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Evaluation of the Effects of Methanolic Extract of Alligator Pepper (*Aframomum melegueta*) on Serum creatinine, Blood Urea Nitrogen and Electrolytes in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the study, wrote the protocol and first draft of the manuscript. Author MIE performed the statistical analysis, and wrote part of the manuscript. Author YNO did the literature search and also wrote part of the manuscript. Author JTE managed the animals, analyses of the study and collected all data. All authors read and approved the final manuscript.

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ABSTRACT

The effect of methanolic extract of Alligator pepper (*Aframomum melegueta)* (AME) on the serum creatinine, blood urea nitrogen (BUN) and electrolytes were investigated in Wistar albino rats. The extraction was done by cold maceration of pulverized seeds in absolute methanol for 48 hours. The extract was filtered and concentrated *in vacuo* in a rotary evaporator at 40ºC. Twenty four male

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albino Wistar rats were randomly assigned into four groups of six animals each. Group A served as the control and received 10 ml/kg of 5 % Tween 20 while Group B-D received 100, 200 and 400 mg/kg respectively, of the AME for 21 days. Twenty four hours later, blood was collected from the rats through direct cardiac puncture. The separated serum was used to determine the serum creatinine, BUN and electrolyte assay. The extract produced no significant ($p > 0.05$) difference in mean serum creatinine, BUN and electrolytes level in the treated rats when compared to the control group rats. In conclusion the extract did not exhibit any adverse effect on the kidney at the doses used and for the period of experiment.

Keywords: Aframomum melegueta; creatinine; blood urea nitrogen; electrolyte.

1. INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made large contribution to human health and well being. Despite the achievements recorded in drug discovery and development from plant sources, phytomedicine continues to be highly valuable for developing synthetic drugs [1,2]. According to the World Health Organization (WHO), about 80% of the world's population depends wholly or partly on pharmaceuticals of plant origin [3]. Thus, the need for toxicity evaluation of these medicinal plants, cannot be underscored [4], such research could advance the utilization of these indigenous herbal medicine for orthodox treatment [5] or to serve as new dietary supplements which consist of minerals that may be used for the treatment of organ related diseases. Kidney is the major organ of excretion of drug and its metabolite from the system. Some of the toxic effect of drugs manifest clinically in the kidney by either enhanced or reduced reabsorption of electrolyte by the kidney tubules [6].

Aframomum melegueta Schum (*Zingiberaceae*) also known as alligator pepper (indigenous names include: Atare in Yoruba, Ose-oji in Igbo and Citta in Hausa) is used in Nigeria and some other parts of West Africa, as a spicy during entertainment and have a wide range of folkloric uses in traditional medicine. They are used as a remedy for treating stomach ache, diarrhoea and snakebite [7,8]. The seed extract have been evaluated for antinociceptive, anti-ulcer, anti-microbial, anti-inflammatory, anti-oxidant and sexual performance enhancing activities [7-11]. There is paucity of information on the effect on the kidney tubule. The present study was aimed at evaluation of the serum electrolyte and renal function markers of methanolic extract of alligator pepper seed.

2. MATERIALS AND METHODS

2.1 Plant

The freshly harvested fruit of *A. melegueta* were bought from Ndoro market, Oboro in Ikwuano LGA of Abia State in the month of July 2013 and were authenticated by Dr. I. C. Okwulehie of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike and the voucher specimen catalogued MOUAU/CVM/VPP/2013/03 was kept in the departmental herbarium for reference purpose.

2.2 Preparation of the Extract

The dehaulled, dried and pulverized seeds of *A. melegueta* were extracted by cold maceration method for 48 hours at room temperature using absolute methanol in a Winchester bottle. The *A. melegueta* extract (AME) was filtered with Whatmann No 1 filter paper. The filtrate was concentrated *in-vacuo* using vacuum rotary evaporator at 40ºC and was later concentrated to dryness in a hot air oven at 40ºC. The extract was stored in a refrigerator at 4ºC throughout the duration of this study.

2.3 Determination of the Yield of the AME

An empty clean and dry beaker was weighed and later the extract was poured into it. The beaker was weighed after the extract has been concentrated to constant weight. The weight of the extract was calculated as follow:

The percentage yield of extract (%) w/w =

$$\frac{weight\ of\ beaker\ and\ extract - wieght\ of\ empty\ beaker}{weight\ of\ plant\ material} \times \frac{100}{1}$$

2.4 Animals

Twenty four male Wistar albino rats weighing about 120 - 170 g were obtained from Department of Zoology, University of Nigeria, Nsukka and kept in the Animal house of the Biochemistry Department, Michael Okpara University of Agriculture, Umudike. The animals were allowed access to feed and water *ad libitum* and kept two weeks for acclimatization before the commencement of the experiment. The animals were kept in well ventilated aluminium cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the *Guide for the care and use of laboratory animals* [12]. The experiment was approved by the University Animal Ethics Committee with ref. MOUAU/CVM/EAEC/2013/201.

2.5 Phytochemical Spot Test

The AME was tested for the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenes/sterols, carbohydrates, and starch using the standard procedures as described by Trease and Evans [13].

2.6 Experimental Design

Twenty four male albino Wistar rats were randomly assigned into four groups of six animals each. Group A served as the control and received 10 ml/kg of 5 % Tween 20 (used in extract dissolution) while Group B-D received 100, 200 and 400 mg/kg respectively, of the AME. The animals were treated orally daily for 21 days with the aid of stomach tube and were observed daily for changes and other signs of toxicity and death throughout the period of study. Twenty four hours after the last treatment blood sample obtained through direct cardiac puncture was used to assay for effects of AME on serum electrolyte, urea and creatinine levels.

2.7 Body Weight and Body Weight Gain

Individual body weights were recorded at study initiation (day 0), and weekly thereafter. Mean body weight gains were calculated for each group at each interval and for the overall (days 0–21) testing interval. Animals were also weighed immediately prior to sacrifice (fasted body weight) for calculation of relative organ weight.

2.8 Biochemical Analysis

After 21 days of extract administration the rats were fasted overnight, only tap water was made available *ad libitum*. Blood samples were collected through direct cardiac puncture under chloroform anesthesia using a 5 ml syringe into plain sample bottles. The blood sample was centrifuged at 4000 RPM for 5 minutes in order to separate the serum. The clean sera were aspirated and stored frozen for serum biochemical analysis. Serum creatinine, urea and electrolytes (Na^+, K^+ Cl^-, HCO^-), were determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturer's instructions.

2.9 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The methanolic extraction of the dried seed of *A. melegueta* yielded 8.34 % w/w dry extract which was oily and dark-brown in colour.

The phytochemical spot test showed that the extract contained saponins, tannins, terpenes/sterols, glycosides, alkaloids, flavonoids.

The result of the effect of AME treatment on the body weight and percentage weight gain is presented in Table 1. The extract did not produce any significant ($p > 0.05$) difference in the body weight and percentage weight gain in the treated rats, when compared to the negative control group rats.

The result of the relative kidney weight of AME treated rats is presented in Fig. 1. The extract did not produce any significant ($p > 0.05$) difference in the relative kidney weight of treated rats, when compared to the negative control group rats.

The effect of AME on the mean serum creatinine, urea and electrolytes of the treated rats is presented in Tables 2 and 3. The extract did not produce any significant ($p > 0.05$) difference in

the mean serum creatinine, urea and electrolytes of treated rats, when compared to the negative control group rats.

3.2 Discussion

Kidney is the main organ by which drug and their metabolites are eliminated from the body. It is the target for drug toxicity. The main function of the kidney is to maintain the constancy of the "interior environment" by eliminating waste products and by regulating volume, electrolyte content and pH of the extracellular fluid in the face of varying environmental demand [6].

The present work evaluated the subacute effect of AME on the serum creatinine, urea and electrolytes levels in treated rats. The doses used in this study were based on report of previous studies on *A. melegueta* [7-11]. The result of the experiment showed that the extract has no adverse effect on kidney at the doses used and for the period of the experiment, when compared to the negative control. Increasing the dose may elicit some adverse effects. Toxicity depends on the dose of the intoxicant [14].

The serum creatinine, blood urea nitrogen and electrolyte are used to evaluate the functional capacity of the kidney [15]. The result of the effect of AME on serum blood urea nitrogen level of the treated rats may suggest that the extract has no the kidney function and protein catabolism. Creatinine is metabolic waste product of creatine and creatine phosphate. It endogenous production is constant and directly proportional to muscle mass of the individual. Its serum level is an index of renal function and measures the glomerular filtration rate. Creatinine is readily filtered and does not undergo any significant tubular reabsorption. The serum level of creatinine increases when the formation or excretion of urine is impaired [16]. In this present study there was no significant ($p > 0.05$) difference in the body weight of all the AME treated groups when compared to the control group, which indicate that the muscle mass were not affected.

The result of the effect of AME on serum blood urea nitrogen level of the treated rats may suggest that the extract has no effect on the kidney function and protein catabolism. Urea is the major nitrogen-containing metabolic waste product of protein catabolism in mammals. It is formed from exogenous or endogenous protein from the breakdown of cell in the body [16]. More than 90% of the urea is excreted by the kidneys, and the remainder is lost through the gastrointerestinal tract and skin. The measurement of BUN is used to evaluate renal function [15].

In the present study the extract produced no significant ($p > 0.05$) difference in all the serum electrolyte levels measured when compared to the negative control. This may suggest that the extract may not have any electrolyte reabsorption, secretion and diuretic effect in the kidney tubule.

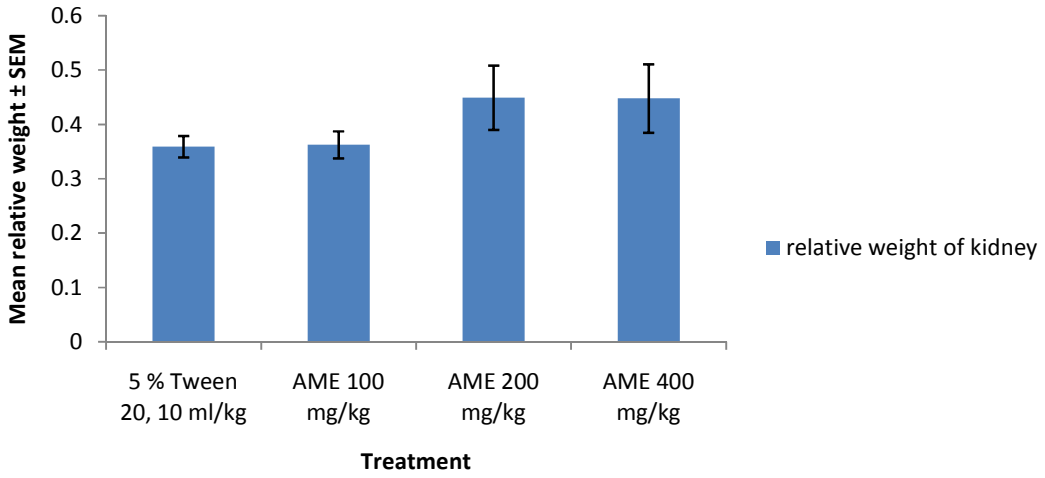

Fig. 1. Effect of AME on the relative kidney weight

Table 1. Effect of AME on the relative kidney weight

Group	Mean body weight in gram ± SEM (percentage increase in weight compared to day 0)			
	Day 0	Day 7	Day 14	Day 21
5 % Tween 20, 10 ml/kg	137.14±4.09	153.96±1.41 (11.74)	169.12±2.05 (15.32)	174.02±2.80 (17.49)
AME 100 mg/kg	124.24±11.37	140.13±12.50 (12.14)	150.94±12.16 (21.33)	159.54±13.31 (28.63)
AME 200 mg/kg	128.38±6.61	142.52±5.54 (11.57)	143.94±5.07 (11.71)	146.18±4.66 (16.54)
AME 400 mg/kg	139.38±10.14	151.15±10.71 (8.92)	155.58±9.14 (10.22)	160.55±7.36 (15.98)

No statistical difference (P > 0.05) compared with control group

Table 2. The effect of AME on the kidney function test of the treated rats

Group	Urea (mg/dl)	Creatinine (mg/dl)
5 % Tween 20, 10 ml/kg	15.96±3.72	0.56±0.08
AME 100 mg/kg	19.38±4.60	0.20±0.08
AME 200 mg/kg	27.22±2.73	0.92±0.28
AME 400 mg/kg	21.50±3.17	0.51±0.05

No statistical difference (P > 0.05) compared with control group

Table 3. The effect of AME on the mean serum electrolyte of the treated rats

Group	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Carbonate (mmol/l)
5 % Tween 20, 10 ml/kg	111.01±28.64	9.89±4.43	166.10±6.85	36.73±1.02
AME 100 mg/kg	98.56±20.18	3.00±2.04	167.23±26.88	33.98±3.19
AME 200 mg/kg	109.93±28.65	8.78±1.90	145.76±27.56	33.19±4.66
AME 400 mg/kg	114.44±11.85	6.11±2.30	162.71±6.85	36.55±2.00

No statistical difference (P > 0.05) compared with control group

4. CONCLUSION

In conclusion, the extract did not exhibit any adverse effect on the kidney at the doses used and for the period of experiment, but may exhibit toxic effect at higher dose and/or more prolonged period treatment.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declarations of Helsinki and Michael Okpara University of Agriculture, Umudike, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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