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Nephroprotective Effects of *Annona Muricata* Leaf Extract on Adenine-Induced Renal Failure in Male Wistar Rats

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Abstract

Renal failure is a prevalent disease in Africa, partly as a result of indiscriminate consumption of various medical herb preparations such as *Annona muricata,* which is locally used for several ailments like inflammation, bacterial and parasitic infections, diabetes, liver diseases amongst others. The present study was aimed at investigating the effect of *Annona muricata* leaf (AML) extract on adenine-induced renal failure in male Wistar rats. Thirty-five rats were divided into five groups, with seven rats per group. Each of the rats in groups II-V were given 50mg/kg adenine for induction of renal failure for a period of 14 days. The rats in groups III-V were treated with 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively following induction of renal failure, while group II was left untreated for a period of 28 days totaling 42 days all together. Rats in group I served as Control. Urinalysis was done prior to the end of the experiment. The animals were euthanised and blood samples collected for assessment of haematocrit, plasma electrolytes, urea and creatinine. Significant sodium reduction was observed in co-administration of 50mg AML and 50mg adenine, and 50mg adenine alone. Significantly increased potassium was noticed in co-administration 50mg AML and 50mg adenine, and 50mg adenine alone. Significant increase was noted in both urea and creatinine levels of co-administration of 50mg or 100mg AML and 50mg adenine. Haematocrit was

only significantly increased in the 200mg AML administration group. There was global proteinuria in all AML intervention groups which was seemingly dose dependent.AML, if administered at a high dose has a nephron-protective effect against acute renal failure perhaps due to modulation in some ion channels in renal tubules.

Keywords: Annona muricata, renal failure, nephro-protection, adenine.

Introduction

Annona muricata (Soursop) is a widely consumed edible and medicinal plant with several therapeutic importances especially in Nigeria, and mostly among local communities with limited access to standard health care system (Abdullah *et al.*, 2017). In certain African countries for instance, up to 90% of the population still relies exclusively on plants, as a source of medicines (Hostettmann *et al.*, 2000; Adewole and Caxton-Martins, 2006; Oreagba *et al.*, 2011; Owolabi *et al.*, 2013; Aranti *et al.*, 2016). *Annona muricata* has also been widely studied in the last decades due to its therapeutic potentials (Md Roduan *et al.*, 2017). The medicinal uses of the Annonaceae family were reported long time ago, and since then, this specie has attracted several attentions due to its bioactivity.

Intensive chemical investigations of the leaves and seeds of this specie have resulted in the isolation of a great number of bioactive ingredients such as acetogenins, a class of polyketide natural products. The isolated compounds have been shown to possess some biological activities, such as antitumor, antiparasitic and pesticidal properties (Gleye *et al.*, 1997; Alali *et al.*, 1999; Champy *et al.*, 2009). Available scientific studies on A. muricata have identified acetogenins as one of the principal bioactive compounds (Badrie and Schauss, 2009; Moghadamtousi *et al.*, 2015). However, report about the toxicity effect of the plant (both fruit and leaves) have called for sense of caution that may hinder its therapeutic uses especially in local communities so as to avoid untoward effects of its usage. At the same instance, the incidence of kidney damage in developing countries associated with indiscriminate use of medicinal plants has become a serious problem that constitutes threat to the whole quality of life and health, especially in areas with very limited resources of health facilities (Uchino *et al.*, 2005; Nejat *et al.*, 2012).

In view of the above, the present study was aimed at investigating the effects of *Annona muricata* leaf extract on adenine-induced acute renal failure in male Wistar rats.

Materials and Methods

Source of plant

Green *Annona muricata* leaves were purchased from a vendor at a herb outlet market (Oja Oba market) in Ilorin, Kwara State, Nigeria. It was validated at the herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Adenine and Urinalysis strip

Adenine was a product of Alpha Chemika, Maharashtra, India. Urine strip for urinalysis was a product of Samay Pur, New Delhi, India.

Plant extraction

Five hundred grams (500g) of the leaves of *Annona muricata* were sliced, oven-dried at 70^0C for 48 hours and pulverized in a blender (Mikachi Blender, Model MK-1830, China). The resulting powder (90g) was dissolved in 500 mL of distilled water and kept for 48 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, England). The resulting filtrate was concentrated on a steam bath to give a yield of 10.42g (11.6%). Calculated amounts were reconstituted in distilled water to give desired doses of 50, 100 and 200 mg/kg body weight.

Determination of the Bioactive components

The bioactive components in *Annona muricata* include annonaceous acetogenins, phenols and alkaloids with annonaceous acetogenins the predominant of all.

Animal care and ethical approval

Thirty five (35) male albino Wistar rats with an average weight of 200g ± 40g were used in this study. These rats were purchased from the Department of Veterinary Medicine, University of Ilorin, and housed in metabolic cages under standard laboratory conditions in the animal house of the Faculty of Basic Medical Sciences. They were allowed free water and feed *ad libitum.* The research was approved to be in compliance with internationally accepted laboratory animal use and care guidelines, and the guidelines for the institution research ethical review committee by Ethical Review Committee of College of Health Sciences, University of Ilorin, Ilorin Nigeria.

Induction and Treatment plan

The rats were randomly distributed into five groups of seven rats each (n=7) as follows: Group I (Control) rats were given rat pellets (60% cornflour, 20% fish meal, 10% wheat flour, 7% oil seed cake, 2% bone meal and 1% salt) and received 2ml of 0.9% NaCl daily throughout the duration of the experiment. The rats in Groups II to V were given rat pellets mixed with 50 mg/kg adenine daily for 14 consecutive days to induce chronic renal failure which was confirmed by clinically manifesting reduced urinary output, loss of appetite, generalized body swelling, anaemia and derangement in urine analysis.

Following this, groups III, IV and V were treated for a duration of 28 days with 50mg/kg (low dose), 100mg/kg (moderate dose), and 200mg/kg (high dose) of the extract respectively while group II was left untreated totaling 42 days all together.

Determination of analytes

Urinalysis was done on days 14 and 28. Following this, the animals were euthanised, and blood samples collected from the right ventricle through cardiac puncture in EDTA and heparin bottles for haematocrit and plasma electrolytes, urea and creatinine analyses respectively using

ion selective electrode, urease spectrophotometer, and Jaffe spectrophotometer methods.

Statistical analysis

The data collected at the end of the experiment were expressed as mean, standard error of mean (MEAN ± SEM). Statistical analysis was done with statistical package for social sciences (SPSS) version 20 using analysis of variance (ANOVA). Values were considered statistically significant at $p<0.05$.

Results

Effects of Leaf Extract of *Annona Muricata* on haematocrit, Plasma Electrolytes, Urea and Creatinine Levels in Male Wistar Rats

Groups	Na^+ (SEM) mmol/L	K^+ (SEM) mmol/L	Urea (SEM) mmol/L	Creatinine (SEM) µmol/L	Haematocrit (SEM) %
I (control)	135.20 ± 9.85	3.85 ± 0.70	2.98 ± 0.67	83.27 ± 3.42	42.10±0.25
II Adenine (50mg/kg)	41.93 ± 0.72*	8.20 ± 0.50*	9.02 ± 0.23*	131.20 ± 0.70*	21.32±1.62*
III AML (50mg/kg) +Adenine (50mg/kg)	106.50 ± 3.61*	6.26 ± 0.62*	4.01 ± 0.31*	91.31 ± 6.68*	25.73±1.09*
IV AML (100mg/kg) +Adenine (50mg/kg)	128.70 ± 3.75	3.91 ± 0.48	5.96 ± 0.71*	90.31 ± 3.62*	31.08±0.41*
V AML (200mg/kg) +Adenine (50mg/kg)	139.70 ± 4.75	3.82 ± 0.48	3.01 ± 0.61	79.62± 1.62	46.37±1.64*

Value of $p<0.05$ was considered as level of statistical significance

Changes in plasma Na^+

There was significant ($P<0.05$) reduction in plasma sodium level in the groups given 50mg/kg and 100mg/kg of AML, and even a more significant reduction in 50mg/kg adenine only administered group when compared to the control. However, extract of 200mg/kg AML caused increase in the

blood level of sodium but not at significant (P>0.05) level when compared to the control.

Changes in plasma K^+

There was significant (P<0.05) increase in plasma potassium level in groups given combined 50mg/kg of AML and 50mg/kg adenine, and in group given only 50mg/kg adenine compared to the control. However, no significant (P>0.05) changes was noted in groups given 100mg/kg and 200mg/kg of AML.

Changes in plasma Urea

There were significant (P<0.05) increases in group given only 50mg/kg of adenine, and in groups administered 50mg/kg and 100mg/kg of AML. However, no significant (p>0.05) change was observed in 200mg administered AML compared to the control, though there was decrease.

Changes in plasma Creatinine

There were significant (P<0.05) increases in plasma creatinine levels in groups given only 50mg/kg of adenine, and in groups administered 50mg/kg and 100mg/kg of AML when compared to the control group. But there was no significant (p>0.05) change in 200mg/kg AML administered group.

Haematocrit

There was significant (p<0.05) decrease in haematocrit of all the groups except the group administered 200mg/kg AML that was statistically not significant (p>0.05).

Urinalysis

The urinalysis result summarily showed that protein was positive (+) with a pH of 5.5 in the control group. Groups administered 50mg/kg AML was positive for bilirubin (2+), and protein (3+) with a pH of 4.0; 100mg/kg AML was positive for protein (2+) with pH of 5.0; and the 200mg/kg AML was positive for bilirubin (+), and protein (+) with a pH of 5.5. The urine assessment in the 50mg/kg adenine only administered group was positive for bilirubin (3+), and protein (3+) with a pH of 3.5.

Discussion

Annona muricata is a widely consumed edible plant with medicinal properties locally used for several ailments. The current study on Renal failure showed that there was global hyponatraemia in all the test groups which was only redeemed in the 200mg/kg AML. Hyponatraemia in this study precipitated significant morbidity in the affected animals. The loss of sodium from the body could have led to excessive fluid loss, thus making the body to be dehydrated. As the fluid loss becomes intense and more than what is consumed, the body further becomes more dehydrated which is consistent with the report of Peri *et al.*, 2019. Invariably, when there is no adequate body fluid it becomes difficult for the body to process potassium adequately. This perhaps made potassium to build up in blood as prominently seen in group III, and even more worse in group II in this study leading to hyperkalaemia in which there was over two times increase in potassium level which was a life threatening condition for the animals as noted in their deteriorated clinical status.

Additionally, hyperkalaemia was also noticed in only adenine administered rats,

and combination of 50mg AML and adenine administered rats. Kidneys play a crucial role in maintaining potassium homeostasis. The study also demonstrated remarkable uraemia in all the groups which also reflected in the pH of urinalysis results except in the group administered 200mg/kg of AML, though there was increase but not significant as presented in words in the text above. There was also global hypercreatinemia in all the groups except in the group administered 200mg/kg of AML which demonstrated unremarkable decrease. Because of disproportionate increase in urea to creatinine perhaps due to enhanced proximal tubular reabsorption following enhanced sodium and water. This similarly reflected in the urea creatinine ratio which is often considered to be predictive of prerenal injury.

In a similar manner, there was global reduction in the haematocrit levels of all the groups except in the group administered 200mg/kg which demonstrated remarkable increase. The observed anaemia in the affected rats could have been due to intense cellular damage to the kidneys with affectation of capacity to produce erythropoietin, a hormone involved in erythropoiesis (Chang *et al.*, 2013, Shih *et al.*, 2018).

In this study, *A. muricata* caused an elevation in the plasma potassium level of treated animals, this report is similar to a research on hexane extraction of A. muricata which also caused elevation in potassium level (Lannuzel *et al.*, 2002; Nwokocha *et al.*, 2012; Höllerhage *et al.*, 2015). This study also showed that *A. muricata*, if given at high dose has protective potential in stabilizing sodium level in the event of renal toxicity hence the protective effect on sodium levels can be said to be dose dependent as noted in this study. This was in tandem with a report which revealed that A. muricata hydroalcoholic extract negatively influenced the uptake of 99mTc-DMSA in bladder, kidney and blood of rats (Holanda *et al.*, 2014; Adaramoye *et al.*, 2019). The work also revealed that AML caused observable derangement in the plasma urea and creatinine levels of low and moderate doses of AML. However, this situation was reversed in the high dose (200mg/kg) AML administered rats. This was an indication that AML given at high dose, perhaps acted at the tubular level by influencing secretion of these substances into the tubular fluid inspite of the renal toxicity the animals were exposed to which could not be redeemed appreciably by other doses of AML in this current study. The activity of AML could be attributed to antiapoptotic property of acetogenins, which is its principal agent for cellular homeostasis (Torres *et al.*, 2012; Sun *et al.*, 2014; Antony and Vijayan, 2016). It is of importance to also mention that the remarkable high levels of plasma urea and creatinine observed in the group of rats administered only adenine, though expected due to the overwhelming infiltration of its renal damaging effect accompanied with features such as protrusion of eyeballs, vomiting, anorexia and gross inactivity. This was in concordance with a previous study that implicated fruit pulp extract to be toxic (Höllerhage *et al.*, 2015) however leaf extract was used in this experiment. It

could be inferred from the urine analysis of all the groups that tubular handling of protein following its filtration was not accompanied by absolute tubular reabsorption, although, the qualitative assessment of proteinuria observed 200mg/kg AML is in proximity with that of control rats in this study. This could have been due to leakages in some protein channels in the renal tubules, and apparently more prominent in low and moderate doses of AML administrations. The global proteinuria observed in this study in the AML intervention groups seemingly was also dose dependent and ditto for the urine pH. The urine analysis understandably, was however, worst in the 50mg/kg adenine only administered group due to the overwhelming toxic effect of adenine on the tubular function. *Anonna muricata* reflected protective effect perhaps due to its constituents, especially acetogenins which possesses a powerful anti-inflammatory property. This must have been involved in the pathway of the mechanism associated with ameliorating effect inherent in the plants. Failure of the low dose of the plant was unabating and could not return the animals back to normal homeostasis.

Conclusion

AML, if given at high dose could proffer a nephron-protective effect against acute renal failure possibly through alteration of some ion channels in renal tubules.

Conflict of Interest Statement: The authors have no conflict of interest to disclose

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