19]. The increase in organ relative weight could be attributable to induction of xenobiotic enzymes leading to increased proteins synthesis. The induction of these enzymes frequently results in an increased of the mean of organ relative weight following an exposure to xenobiotic [20]. One may also argue that these changes could be toxicologically significant especially for the liver [21]. This result is similar to that reported by Sewani-Rusike *et al.* [8] on the activity of the hydro-ethanol extract 70% of *G. kola*. Histological sections performed on the heart, liver, testes and kidneys showed no histological lesions.

''Natural bio juice'' at 1.7 and 10 mL/kg decreased significantly ($p < 0.0001$) rats platelet counts after 28 days. Achieving the blood count is very important in toxicological studies. The hematopoietic system is one of the main targets of toxic substances, and consequently, an important parameter in the pathophysiology of human or animal [22-25]. Platelets have a crucial role in the coagulation process, they maintain a balance between bleeding and obstruction of vessels (thrombus) and are used to prevent bleeding inside the body. The decrease in platelets sign a thrombocytopenia and may result in bleeding risk. The platelet decrease observed with *"Natural bio juice"* was reported by El Haouari and Rosado [26] who have seen this activity in some plant families including Asteraceae, Rutaceae, Fabaceae, Lamiaceae, Zygophyllaceae, Rhamnaceae, Liliaceae, and Zingiberaceae family. Similarly, they linked this activity to the presence of phytochemicals groups such as polyphenols and flavonoids which are found in the *''Natural bio juice''* [27].

On toad heart, ''*Natural bio juice''* has increased the cardiac amplitude (positive inotropic) and a significantly decrease ($p < 0.001$) the cardiac frequency (negative chronotropism). These effects may be due to cardiac glycosides contained in *''Natural bio juice''*. Cardiac glycosides increase the contractile force (positive inotropic effect) by increasing the intracellular calcium during systole. ''Natural bio juice'' chemicals bind to a specific site of the Na-K-ATPase. Depolarization occurs at the opening of fast sodium channels. In addition, calcium flux induces the release of calcium from the sarcoplasmic reticulum, producing contraction [27]

V. CONCLUSION

Our results have shown that *''Natural bio juice''* can be toxic to human liver, blood and heart after a long-term administration. Cardiac disorders are characterized by an increase in the force of contraction and a significant decrease in the heart frequency. Therefore, some caution should be taken when administering *''Natural bio juice''* for long periods. These toxics effects of *''Natural bio juice''* can be attributed to it contain in cardiac glycosides.

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AUTHORS CONTRIBUTIONS

All authors have participated in the study especially in data collection

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Table 1: Effect of ''*Natural bio juice''* on body weight of rats

Parameters	Control	Extract dose	
		500 mg/kg	1000 mg/kg
0	198.5 ± 9.58	197.8 ± 8.05	203.4 ± 17.22
1	209.5 ± 8.66	200.8 ± 9.74	197.6 ± 17.86
2	214.6 ± 8.41	203.1 ± 10.54	199.0 ± 17.88
3	220.8 ± 8.80	204.6 ± 9.88	202.4 ± 15.79
4	215.0 ± 9.99	206.6 ± 11.07	201.4 ± 13.61

Each value represents the mean ± SEM n (the number of animals per group) = 6

Table 2: Effect of ''*Natural bio juice''* on relative organ weight

Organs	Control	Extract dose	
		500 mg/kg	1000 mg/kg
Heart	0.42 ± 0.02	0.35 ± 0.01	0.37 ± 0.02
Rate	0.22 ± 0.02	0.19 ± 0.02	0.19 ± 0.01
Testis	**1.16 ± 0.20**	**0.97 ± 0.19**	**0.78 ± 0.22***
Liver	3.04 ± 0.10	3.19 ± 0.09	3.21 ± 0.09
Kidney	0.57 ± 0.02	0.61 ± 0.01	0.03

Each value represents the mean ± SEM. n (the number of animals per group) = 6. *$p<0.05$ significant difference as compared the control.

Table 3: Effect of ''*Natural bio juice''* on hematological parameters.

Parameters (unit)	Control	Extract dose	
		500 mg/kg	1000 mg/kg
WBC (10^3/UL)	6.53 ± 0.97	5.73 ± 0.54	4.94 ± 0.34
Haemoglobin (g/dL)	14.13 ± 0.41	14.05 ± 0.30	14.28 ± 0.20
RBC (10^6/UL)	8.27 ± 0.15	8.30 ± 0.18	8.40 ± 0.05
Haematocrit (%)	42.95 ± 1.19	42.16 ± 1.12	43.10 ± 0.56

MCV (fL)	51.91 ± 0.67	50.88 ± 0.63	51.34 ± 0.42
MCH (pg)	17.01 ± 0.26	16.86 ± 0.13	16.94 ± 0.16
MCCH (g/dL)	32.85 ± 0.11	33.30 ± 0.38	33.08 ± 0.15
Platelet (10^3/UL)	**696.83 ± 60.13**	**717.16 ± 72.75***	**798.80 ± 66.02****

Each value represents the mean ± SEM. n (the number of animals per group) = 6. ** p <0.001 significant difference in the dose 1000 mg/kg compared with the control. * p <0.05 significant difference as compared to control.

Table 4: Effect of ''*Natural bio juice''* on biochemical parameters.

Parameters (Unit)	Control	Extract dose	
		500 mg/kg	1000 mg/kg
Urea (g/L)	0.21 ± 0.09	0.20 ± 0.01	0.19 ± 0.01
Creatinine (mg/L)	7.66 ± 0.49	7.16 ± 0.16	7.20 ± 0.20
ASAT (UI/L)	109.00 ± 23.96	83.66 ± 7.89	91.80 ± 6.86
ALAT (UI/L)	58.00 ± 13.54	48.16 ± 3.89	54.00 ± 3.64
ɣGT (UI/L)	3.50 ± 0.22	3.50 ± 0.56	2.60 ± 0.60
Alkaline phosphatase (UI/L)	174.50 ± 13.05	141.50 ± 11.18	164.40 ± 13.70
Glucose (mg/dL)	88.66 ± 5.76	100.83 ± 4.67	106.00 ± 11.52
Na^+ (mmol/L)	**427.08 ± 61.72**	**416.66 ± 57.44*****	**307.29 ± 51.52*****
Cl^- (mmol/L)	9.96 ± 2.16	15.75 ± 2.66	14.42 ± 1.45
K^+ (mmol/L)	15.40 ± 2.10	18.44 ± 1.37	13.22 ± 1.99
Ca^{2+} (mmol/L)	6.92 ± 2.37	3.93 ± 0.94	2.89 ± 0.42

Each value represents the mean ± SEM. n (the number of animals per group) = 6. *** p <0.0001 significant difference as compared with the control.

Table 5: Effect of ''*Natural bio juice''* on the frequency and amplitude of the heart

Parameters	Control	Extract dose			
		0.1 mg/ml	1 mg/ml	10 mg/ml	100 mg/ml
Frequency	71.20 ± 3.51	68.80 ±3.95***	65.40 ± 4.38**	58.80 ± 5.33	47.60 ±5.00***
Amplitude	0.76 ± 0.12	0.79 ± 0.12	0.81 ± 0.12	0.84 ± 0.14	0.95 ± 0.17

Each value represents the mean ± SEM. n (the number of reply) = 5. *** p <0.0001 significant difference as compared to the control. ** p <0.01 significant difference as compared to the control.