Effects of Annona Muricata Linn. Water Leaf Extract in Melamine-Induced Renal Impairments in Male Rats

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Abstract: **The ameliorative effects of *Annona muricata* water leaf extract on renal impairments caused by induction of melamine were studied in male albino rats. Forty (40) albino rats were randomly distributed into various groups I to V (n=8). Group I (vehicle control) got 7ml/kg b.w of 10% tween 80, Groups II to V got 1000mg/kg body weight of melamine for 10 days. Group III was then given standard reference drug (100mg/kg b.w) allopurinol. While doses of 300 and 500mg/kg b.w of water leaf extracts were administered to Groups IV and V. Phytochemical analysis of water extracts yielded alkaloids (70mg/g), flavonoids (40mg/g), tannins (1.35mg/g), oxalates (0.66mg/g), Saponins (7.90mg/g), Cyanogenic glycosides (1.73mg/g), and phytates (1.34mg/g). Oral acute toxicity test gave zero mortality up to 5000mg/kg b.w for water extracts. Treatment with water extracts at 300mg/kg b.w resulted in significant (P<0.05) decrease in serum creatinine and urea while 500mg/kg b.w only resulted in significant (P<0.05) decrease in creatinine when compared to melamine-induced renal impaired rats that were not treated. The findings imply that renal impairment caused by melamine could be ameliorated at low dose of leaf extract of *Annona muricata.* Hence the leaf could be effective in the management of renal impairment.**

Keywords: **melamine, toxicity, phytochemicals, Annona, renal.**

I. INTRODUCTION

Nephrotoxic substances induce inflammation in glomerulus, proximal tubules, surrounding cellular matrix, and then fiberize the kidney tissue and one of such substances is melamine. Melamine is a white solid organic and highly nitrogenous compound when added to food it falsify or increase the protein content because melamine has about 66.6% nitrogen [1] and even standard tests such as kjeldahl method of protein analysis will indicate the products as a nitrogen rich compound [2]. This makes adulteration to become tempting and easy, knowing that no immediate laboratory technique is readily available to identify melamine contaminated foods which could endanger individuals.

Melamine forms multiple hydrogen bonds interaction with uric acid to form crystals in the kidney [3]. These crystals are insoluble complexes that precipitate in the kidney to cause renal diseases through blocking of renal tubules, impairment of urine filtration, damaging of tubules via dilation of the tubules and cutting walls of tubules, which in turn leads to bleeding [4]. This could lead to a variety of toxic diseased conditions such as nephrolithiasis (kidney stone), chronic kidney inflammation, and bladder carcinoma among others [5].

Medicinal herbs have been identified and used as treatments in medicines before historic times. The traditional use of *A. muricata* is known worldwide in folk medicine systems. *A. muricata* (soursop) is widely used in traditional Indian medicine for the treatment of kidney troubles, fever, nervousness, ulcers and wounds' and possesses antispasmodic, antidysenteric, and parasiticidal activity ([6], [7], and [8]).

However, one ready natural source that might apparently provide relief and alternative therapy might be derived from herbs or medicinal plants. With the vast ethnomedicinal value attributed to *A. muricata* traditionally, the need to determine the effects of this plant leaf on renal impairment is apt.

II. MATERIALS AND METHODS

A. Materials:

The animals used for the study were (40) albino rats purchased from University of Nigeria Nsukka (UNN), Nigeria. The leaves under investigations were that of *Annona muricata*, collected from its trees around Afikpo, in Ebonyi State, South-East region of Nigeria. The leaves (with voucher number KennedyFHI1493) were authenticated by Plant taxonomist in the Forestry Department of Michael Okpara University of Agriculture, Umudike, Abia State Nigeria. All chemicals and reagents used were of standard analytical grade.

1) Preparation of Plant Materials:

Water extraction was carried out using cold maceration according to the method applied by [9]. Sixty (60) grams of powdered leaves was soaked in 500ml of distilled water and allowed to stand for twenty four (24) hours at room temperatures (25^0C). It was then made into slurry by blending and then filtered using cheese cloth and then whatmann filter paper No. 42. The filtrate was concentrated in an oven at 50°C.

B. Animal Studies

Healthy abino rats aged 8-10 weeks old were used for this research. The animals were kept for 14 days under 12 hours light and dark cycle, given water *ad libidum* and fed with rat chow. All experimental procedures used complied with standard principle for laboratory animal use and care [10] after approval was obtained for commencement of the study.

1) Induction of melamine and treatments

Forty (40) albino rats were selected randomly into Groups I to V (n=8). Group I (vehicle control) received 7ml/kg b.w of 10% tween 80, Groups II (melamine only) to V were administered 1000mg/kg b.w of melamine for 10 days. Treatments with extracts and standard reference drug were done after confirmation of toxicity. Group III got standard reference drug (100mg/kg b.w) allopurinol. Group IV and V were administered 300 and 500mg/kg b.w of water leaf extracts respectively after 10 days of melamine induction. Animals in Groups II to V were later sacrificed on day 22 after treatments.

2) Acute Toxicity Test (as described by [11].

Method was conducted in 2 phases of three groups and three rats per group. First, they were administered water extracts at 10,100 and 1000mg/kgbw for phase one. Phase two consisted of three groups of three rats each and they received 1600, 2900 and 5000mg/kgbw of same extract. Animals were then observed for 24, 48, and 72 hours for possible changes and death.

3) Serum Analysis

Creatinine, urea, uric acid calcium, potassium, magnesium, and sodium were analyzed from the serum obtained from the rats using standard reagent analytical Randox kits (Randox Laboratories Ltd, UK).

C, Phytochemical Studies

Selected phytochemical determinations were carried out on extracted leaf samples; Flavonoid and Alkaloid [12], Reducing Sugars [13], Saponin and Cyanogenic Glycosides[14], Tannin [15], Phytate [16] and Oxalate [17].

D. Statistical Analysis

SPSS software (20.0) was used to analyze data of biochemical analysis using one way ANOVA. Then descriptive and multiple comparisons were obtained using LSD (Post Hoc Test) to determine the mean difference which was taken to be significant at $P<0.05$.

III. RESULTS

In the water extract alkaloids is highest, then flavonoids, saponin and the least is reducing sugars. (Table 1). The acute toxicity dose that is capable of causing morbidity or mortality through the administration of water leaf extract of *A. muricata* is > 5000mg/kg body weight.

Treatment with 300 and 500mg/kg b.w of *A. muricata* water leaf extracts to melamine-induced renal impaired groups IV and V respectively caused a significant ($P<0.05$) decrease in serum creatinine concentration compared to group II animals that received 1000mg/kg b.w of melamine while there was a non significant ($p>0.05$) difference in serum uric acid concentration compared to group II rats administered 1000mg/kg b.w. Treatment of group IV with 300mg/kg b.w of caused a significant ($P< 0.05$) decrease in serum uric acid concentration compared to group II animals that received 1000mg/kg b.w. (Table 2). Administration of 300 and 500mg/kg b.w of water extracts resulted in a nonsignificant ($p> 0.05$) decrease in serum calcium and sodium ion concentration compared to animals that received 1000mg/kg b.w of melamine in group II. (Table 3).

Table 1. Phytochemical Constituents of Anonna *Muricata* in Mg/G Dry Weight.

Phytochemicals	Water extract (mg/g)
Flavonoid	40.0 ± 10.0
Alkaloid	70.0 ± 10.0
Saponin	7.90 ±0.50
Tannin	1.35 ± 0.21
Reducing sugars	0.02 ± 0.03
Cyanogenic glycoside	1.73± 0.21
Phytate	1.34 ± 0.41
Oxalate	0.66 ± 0.22

Data obtained were determined in duplicate and result presented as Mean±SEM on dry weight basis.

Table 2. Serum Concentration of Kidney Function Parameters in Albino Rats

Groups	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
Group I	0.81±0.11	5.35 ± 0.31	28.37 ± 1.82
Group II	2.35 ± 0.27*	9.36 ± 0.54*	63.37 ±6.55*
Group III	1.00 ± 0.30**	8.49 ± 0.31*	27.84 ± 6.04**
Group IV	0.85 ± 0.23**	9.37 ± 0.56*	43.15 ± 7.69**
Group V	0.53 ± 0.13**	9.29 ± 0.27*	51.32 ± 10.06*

Values were presented as Mean ±SEM and analyzed using one way ANOVA LSD Post Hoc Test.

*P<0.05 significant compared with Group I, ** P< 0.05 compared with Group II

Table 3. Serum Concentration Of Selected Electrolytes In Albino Rats

Groups	Calcium ion (mEq/l)	Sodium ion (mEq/l)	Potassium ion (mEq/l)	Magnesium ion (mEq/l)
Group I	4.16 ± 0.16	146.87 ± 7.84	3.08 ± 0.18	2.66 ± 0.13
Group II	5.43 ± 0.32*	184.81 ± 15.24*	3.39 ± 0.09	3.27 ± 0.46
Group III	5.04 ± 0.41	178.89 ± 9.34*	3.80 ± 0.39	2.13 ± 0.15**
Group IV	4.91 ± 0.29	161.17 ± 12.51	4.34 ± 0.55*	2.58 ± 0.30
Group V	5.35 ± 0.41*	184.30 ± 8.27*	4.27 ± 0.25*	3.89 ± 0.64*

Values were presented as Mean ±SEM and analyzed using one way ANOVA LSD Post Hoc Test.

*P<0.05 significant compared with Group I , ** P< 0.05 compared with Group II

IV. DISCUSSION

The phytochemical constituents of water leaf extracts of *A. muricata* revealed that alkaloid gave the highest value compared to other phytochemicals under investigation and the value is lowered than that reported by [18] and this might be due to differences in solvents used for extraction. Many drugs are structural modification of alkaloids and used as psychoactive substances and pain reliever [19]. The value of flavonoid obtained is higher than that reported by [20] of same plant. This could be due to differences in methods used in analysis. Flavonoids such as kaempferol, myricetin and quercetin and planar flavonoids have tendency to exhibit antixanthine oxidase activity [21]. Saponin is actively more extracted in water and reported to have tendency to react with cholesterol rich membrane of cancer cells to limit their growth and viability [22]. The antinutrients value of tannin is lower than that reported by [18] probably due to different solvents combination used in extraction. Cyanogenic glycoside and phytate values were higher than that reported by [23] for the seed of *A. muricata*. This could be because different levels of phytochemical constituents are distributed differentially in different part of plants [24]. Phytate has tendency to complex minerals or electrolytes to reduce their apparently high concentration [25]. Oxalate also has ability to bind minerals and affect nutrients uptake in organism..

Acute toxicity test of water showed neither mortality nor visible clinical presentations in the animals even at dose 5000mg/kg b.w. This result agrees with that reported by [26]. The treatment doses were not greater than one-tenth of cut off 5000mg/kgb.w in accordance with fixed dose method of OECD, (2000) guidelines. Administration of the water extracts of *A. muricata* to the melamine-induced renal impaired animals at 300mg/kgb.w cause a significant (P<0.05) decrease in creatinine and urea concentration compared to animals administered melamine only. This result was corroborated by previous study by [27] with photomicrographic evidence that showed mild tubular dilation and few collapse vessels in the kidney of the treated renal impaired rats treated with 300mg/kgbw. The reduction in creatinine and urea could be due to the ability of extract to enhance elimination of creatinine and urea in the urine through increase urine production and synergistic effects of the phytochemical constituents.

However, at increasing dose (500mg/kgb.w), the extract caused a significant decrease in creatinine, a non significant decrease in uric acid, urea, calcium, sodium and nonsignificant increase in potassium and magnesium when compared with group administered melamine only. The nonsignificant decrease in magnesium and calcium at 300mg/kgbw can be ascribed to the fact that antinutrient such as phytate could not sufficiently bind to these electrolytes to reduce their concentrations. The concentrations of uric acid were non-significantly reduced possibly owing to the presence of occlusion interfering with secretion/elimination processes. Reference [27] in a previous study using ethanol extract of *A. muricata* leaf for treatment opined that occlusion occurred because of persistent tubular dilations that arose from melamine induction and coupled with many collapse vessels that characterized melamine-induced renal impairment of the animals treated with 500mg/kgbw of extract.

V. CONCLUSION

The tendency of *Annona muricata* leaf to significantly reduce elevated concentration of creatinine and urea is an indication of its potentials to ameliorate renal disorder. Therefore, these findings suggest that *Annona muricta* leaf could be effective in the management of renal disorder. For further study, Identification and isolation of active compounds in the plants that cause the reduction can be done.

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