

Original Research Article

Evaluation of the effects of methanolic extract of alligator

pepper (*Aframomum melegueta*) seed on serum electrolyte and renal function markers of serum creatinine, blood urea nitrogen and electrolytes in Wistar Albino rats.

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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 albino rats. The extraction was done by cold maceration of pulverized seeds of alligator pepper in absolute methanol for 48 hours. The extract was filtered and concentrated *in vacuo* in a rotary evaporator at 40 °C. Twenty male albino Wistar rats were randomly divided allocated into four groups of five animals in each. Group A served as the control and received 10 ml/kg of 5 % Tween 20 while Group (B- to D) received 100, 200 and 400 mg/kg, respectively, of the AME. The animals were dosed daily 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

27 toxicity evaluation of these medicinal plants, cannot be underscored [4], such research could advance the
28 utilization of these indigenous herbal medicine for orthodox treatment [5] or to serve as new dietary
29 supplements which consist of minerals that may be used for the treatment of organ related diseases.
30 Kidney is the major organ of excretion of drug and its metabolite from the system. Some of the toxic effect
31 of drugs manifest clinically in the kidney by either enhanced or reduced reabsorption of electrolyte by the
32 kidney tubules [6].

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

41

42 **2. Materials and methods**

43 **2.1. Plant**

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

49 **2.2. Preparation of the extract**

50 The dehauled, dried and pulverized seeds of *Aframomum A. melegueta* were extracted by cold
51 maceration method for 48 hours at room temperature using absolute methanol in a Winchester bottle.
52 The *Aframomum A. melegueta* extract (AME) was filtered with Whatmann No 1 filter paper. The filtrate was
53 concentrated *in-vacuo* using vacuum rotary evaporator at 40 °C and was later concentrated to dryness in

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54 | a hot air oven at 40 °C. The extract was stored in a refrigerator at 4 °C throughout the duration of this
55 | study.

56 | 2.3. Determination of the yield of the AME

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

60 | The percentage yield of extract (%) w/w =
$$\frac{\text{weight of beaker and extract} - \text{weight of empty beaker}}{\text{weight of plant material}} \times \frac{100}{1}$$

61 | 2.4. Animals

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

Comment [DAHE1]: Twenty or twenty four

70 | 2.5. Phytochemical spot test

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

74 | 2.6. Experimental design 2

75 | Twenty male Wistar albino Wistar rats were randomly divided allotted into four groups of five animals each.
76 | Group A served as the control and received 10 ml/kg of 5 % Tween 20 while Group B-D received 100,
77 | 200 and 400 mg AME/kg, respectively, of the AME. The animals were dosed treated daily for 21 days and
78 | were observed daily for changes and other signs of toxicity and death throughout the period of study.

Comment [DAHE2]: Please, mention the route of treatment

Comment [DAHE3]: Twenty or twenty four

79 Twenty four hours after the last treatment blood obtained through direct cardiac puncture was used to
80 assay for effects of AME on serum electrolyte, urea and creatinine levels.

81 2.7. Body weight and body weight gain

82 Individual body weights were recorded at study initiation (day 0), and weekly thereafter. Mean body weight
83 gains were calculated for each group at each interval and for the overall (days 0–21) testing interval.
84 Animals were also weighed immediately prior to [killing-scarification](#) (fasted bodyweight) for calculation of
85 relative organ weight.

86 2.8. Biochemical analysis

87 After 21 days of extract administration the rats were fasted overnight, only distilled water was made
88 available *ad libitum*. Blood samples were collected through direct cardiac puncture under chloroform
89 anesthesia using a 5ml syringe into plain sample bottles. The blood sample was centrifuged at 4000 ~~rpm~~
90 [RPM](#) for 20 ~~mins~~ [minutes](#) in order to separate the serum. The clean sera were aspirated and stored frozen
91 for serum biochemical analysis. Serum [creatinine, urea and](#) electrolytes (Na^+ , K^+Cl^- , HCO_3^-), ~~urea and~~
92 ~~creatinine~~ were determined using standard ready-to-use kits (Randox Ltd, UK) following the
93 manufacturer's instructions.

96 2.9. Statistical analysis

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

101 3. RESULTS AND DISCUSSION

102 3.1 Results

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

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

Comment [DAHE4]: Please, support your result by photos

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

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

113 The effect of AME on the mean serum creatinine, urea and electrolytes of the treated rats is presented in
114 Table 2 and 3. The extract did not produce any significant ($p > 0.05$) difference in the mean serum
115 creatinine, urea and electrolytes of treated rats, when compared to the negative control group rats.

116

117

118 Table 1. Effect of AME on the relative kidney weight

Group	Mean body weight in gram $\pm$ 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 $\pm$ 4.09	153.96 $\pm$ 1.41 (11.74)	169.12 $\pm$ 2.05 (15.32)	174.02 $\pm$ 2.80 (17.49)
AME 100 mg/kg	124.24 $\pm$ 11.37	140.13 $\pm$ 12.50 (12.14)	150.94 $\pm$ 12.16 (21.33)	159.54 $\pm$ 13.31 (28.63)
AME 200 mg/kg	128.38 $\pm$ 6.61	142.52 $\pm$ 5.54 (11.57)	143.94 $\pm$ 5.07 (11.71)	146.18 $\pm$ 4.66 (16.54)
AME 400 mg/kg	139.38 $\pm$ 10.14	151.15 $\pm$ 10.71 (8.92)	155.58 $\pm$ 9.14 (10.22)	160.55 $\pm$ 7.36 (15.98)

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

120

121 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 $\pm$ 3.72	0.56 $\pm$ 0.08
AME 100 mg/kg	19.38 $\pm$ 4.60	0.20 $\pm$ 0.08
AME 200 mg/kg	27.22 $\pm$ 2.73	0.92 $\pm$ 0.28
AME 400 mg/kg	21.50 $\pm$ 3.17	0.51 $\pm$ 0.05

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

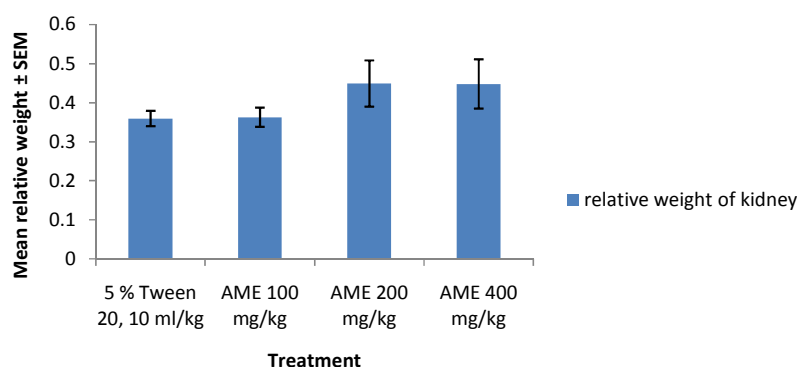
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Table 3. The effect of AME on the mean serum electrolyte of the treated rats

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



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Figure 1. Effect of AME on the relative kidney weight

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](#), [creatinine and urea](#) levels in treated rats. The doses used in this study were based on report of previous studies on [Aframomum 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. Its endogenous production is constant and directly proportional 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 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 gastrointestinal 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. ~~Diuretic agent (furosemide) act by inhibiting sodium chloride and water reabsorption at the loop of Henle of the nephron, which may lead to hyponatraemia or hyperkalaemia [17].~~

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.

171 **Conflict of interest statement**

172 We declare that we have no conflict of interest.

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176 **Reference**

- 177 1. AdeneyeAA,BeneboAS. Pharmacological evaluation of a Nigerian polyherbal health tonic tea in
178 rat.African Journal of Biomedical Research.2007;10(3):249-255.
- 179 2. Ogunka-NnokaCU, UwakweAA,NnabuikeCJ. Effects of Ethanolic /Potash Extract of Sorghum Bicolor
180 Leaf Sheath on Serum Electrolytes, Liver and Kidney Indicative on Albino Rats. Journal of Natural
181 Sciences Research. 2012;2(4):66-70.
- 182 3. World Health Organization. Guidelines for the Assessment of Herbal medicines.WHO expert committee
183 on specification for pharmaceutical preparations.Technical Report series.No. 863 Geneva, 1996.
- 184 4. Jarnovska D, KubikovaK, KokoskaL.Screening for Antimicrobial Activity of some Medicinal Plant
185 Species of Traditional Chinese Medicine.Czechoslovakian Journal of food Science. 2003;21:107-
186 111.
- 187 5. ParekJ, ChandaS.In vitro Antimicrobial activities of Extract of LaunareaprocumbensRoxb (Labiatae),
188 vitisvinifera L. (vitaceae) and cyprenusrotundus L. (cyperaceae).African Journal of Biomedical
189 Research.2006;9:89-93.
- 190 6. RangHP, DaleMM, RitterJM, MooreRK. Pharmacology 6th edition, Edinburgh:Churchill livingstone;
191 2007.
- 192 7. Umukoro S, Ashorobi RB. Further studies on the antinociceptive action of aqueous seed extract of
193 *Aframomummelegueta*. Journal of Ethnopharmacology, 2007;109:501–504.
- 194 8. IlicN, SchmidtBM, PoulevA, RaskinI. Toxicological evaluation of grain of paradise
195 (*Aframomummelegueta*) [Roscoe] K. Schum.Journal of Ethnopharmacology.2010;127:352-356.

- 196 9. GalalAM. Anti-microbial activity of 6-paradol and related compounds.International Journal of
197 Pharmacognosy. 1996;31:64–69.
- 198 10. KamtchouingP, MbongeGYF, DimoT, WatchoP, JatsaHB, SokengSD.Effects of
199 Aframomummelegueta and Piper guineense on sexual behaviour of male rats.Behavioural
200 Pharmacology. 2002;13:243–247.
- 201 11. OnojaSO, OmehYN, EzejaMI,ChukwuMN.Evaluation of the In Vitro and In Vivo Antioxidant Potentials
202 of *Aframomummelegueta* Methanolic Seed Extract.Journal of Tropical Medicine,2014; Article ID
203 159343, 6 pages.
- 204 12. DHHS, Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources
205 Commission on life Sciences, National Research Council, National Academy Press Washington,
206 D.C., 1985.
- 207 13 EvansWC.Trease and Evans Pharmacognosy. 16th ed. Edinburgh:W. B. saunders Elsevier,
208 2009;16:58-302.
- 209 14. BraideVB,AnikaSM.Environmental toxicology. 1st ed. Enugu: SNAAP press Ltd; 2007.
- 210 15. SunheimerRL, GravesL. Clinical Laboratory Chemistry.New York:Pearson Education.2011;154.
- 211 16. BrarRS, SandhuHS, SinghA. Veterinary Clinical Diagnosis by Laboratory Methods.New Delhi: Kalyani
212 Publishers.2011;93-97.
- 213 17. AsuzuIU, AnikaSM.Textbook of Veterinary Pharmacology.Enugu:Fourth Dimension
214 Publishers.2012;178-189.

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