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Biochemical influence of *Annona muricata* on the basal renal profile of healthy adult Wistar rats

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Numerous ethnopharmacological benefits have been attributed to *Annona muricata*, including, wound healing, anti-arthritic, hypolipidemic, anti-inflammatory, anti-nociceptive, anti-hypertensive, hepatoprotective and antineoplastic effects. This study is a baseline investigation of the influence of *A. muricata* on renal profile of healthy adult Wistar rats. A total of 144 adult male Wistar rats of average weight (100 - 150 g) were randomly divided into six groups (1-6): Group 1 served as control (0 mg/kg), Groups 2, 3, 4, 5 and 6 were orally treated doses of methanol extracts of the leaf, stem-bark, fruit-pulp or root-bark of *A. muricata* (each) at 100, 200, 400, 600 and 800 mg/kg body weight respectively, for a total of twenty-eight days consecutively. At the end of the experimental period, biochemical assays were done, including, plasma electrolytes and plasma urea/creatinine. The results of rats treated with different parts of *A. muricata* exhibited various changes. The changes observed in plasma electrolytes and non-protein nitrogen compounds did not give information of any significant deleterious effects for the rats treated with leaf and fruit-pulp extracts, as there were relative decreases in plasma urea and creatinine levels at the highest administered dose (800 mg/kg) following an initial increase (600 mg/kg). Conversely, the plasma urea and creatinine levels of rats treated with stem-bark and root-bark extracts significantly increased at 800 mg/kg, when compared with control. The fruit-pulp caused a significantly increased plasma urea/creatinine, while the root-bark extract led to a decrease, both at 800 mg/kg. The study indicates that *A. Muricata* possess relatively safe toxicity influence on renal functions.

Keywords: *Annona muricata*, Biochemical assays, Extracts, Kidney, Plasma.

1. Introduction

Studies on *Annona muricata*, reveal many ethnopharmacological benefits including, wound healing (Agu *et al*., 2017), anti-arthritic, anticonvulsant (Moghadamtousi *et al*., 2015; N'gouemo *et al*., 1997), anti-diabetic and hypolipidemic (Adeyemi *et al*., 2009; Ahalya *et al*., 2014); anti-inflammatory and anti-nociceptive (De Sousa *et al*., 2010; Hamizah *et al*., 2012), antioxidant, anti-hypertensive (Agu and Okolie, 2017; Agu *et al*., 2018; George *et al*., 2015), antiparasitic (Jaramillo *et al*., 2000), anti-plasmodial (Ménan *et al*., 2006), hepatoprotective (Arthur *et al*., 2012), gastroprotective, and antineoplastic effects (Agu and Okolie, 2017; Agu *et al*., 2018). These benefits have been attributed to its phytochemical constituents, and the major phytochemicals reported to be present in *Annona muricata* are alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids and proteins (Agu and Okolie, 2017; N'gouemo *et al*., 1997). Also, among the phyto-active chemical constituents found in *A. muricata*, the alkaloids (reticulin, coreximine, coclarine and anomurine) (Edeoga *et al*., 2005) and essential oils (beta-caryophyllene, delta-cadinene, epi-α-cadinol and alpha-cadinol) (Leboeuf *et al*., 1982; Kossouoh *et al*., 2007) stand out.

However, not much has been reported about the ethnomedicinal benefits of *Annona muricata* on renal functions. In this experiment, the possible influence of *Annona muricata* on the renal profile of healthy adult Wistar rats was investigated by assaying some basal biochemical parameters.

2. Materials and Methods

2.1 Experimental rats

Adult albino male Wistar rats weighed between 100 g -– 150 g were used. The rats were purchased in the Department of Anatomy, University of Benin, Benin City, and taken to the Department of Biochemistry animal house where they were acclimatized for one week before the

study. They were fed standard rat chow and water *ad libitum*. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the College of Medicine, University of Benin (CMR/REC/2014/57), and was strictly adhered to.

2.2 Preparation of *Annona muricata* crude extracts

A large quantity of the plant samples (fresh leaf, stem-bark, fruit-pulp, and root-bark) was collected from trees in household gardens in Benin City and around the University of Benin vicinity, Edo state, Nigeria. The plant was identified in the Department of Plant Biology and Biotechnology, University of Benin, authenticated and a voucher specimen number UBHa 0205 was deposited at the Herbarium of the same Department. The samples (leaves and stem-bark) were washed and pulverized separately after drying at room temperature (about 25 ^{o}C) for 4 weeks. Each pulverized plant sample was macerated in methanol for 48 hours after which it was filtered through cheesecloth. The obtained extracts were then concentrated *in vacuo* using a rotary evaporator to obtain viscous gels which were air-dried to gel-like solids. The gel-like crude methanol extracts for both plant parts were reconstituted to obtain a stock solution using distilled-deionized water as solvent. Each reconstituted crude extract was stored in small-capped plastic containers in a refrigerator at -4 ^{o}C until used.

2.3 Administration of extracts

The extracts were administered with the aid of a gavage, acting as an oro-gastric tube. Utmost care was taken not to inflict oral or oesophageal injuries on rats.

2.4 Sub-chronic toxicity assessment

During the period of usage, extracts were administered to the rats based on calculated doses per weight of rat (*i.e*. equivalent volume). These dose calculations were done weekly per weight of rats for the sub-chronic studies, as the weekly weights of the rats per group were recorded *i.e*. day 0, day 7, day 14, day 21, and day 28. Untreated rats (Group 1) served as the control and was administered 2 ml of distilled water (Agu *et al*., 2017; Agu, 2016; Pongri and Igbe, 2017)

2.5 Experimental protocol for sub-chronic toxicity studies

Various methanol extracts of the plant parts (fruit-pulp, leaf, stem-bark, and root-bark) were administered at increasing doses from 100 mg/kg (Group 2), 200 mg/kg (Group 3), 400 mg/kg (Group 4), 600 mg/kg (Group 5) and 800 mg/kg (Group 6). The Group 1 rats were given 2 ml of distilled water (0 mg/kg) and served as the control. Each group had six (6) rats each.

2.6 Plasma electrolyte, urea, and creatinine determination

At the end of the 28th day of experimental treatments, the rats were weighed and then euthanized. Blood samples were collected with sterile syringes into heparinized sample bottles, for biochemical analysis. Potassium and sodium were determined according to the method described by Tietz (1987), using a flame photometer (Eppendorf Flame Photometer AFM 5051, Germany). Chloride was determined according to the method described by Skeggs and Hoschstrasser (1964). Bicarbonate concentration was determined according to the method described by Van-Slyke *et al*., (1925), while the determination of urea was by the method described by Weatherbum (1967) (Urease Berthelot method). The determination of Creatinine was in accordance with the protocol described by Bartels and Bohmer (1972). Chloride, bicarbonate, urea, creatinine, and protein assays were done using standard assay kits from Randox® (Randox Laboratory, UK).

2.7 Statistical analysis

Data were entered into the Microsoft Excel spreadsheet (v.10) prior to descriptive analysis. The data are presented as mean ± SEM and were analyzed using Duncan's multiple range analyses of variance, ANOVA. Correlation analyses were done using Pearson's correlation (p = 0.05) of the Statistical Package for Social Sciences, SPSS®, Version 21.0, IBM Corp., Armonk, NY, USA. Values of $p < 0.05$ were considered significant.

3. Results and Discussion

3.1 Results

Effects of the various *Annona muricata* extracts on the renal function profile of the rats revealed that for the group administered fruit-pulp extracts, plasma [Na^+] decreased with a significant increase at the highest dose, plasma [K^+] increased but decreased close to the control value, while bicarbonate ion decreased significantly at the highest administered dose compared to the control value (Table 1a). Plasma urea level decreased but increased with the highest administered dose, compared to the control ($p > 0.05$). Plasma creatinine showed a similar pattern with a much lower level at the highest administered dose compared to the control ($p < 0.05$) (Table 1b). For the group administered leaf extracts, plasma [Na^+] and bicarbonate ions both showed relatively stable trends but increased at the highest dose ($p <$

0.05), while plasma [K^+] did not show any significant change (Table 2a). Plasma urea and creatinine levels showed increases at lower doses but decreased at the highest administered dose ($p < 0.05$) (Table 2b). For the group administered stem-bark extracts, plasma [Na^+] decreased but was later observed to increase to a near-normal level (Table 3a). Plasma [K^+] did not show any significant change, whereas, plasma bicarbonate ion decreased ($p > 0.05$). The plasma urea and creatinine levels increased significantly, for the administered doses compared to the control (Table 3b). The group administered the root-bark *A. muricata* extract, (Tables 4a and 4b), showed increases in plasma [Na^+] and [K^+], increased bicarbonate ion, decreased plasma urea, and a significantly increased plasma creatinine.

The fold change of the urea/creatinine ratio (Fig.1) showed that the fruit-pulp extract significantly influenced an increase in the plasma urea/creatinine level in the rats especially at the highest concentration, while there was a significantly reduced urea/creatinine level with the root-bark extract treatment.

Table 1a: Electrolytes of Wistar rats treated with fruit-pulp extracts of *Annona muricata*.

GROUPS	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (0 mg/kg)	104.33±3.00[a]	18.83±2.00[a]	63.83±5.00[a]	20.00±2.11[a]
Group 2 (100mg/kg)	99.00±4.00[b]	18.28±2.00	74.16±6.00	21.00±3.00[b]
Group 3 (200mg/kg)	99.83±6.00	24.43±0.30	74.33±3.00 [b]	19.00±2.00
Group 4 (400mg/kg)	109.50±3.00	25.11±1.0[b]	66.16±4.00	19.83±2.00
Group 5 (600mg/kg)	101.16±1.00	23.60±1.00	69.33±4.00	20.00±1.00
Group 6 (800mg/kg)	112.50±3.00[c]	19.68±1.00	58.16±3.00[c]	16.00±2.00 [c]

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 1b: Urea, creatinine and tissue protein of rats treated with fruit-pulp extracts of *Annona muricata*.

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (0mg/kg)	30.00±5.00 [a]	0.25±0.02 [a]	2.13±2.17 [a]	120.00±2.00[a]
Group 2 (100mg/kg)	29.33±1.00	0.28±0.03 [b]	2.25±2.30 [b]	104.75±0.60 [b]
Group 3 (200mg/kg)	24.50±6.00 [b]	0.21±0.01	2.11±2.20	116.67±0.80
Group 4 (400mg/kg)	24.50±5.00 [b]	0.21±0.04	2.21±2.22	116.67±0.60
Group 5 (600mg/kg)	26.00±2.00	0.23±0.02	2.20±2.20	113.04±0.50
Group 6 (800mg/kg)	32.00±3.00 [c]	0.20±0.03 [c]	2.03±2.13 [c]	160.00±0.40 [c]

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 2a: Electrolytes of Wistar rats treated with leaf extracts of *Annona muricata*.

GROUPS	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (0 mg/kg)	104.00±3.00[a]	23.68±0.30[a]	74.16±0.90[a]	18.33±0.50[a]
Group 2 (100mg/kg)	103.50±2.00	23.53±0.40	84.50±2.00[b]	24.00±1.00
Group 3 (200mg/kg)	103.50±3.00	19.68±0.70 [b]	81.83±0.60	24.00±0.70
Group 4 (400mg/kg)	103.83±2.00	22.50±3.00	76.00±4.00	23.67±2.00
Group 5 (600mg/kg)	109.66±1.00 [b]	25.63±0.90 [c]	79.67±2.00	17.33±2.00
Group 6 (800mg/kg)	109.66±3.00 [b]	24.31±1.00	71.83±6.00 [c]	24.17±1.00 [b]

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 2b: Urea, creatinine and tissue protein of rats treated with leaf extracts of *Annona muricata*.

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (0 mg/kg)	25.00±1.00[a]	0.20±0.04[a]	1.35±0.06[a]	125.00±0.01[a]
Group 2 (100mg/kg)	20.67±2.00[b]	0.25±0.04	1.37±0.04	82.68±0.02[b]
Group 3 (200mg/kg)	27.67±1.00	0.23±0.04	1.40±0.06[b]	120.30±0.02
Group 4 (400mg/kg)	28.33±2.00[c]	0.27±0.03[b]	1.33±0.05[c]	104.93±0.02
Group 5 (600mg/kg)	27.83±0.90	0.25±0.04	1.35±0.04	111.32±0.02
Group 6 (800mg/kg)	23.17±2.00	0.22±0.33	1.33±0.02[c]	105.32±0.03

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 3a: Electrolytes of Wistar rats treated with stem-bark extracts of *A. muricata*.

GROUPS	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (0 mg/kg)	107.50±4.00[a]	22.48±1.00[a]	59.33±2.00[a]	25.83±1.00[a]
Group 2 (100mg/kg)	100.83±2.00	20.52±1.00	76.00±6.00[b]	17.33±2.00
Group 3 (200mg/kg)	93.33±5.00[b]	22.12±2.00	69.00±4.00	24.17±2.00
Group 4 (400mg/kg)	93.33±6.00[b]	20.40±0.50	61.67±5.00	15.83±3.00[b]
Group 5 (600mg/kg)	108.33±6.00	17.58±2.00[b]	63.00±4.00	17.83±2.00
Group 6 (800mg/kg)	106.33±4.00	19.33±0.70[bc]	63.00±4.00	21.00±4.00

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan;s multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 3b: Urea, creatinine and tissue protein of rats treated with stem-bark extracts of *A. muricata*.

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (0 mg/kg)	31.33±2.00[a]	0.15±0.02[a]	1.83±0.08[a]	208.87±0.05[a]
Group 2 (100mg/kg)	45.00±4.00	0.23±0.03	1.88±0.08	195.65±0.06
Group 3 (200mg/kg)	50.33±4.00[b]	0.23±0.03	1.88±0.06	218.83±0.04[b]
Group 4 (400mg/kg)	44.00±2.00	0.23±0.03	1.95±0.06	191.30±0.04
Group 5 (600mg/kg)	44.67±4.00	0.25±0.02[b]	2.32±0.20[b]	178.68±0.01[c]
Group 6 (800mg/kg)	43.67±2.00	0.20±0.00	1.92±0.05	218.35±0.02

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 4a: Electrolytes of rats treated with root-bark extracts of *Annona muricata*.

GROUPS	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Bicarbonate (mmol/L)
Group 1 (0 mg/kg)	105.50±4,00[a]	18.65±0.80[a]	57.17±6.00[a]	14.50±1.00[a]
Group 2 (100mg/kg)	106.83±3.00	17.53±0.60	59.83±4.00	24.00±4.00
Group 3 (200mg/kg)	99.17±5.00[b]	20.07±1.00 [b]	58.17±3.00	27.50±4.00[b]
Group 4 (400mg/kg)	117.17±1.00[c]	19.72±2.00	52.33±3.00[b]	19.33±3.00
Group 5 (600mg/kg)	107.17±4.00	17.67±1.00	69.00±4.00	25.50±3.00
Group 6 (800mg/kg)	108.83±3.00	16.28±1.00	74.50±6.00[c]	22.33±2.00

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

Table 4b: Urea, creatinine and tissue protein of rats treated with root-bark extracts of *A. muricata.*

GROUPS	Urea (mg/dl)	Creatinine(mg/dl)	Tissue Protein (g/dl)	Urea/creatinine ratio
Group 1 (0 mg/kg)	51.33±0.33[a]	0.17±0.03[a]	1.82±0.08[a]	301.94±0.25[a]
Group 2 (100mg/kg)	43.33±2.00	0.25±0.02	2.00±0.20[b]	173.32±0.10
Group 3 (200mg/kg)	41.33±3.00[b]	0.28±0.03	1.77±0.05[c]	147.61±2.00
Group 4 (400mg/kg)	46.67±3.00	0.33±0.33[b]	1.82±0.06	141.42±2.00
Group 5 (600mg/kg)	50.33±4.00	0.25±0.02	1.83±0.04	201.32±3.00
Group 6 (800mg/kg)	43.17±2.00	0.27±0.03	1.88±0.06	159.89±1.00[b]

Values are represented as mean ±SEM (n=6). Means with different superscripts are significantly different ($p < 0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (Group 1) taking the superscript 'a' alphabet.

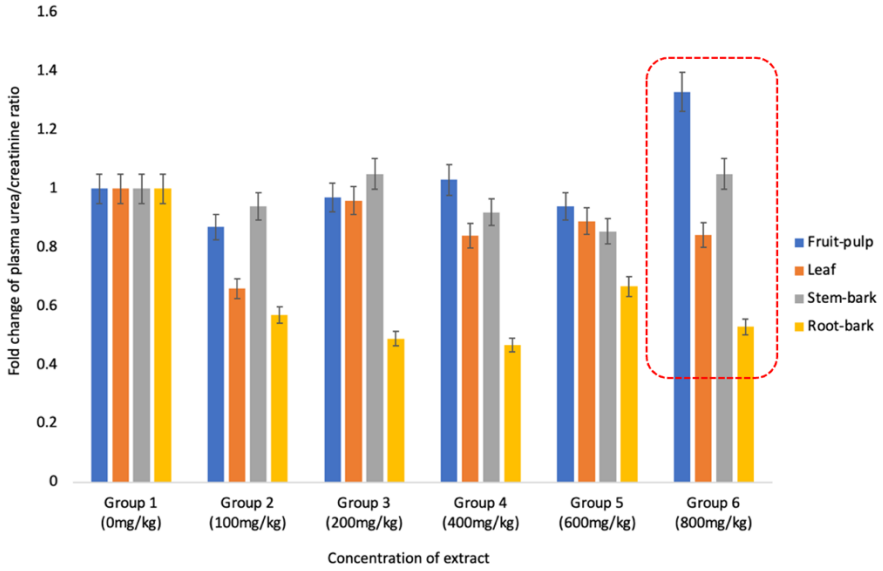

Figure 1: Fold change of the plasma urea/creatinine ratio of the extracts administered at different concentrations.

3.2 Discussion

Blood urea and creatinine levels are important biomarkers of renal function that are routinely measured to assess the renal status, such that, an increased level of these metabolites in the plasma could be indicative of renal incompetence to clear these metabolites from the blood for onward excretion through the urine (Oladele *et al*., 2019). Increases in the blood levels of these metabolites may also be indicative of dehydration resulting in hemo-concentration or post-nutritional status following a high protein profile. Muscular dystrophy is not left out of the possibilities that could lead to an increased creatinine blood level. Thus, accessing how plant extract, plant natural chemical and drugs could influence the profile of these metabolites could give an insight into their possible toxicological influence on the status of the aforementioned physiologic and pathologic conditions.

In this experiment, graded doses of *A. muricata* for the fruit-pulp, leaf, stem-bark and root-bark, where administered to randomly group adult male Wistar rats that were considered healthy. A group that was not administered any of the *A. muricata* extracts was included to provide a background control for the health status of the experimental animals, to which the other treated groups were compared.

The groups treted with the fruit-pulp and leaf *A. muricata* extracts demonstrated significant increases in the plasma levels of urea and creatinine at lower doses, followed by significant decreases at the highest dose (800 mg/kg), compared to the control group (0 mg/kg). There was a significantly increased plasma creatinine level at the highest doses (800 mg/kg) for the groups administered the *A. muricata* stem-bark and root-bark. These observations are in agreement with the findings of Natacha *et al*. (2018) who had earlier reported significant increases in plasma urea and plasma creatinine concentrations, with a corresponding decrease in the concentration of uric acid following the administration of *A. muricata* leaf doses greater than 100 mg/kg; based on which they suggested a possible perturbation of renal functions. Plasma sodium and potassium electrolyte levels demonstrated increases and decrease at the highest *A. muricata* dose (800 mg/kg), respectively ($p < 0.05$), for the groups administered all the plant parts; fruit-pulp, leaf, stem-bark, and root-bark.

The determined fold change of the urea/creatinine ratio (Fig.1) revealed that the fruit-pulp extract relatively caused an increased plasma urea/creatinine level in the rats ($p < 0.05$), especially at the highest concentration (800 mg/kg), while the root-bark extract treatment led to a significant decrease.

Arthur *et al*. (2011) had suggested that consuming *A. Muricata* for a long time may be relatively non-toxic, kidney function should be monitored while avoiding the usage during pregnancy. Thus, *A. muricata* possesses a relatively safe toxicity influence on renal functions.

4. Conclusion

The study showed that oral administration of the different plant parts of methanol extracts of *A. muricana* altered the kidney profile of the Wistar rats. The alterations observed in plasma electrolytes and non-protein nitrogen compounds did not give information of any significant deleterious effects for the rats treated with leaf and fruit-pulp extracts. Conversely, the significant increase observed at the highest dose used in this study for the plasma urea and

creatinine levels of rats treated with stem-bark and root-bark extracts suggests that the extract may not be completely safe at higher doses. This study indicates that *A. muricata* possesses relatively safe toxicity at lower doses which may have an influence on renal functions. However, experimental investigations are necessary to make further validations.

Conflict of Interest

The author declares that there is no conflict of interest.

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