

Received: 01 May, 2023

Accepted: 10 May, 2023

Published: 11 May, 2023

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Keywords: Toxicity; *Aframomum melegueta*; *Garcinia kola*; *Picralima nitida*; Wistar rat

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Research Article

Evaluation of the toxicity of aqueous extracts of *Aframomum melegueta*, *Picralima nitida*, and *Garcinia cola* in Wistar rats

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Summary

In order to determine the risks to human health associated with the use of certain medicinal plants, including *Aframomum melegueta*, *Garcinia kola* and *Picralima nitida* in a preclinical evaluation of the resistance, a pool of these three aqueous extracts was given once daily for ten days by gavage in Wistar rats. Haematological and biochemical analyzes after oral administration revealed a decrease in certain hepatic biomarkers such as glucose, Alanine Aminotransferase (ALT), etc., and renal biomarkers such as urea, creatinine, and creatinine kinase); increase in certain biomarkers such as Aspartate Transaminase (AST), an indicator of kidney and liver capacity.

Introduction

All of humanity has invested in seeking natural substance-based therapy against SARS-CoV-2 during the COVID-19 pandemic, almost all of which did not mention structured clinical trials. The cost of living in countries with limited resources has not allowed the population of the Democratic Republic of the Congo to receive proper treatment and above all to observe the prophylactic measures recommended by the WHO. Traditional medicine offers a wide opportunity for treatment at an affordable price, but for which the scientific evidence has not always been demonstrated. Whereas the

population of these countries resorts to medicinal plants, the effectiveness of which most authors have shown in the treatment of communicable and non-communicable diseases without conducting a structured clinical trial [1-7]. Among these plants, there are *A. melegueta* and *G. kola* [8] which, together with *P. nitida* are investigated in this study. However, Iserin has combined the latter with six other plants including, *Catharanthus roseus*, *Senna occidentalis*, *Rauwolfia vomitoria*, *Tamarindus indica*, *Combretum micranthum*, *Guiera senegalensis*, *Euphorbia hirta*, *Allium sativum*, *Hibiscus sabdarifa* to treat pathology other than COVID-19 [9-11]. However, the obvious enthusiasm for phytotherapy does not take into account aspects

related to toxicity, resulting from a lack of clinical trials with monitoring.

The objective of the study is to contribute to the evaluation of the toxicity of these three plants used in the treatment of human diseases, including COVID-19.

Material and methods

Site of study

The study was carried out at the Zoonoses Department of the National Institute for Biomedical Research (INRB).

Experimental model and laboratory analysis

22 male and female Wistar rats (*Rattus norvegicus*), weighing between 140 and 225 g bred at the INRB were retained in this study.

Choice of plant extracts: The plant materials were collected from herbalists on the market of Kinshasa and taxonomically identified by the Botanists of the Herbarium at the Department of Biology (University of Kinshasa). Air-dried and powdered-plant materials were then submitted to aqueous decoctions (10%) and freeze-dried (Christ-Alpha 1 - 4 LSC, Germany). The mixture sample consisted of the 3 prepared extracts in equal parts.

Animal facilities

Wistar rats of either sex (140 - 225 g body weight) bred at the INRB were used for this study. The animals were kept under a standard condition maintained at 23 °C -25 °C, and given a standard INRB homemade pellet diet.

Laboratory analysis

The assessment of the safety of plant extracts was investigated through a subacute toxicity assay in rats [12]. A mixture of the aqueous extracts from the 3 plants (*A. melegueta*, *G. kola*, and *P. nitida*) on one side and an aqueous extract of *A. melegueta* on the other side, were given once daily for ten consecutive days by oral gavage (500 mg/kg) to Wistar rats.

The control rats (Group I) received 0.5 ml of the vehicle, distilled water alone. Toxic manifestations and mortality were monitored daily. Haematological and biochemical analyzes were carried out on blood samples at the end of the gavages. The experimental protocol was approved by the Animal Ethics Committee of the INRB.

The control rats were subjected to physiological water; two other groups of rats were, one force-fed with a unique extract of *A. melegueta* and the other stuffed with three extracts (*A. melegueta*, *G. kola*, and *P. nitida*).

The high frequency of use of plants from the ginger family, including *A. melegueta* in the treatment of human diseases in DR Congo, has led to the decision to evaluate its toxicity on its own.

A. melegueta has exceptional characteristics that distinguish it from other medicinal plants especially:

(1) Its high concentration of sesquiterpenes and phenylpropanoids, which are responsible for its unique aroma and flavor [13,14]; (2) It had higher antioxidant and anti-inflammatory activity, which can be attributed to its high content of 6-paradol, a bioactive compound found only in *A. melegueta* [15]; (3) It had stronger antimicrobial activity against several strains of bacteria and fungi, which can be attributed to its high content of 6-paradol and other bioactive compounds [14].

The total dose of the products was 500mg. The volume of products used was calculated as follows:

$$V_{max} = \frac{\text{Weight} \times 1ml}{\text{Total dose}}$$

Laboratory analysis

SYSMEX (XN330) hematology and Piccolo XPRESS 21.31 biochemistry analyzers were used according to the manufacturer's instructions with valid reagents.

Statistical analysis

In this study, it was assumed that the biochemical and hematological parameters, as well as the blood ionogram (dependent variables), depended on the types of medicinal plants used.

To verify that, linear regression was used to compare the different parameters to controls.

For comparing the biochemistry, hematology, and blood ionogram parameters of different groups of rats, a p-value threshold of 0.05 was used.

Results

A single case of mortality out of the 22 experimental rats corresponds to 4.5%.

Biochemistry, hematology, and blood ionogram parameters

A significant decrease ($p < 0.05$) in glucose was observed in the rats force-fed with the three extracts and in ALAT in the rats force-fed with Aframomum, unlike the rats force-fed only with *A. melegueta*.

Note also the absence of significant variations in ASAT and total bilirubin levels.

Regarding kidney biomarkers, an increase ($p < 0.05$) in plasma levels of urea, uric acid, and creatinine was observed in rats exposed to *A. melegueta* in comparison with rats in the control group.

Sodium (Na^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) ions were significantly increased in rats treated with the three extracts. In addition, rats treated with *A. melegueta* only experienced an increase in Na^+ , K^+ , Ca^{2+} , and Mg^{2+} ions followed by a decrease in phosphorus levels [Tables 1-3].

**Table 1:** Plasma level of biochemical parameters of experimental rats.

	Group of rats		
	Control	Mixt	Single
Biochemical parameters			
1. Hepatic and pancreatic biomarkers			
Glucose (mmol/l)	91,8 ± 2,12	47,4 ± 2,36*	177,5 ± 10,3*
Total bilirubin (µmol/l)	0,24 ± 0,013	0,23 ± 0,012	0,3 ± 0,0
ALT (UI/l)	122 ± 0,9	74,7 ± 4,3	29,4 ± 3,2*
AST (UI/l)	138,8 ± 9,2	210,8 ± 11,6	134,5 ± 1,1
Amylase	-	-	508,5 ± 2,6
2. Kidney Biomarkers			
Urea (mmol/l)	15,6 ± 1,05	9,1 ± 0,8	14,5 ± 0,2
Creatinine (mol/l)	0,6 ± 0,04	0,22 ± 0,03*	0,35 ± 0,02
Creatinine Kinase (CK)			516 ± 14,8
CRP			4,5 ± 0
3. Lipid biomarker			
Lipid	-	52,8 ± 5,96	-
ICT	-	0,13 ± 0,04	-

Treated vs. controls* : $p < 0,05$.

ALT: Alanine Transaminase; AST: Aspartate Transaminase; CRP: C-Reactive Protein

Table 2: Hemogram of rats.

	Groups of rats		
	Control	Mixt	Single
Hematologic parameters			
WBC	6,6 ± 0,18	3,7 ± 0,12*	5,1 ± 0,44
RBC	8,6 ± 0,15	8,6 ± 0,07	9,8 ± 0,07
Hemoglobin	14,1 ± 0,24	14,2 ± 0,13	16 ± 0,08
Haematocrit	48,2 ± 0,7	49,8 ± 0,38	54 ± 0,54
Platelet	1233 ± 40,7	1078 ± 30,9	973 ± 6,8
MCV	16,3 ± 0,21	16,4 ± 0,07	16,4 ± 0,02
MCHC	29,1 ± 0,12	28,5 ± 0,11	29,5 ± 0,14
Neutrophil	23 ± 0,57	21 ± 0,72	26 ± 0,42
Leucocytes	63 ± 0,95	58,4 ± 0,11	56 ± 0,11
Monocytes	2,6 ± 0,11	3,3 ± 0,05	8,5 ± 0,92
Eosinophil	2,2 ± 0,28	2 ± 0,12	1 ± 0,14
Basophil	9,8 ± 0,4	16,07 ± 0,5*	7 ± 0,4
Blood ionogram			
P ⁻ (mmol/l)	4 ± 0,1	3,5 ± 0,06	3,4 ± 0,02
Mg ²⁺ (mmol/l)	1,02 ± 0,013	1,08 ± 0,01	1,14 ± 0,02

Treated vs. controls* : $p < 0,05$.

MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; WBC: White Blood Cell; RBC: Red Blood Cell

Table 3: Ionogram.

Blood ionogram			
Na ⁺ (mmol/l)	103,8 ± 5,8	114,8 ± 4,8	136,5 ± 0,4
K ⁺ (mmol/l)	4,84 ± 0,3	4,8 ± 0,3	7 ± 0,2
Ca ²⁺ (mmol/l)	10,2 ± 0,08	11 ± 0,11	11,2 ± 0,13

Treated vs. controls* : $p < 0,05$.

Discussion

Mortality rate of rats after gavage

Compared to the obtained results (4.5% mortality due to force-feeding of *A. melegueta*), a previous study by Mozaffari-Khosravi, et al. [16] showed that oral administration of powdered ginger up to 2000 mg/kg to male and female rats was not associated with any mortality.

The mortality of the rat recorded during the investigations could be due to poor force-feeding as well as to the dosage of *A. melegueta* that was used in this study. The intrinsic characteristics of the rat would also explain this mortality.

Biochemical parameters

It has been reported that extracts from the ginger family, in particular *A. melegueta*, interfere with the activities of certain digestive enzymes. [16,17]. In animals with diabetes, apolipoprotein E gene deficiency, or fed a high-fat diet, extracts of the ginger family significantly reduced serum total cholesterol, LDL, VLDL and triglycerides, and increased HDL [18-20].

Ginger has also been found to act on the liver to reduce cholesterol biosynthesis and may stimulate the conversion of cholesterol into bile acids and increase its fecal excretion [20].

On the other hand, Sharma, et al. [21] demonstrated that extracts of gingers increased the activity of pancreatic lipase and amylase when directly in contact with the enzyme. However, Han, et al. [22] recently demonstrated that an aqueous extract of ginger inhibited the hydrolysis of phosphatidylcholine-emulsified triolein by pancreatic lipase *in vitro* and reduced the elevation of plasma levels of rat triacylglycerols after oral administration of a lipid emulsion containing corn oil.

The present study was unable to demonstrate the different variations in lipid levels as well as amylases.

Treatments of rats with ginger powder up to 2000 mg/kg for 35 days did not affect blood glucose, total cholesterol and triglyceride levels, and platelet counts in male and female rats [16].

These results suggest that ginger extracts do not interfere with glucose and lipid metabolism, nor with platelets in a physiological context [23].

Our results of the dosage of hepatic and pancreatic biomarkers showed a significant decrease ($p < 0.05$) in glucose in the rats force-fed with the three extracts and in ALT in the rat force-fed with *A. melegueta*. The decrease in blood glucose is similar to that reported by Tankeu, et al.; Ngo [24,25].

A significant ($p < 0.05$) increase in glucose levels was observed in rats fed at *A. melegueta*.

The specific characteristics of *A. melegueta*, distinct from other extracts of the order *Gingemberaceae* and could explain the increase in glucose in the setting in this study.



Changes in blood biochemical parameters called toxicity markers including ALT, AST, bilirubin, creatinine, and urea are signs indicating the toxicity of a drug [26] and these disorders of toxicity, often appear after a long impregnation of the extract in the organism [27]. Recent studies have demonstrated that ginger exhibits considerable anti-inflammatory, antioxidant, antiplatelet, hypotensive, and hypolipidemic effects *in vitro* and *in vivo* [28,29]. Treatment with ethanolic extract of ginger in isoproterenol-treated rats increased levels of endogenous myocardial antioxidants (catalase, superoxide dismutases, and tissue glutathione), decreased levels of serum marker enzymes [LDH, creatinine kinase, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)] and increased myocardial lipid peroxides.

Oral administration of an aqueous extract of ginger at a dose of 600 mg/kg for 6 days has been reported to significantly increase relative testicular weight, serum testosterone level and testicular cholesterol level in Wistar rats suggesting the androgenic activity of ginger [30,31].

Conclusion

At the end of the investigations aimed at contributing to the assessment of the toxicity of *A. melegueta* (Mondongo) force-fed in Wistar rats, the following results were obtained:

A significant decrease in biochemical parameters such as blood sugar (mixed extracts), ALAT (single extract), and creatinine (mixed). Rats fed with *A. melegueta* experienced a significant increase in blood glucose. In addition, a single case of mortality was observed after force-feeding.

Among the haematological parameters, only WBC showed a significant decrease.

After force-feeding aqueous extracts to Wistar rats, changes in biomarker concentrations such as ALT, creatinine, and glucose reveal the toxicity of these plants (*Aframomum melegueta*, *Garcinia kola*, *Picalima nitida*), indicating that their consumption should be previously controlled.

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