

**Protective Effect of *Annona muricata* leaf extract against Electrolytes imbalance
in Wistar rats induced with Acute myocardial infarction**

ABSTRACT

Annona muricata, a tropical fruit known for its medicinal properties, has been investigated for its potential cardioprotective effects, particularly in treating acute myocardial infarction (AMI), a critical condition caused by blocked coronary arteries. This study investigates protective effect of *Annona muricata* leaf extract against electrolytes imbalance in wistar rats induced with acute myocardial infarction. Thirty (30) wistar rats were randomly grouped into five classes (A, B, C, D and E) of six rats each. Groups A, B and C were pre-treated with 100mg/kg, 200mg/kg and 400mg/kg of *Annona muricata* extract respectively. Group D was administered 10 mg of lisinopril (standard drug), this served as the positive control group while group E was administered 0.2 ml distilled water, and this served as the negative control group. All the rats were intraperitoneally induced with 150 mg of isoproterenol after fourteen days (two weeks) of pretreatment. Serum electrolytes such as potassium and sodium were assayed using standard assay kit. Results showed that rats pre-treated with *Annona muricata* extract showed significant decrease ($p>0.05$) in the serum potassium and sodium levels in a dose dependent manner when compared to the untreated group; thereby suggesting that *Annona muricata* leaf extract may have a role in modulating sodium and potassium levels in animal models and may consequently help to mitigate the effect of cardiovascular disorders especially myocardial infarction.

Keywords: Myocardial infarction, *Annona muricata*, Electrolytes, Health.

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality globally, significantly impacting the quality of life and posing an economic burden [1]. Among these diseases, myocardial infarction (MI), commonly known as a heart attack,

is a prevalent condition characterized by the sudden loss of blood supply to a part of the heart muscle, often leading to significant damage or even death of the affected tissue [2, 3]. Symptoms include chest pain, shortness of breath, and fatigue [4]. Immediate treatment for acute myocardial infarction involves restoring blood flow through percutaneous coronary intervention or thrombolysis [5], and early intervention significantly reduces mortality and improves outcomes [6]. The major cause of MI is coronary artery occlusion, resulting from atherosclerosis—a condition influenced by several modifiable and non-modifiable risk factors, including dyslipidemia, smoking, hypertension, and obesity [7, 8]. Recent interest in natural remedies has grown in an attempt to find new, cost-effective, and accessible options for the prevention and management of cardiovascular diseases [9].

Annona muricata, commonly known as soursop, is a tropical plant that has been extensively studied for its pharmacological properties, including antioxidant [10], anti-inflammatory [11], and anti-hyperlipidemic [12] effects. Its leaves, in particular, contain bioactive compounds such as flavonoids, tannins, alkaloids, and saponins, which have demonstrated potential in promoting cardiovascular health by regulating lipid metabolism and protecting against oxidative stress [13].

Although a number of research has been done on the plant, its effect on electrolyte balance in animal models have not been well explored and documented. Arising from the foregoing, there is need for research to focus on the effect of *Annona muricata* on electrolyte balance in modifying cardiovascular disorders. Hence, this research was aimed to evaluate the effect of *Annona muricata* leaf extract in improving electrolyte balance in wistar rats induced with myocardial infarction.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh green leaves of *Annona muricata* leaves were collected from a farmland in Awka, Anambra State, Nigeria. Identification and authentication of the plant was carried out at the Department of Botany, Nnamdi Azikiwe University, Awka and a voucher specimen was deposited at the herbarium of the Department for future references. The plant material was shredded with a knife and air-dried under shade for 21 days. The dried leaf was pulverized using a laboratory blender and the fine powders obtained was weighed and stored in an air-tight container at room temperature for further use.

Extraction of Plant Materials

The weighed powdered sample (250 g) was then used for the extraction with solvent combination of ethanol and water (7:3) (2500 ml) for 48 hr via maceration. The mixture was decanted and filtered using sterile Whatman paper No. 1. The filtrate was thereafter evaporated to dryness with the aid of rotary evaporator to obtain crude ethanol extract which was carefully preserved for further analysis. The method of Nkafamiya *et al.* [14] was used to calculate the yield (15.16g) of the crude extract using the formula below:

$$\text{Percentage yield} = \frac{\text{mass of crude extract (g)}}{\text{mass of powdered sample (g)}} \times 100$$

Determination of Median Lethal Dose (LD₅₀)

Determination of Median Lethal Dose as described by Lorke [15] was used to determine the LD₅₀ of the extract.

Animal Studies

Procurement of study animals

Wistar albino rats (30) weighing approximately 180 g were purchased from Chris Farm Ltd Mgbakwu, Awka, Anambra State and were brought to the animal house of

the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The rats were kept in standard cages with saw dust as bedding and standard housing conditions of 12:12 light: dark cycles and fed with standard rat pellets and water *ad libitum*. The animals were allowed to acclimatize to the new environment for seven days.

Dose Preparation and Treatment

The hydro-ethanolic leaf extract of *Annona muricata* was prepared with distilled water in three divided dose (100, 200, and 400) mg / kg, lisinopril (10 mg/kg) used as a reference drug and distilled water was used as vehicle for the untreated group. The animals were administered the extract and drug for fourteen consecutive days prior to induction with water *per os* and feed *ad libitum*.

Experimental design

The animals were randomly grouped into five, with six animals in each group, and the treatment was as follows: Groups A, B and C animals were designated as *A. muricata* treatment group and were pre-treated with the ethanol leaf extract at 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively, for 14 days and thereafter 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected subcutaneously at an interval of 24 h on the 15th and 16th day. Group D animals were designated as isoproterenol control and were administered 0.2 ml of 10 mg lisinopril for 14 days and thereafter 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected subcutaneously at an interval of 24 h on the 15th and 16th day while group E animals (designated as vehicle control group) were administered 0.2 ml distilled water for 14 days; and on the 15th and 16th day, 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected subcutaneously at an interval of 24 h [16].

Blood pressure measurement

Measurement of blood pressure of all the experimental animals was carried out the 17th day with the aid of a non-invasive tail cuff blood pressure monitor, the 6-channel CODA blood pressure monitor for rats and mice. Blood pressure parameters (the systolic, diastolic and mean arterial blood pressure) were all determined and recorded.

Collection of Blood Sample

At the end of the experimental period, the animals were anaesthetized with chloroform vapor, and sacrificed. Sterile syringe with needle (5 ml) was used for blood collection through closed cardiac puncture and the sera obtained were used for bioassay studies.

Biochemical assays

Serum Electrolytes

Determination of Sodium (Na) concentration

Method:

Modified method of Maruna, [17] was used for the determination of sodium concentration

Principle:

Sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Procedure:

An Agape sodium kit was used for this assay. 1ml of filtrate reagent (Uranyl acetate 2.1mM and Magnesium acetate 20mM in ethyl alcohol) was added to test tubes labeled test (sample), blank and standard. This was followed by the addition of 50 μ l

of sample, distilled water and standard in the respective test tubes with vigorous mixing for 3 minutes and centrifugation at 1500rpm for 10 minutes after which the supernatant was collected. Then, 1ml of Acid reagent (dilute acetic acid) was added in a new set of test tubes labeled as above with the addition of 50µl of color reagent (mixture of potassium ferrocyanide, non-reactive stabilizers and fillers). The spectrophotometer was zeroed with distilled water at 550nm and the absorbances of the test, blank and standards read. The concentration of the test was calculated thus:

$$\frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank} - \text{Absorbance of standard}} \times \text{Concentration of standard} \left(\frac{\text{mEq}}{\text{l}} \right)$$

Where Concentration of standard = 150meq/L

Determination of Potassium concentration

Method:

Potassium is determined spectrophotometrically through a kinetic coupling system using potassium dependent pyruvate kinase as described by Berry *et al.*, [18].

Principle:

Potassium measurements are used to monitor electrolyte balance in the diagnosis and treatment of disease conditions characterized by low or high blood potassium levels. Pyruvate generated is converted to lactate accompanying conversion of NADH to NAD. The corresponding decrease of optical density at 380nm is proportional to the potassium concentration in the serum.

Procedure:

An Agape linear potassium kit was used for this assay. 1ml of reagent 1 (mixture of LDH, NADH (10mmol/l), sodium azide (0.05%) and stabilizers) was added in three different set of test tubes labeled sample, blank and standard. This was followed by

addition of 25µl of sample to the sample test tube and 25µl potassium/sodium standard (mixture of 100mmol/l of sodium and 6.0mmol/l of potassium) to the standard test tube. The contents were mixed and incubated for 5 minutes at 37°C. This was followed by the addition of 250µl of reagent 2 (mixture of 50U/L pyruvate kinase, 0.05% sodium azide and stabilizers). After mixing and incubation at room temperature, absorbance (A1) was read at 405nm after 1 minute and read again after 3 minutes (A2). The concentration of potassium was calculated thus:

$$\frac{(A2 - A1)Sample}{(A2 - A1)standard} \times Standard\ concentration$$

Where concentration of standard = 6mmol/L.

Ethical Consideration

This research was cleared and approved by the Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University, Awka.

Data Analysis

The results obtained in this research were expressed as Mean ± S.E.M of triplicate determinations. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at p<0.05.

RESULTS

The acute toxicological study of the plant extract on experimental rats is presented in Tables 1a and 1b.

Table 1a: Phase 1 of Acute toxicological study of hydro-ethanol leaf extract of *Annona muricata*

Groups	Number of Animals	Dose (mg/kg)	Number of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 1b: Phase 2 of Acute toxicological study of hydro-ethanol leaf extract of *Annona muricata*

Groups	Number of Animals	Dose (mg/kg)	Number of Death
1	1	1600	0
2	1	2900	0
3	1	5000	1

The plant extract showed a toxic effect at a dose of 5000 mg/kg when one mortality was recorded. The median lethal dose (LD₅₀) which is the dose required to kill half of the members of a tested population after specified test duration is calculated to be 3,808 mg/kg.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where:

D₀ = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produced mortality

$$LD_{50} = \sqrt{(2900 \times 5000)}$$

$$LD_{50} = 3,808 \text{ mg/kg}$$

Table 2: Effect of Ethanol leaf extract of *Annona muricata* on blood pressure and heart rate in isoproterenol-induced myocardial infarction in wistar rats

Group	Treatment	Mean arterial blood pressure (mmHg)	Heart rate (beats/min)
A	Myocardial Infarction plus 100 mg/kg extract sample	105.20 ^d	200 ^d
B	Myocardial Infarction plus 200 mg/kg	119.50 ^c	235 ^c

	extract sample		
C	Myocardial Infarction plus 400 mg/kg extract sample	121.15 ^c	258 ^b
D	Myocardial Infarction plus 10 mg/kg lisinopril	103.50 ^b	196 ^c
E	Myocardial Infarction plus distilled water	135.45 ^a	340 ^a

Values are expressed in mean±STDEV

Columns with different alphabets superscript are significantly different at $P < 0.05$

Table 3: Effect of oral administration of ethanol leaf extract of *Annona muricata* on serum electrolytes (potassium and sodium) in wistar rats induced with acute myocardial infarction

Groups	Treatment	Potassium (mmol/l)	Sodium (mmol/l)
A	Myocardial Infarction plus 100 mg/kg extract sample	5.21±1.20 ^b	120.96±3.66 ^d
B	Myocardial Infarction plus 200 mg/kg extract sample	4.49±1.08 ^c	139.92±3.37 ^c
C	Myocardial Infarction plus 400 mg/kg extract sample	3.64±1.44 ^d	141.11±2.84 ^b
D	Myocardial Infarction plus 10 mg/kg lisinopril	3.93±0.92 ^d	140.06±5.23 ^b
E	Myocardial Infarction plus distilled water	7.02±1.05 ^a	159.23±2.25 ^a

Values are expressed in mean±STDEV

Columns with different alphabets superscript are significantly different at $P < 0.05$

DISCUSSION

The use of medicinal plants in the treatment of several ailments dates back into centuries [19, 20, 21]. Medicinal plants, especially *Annona muricata* has been proven to be a promising plant in the treatment of chronic diseases especially myocardial infarction. This has been partly attributed to the presence of many phytochemicals and other bioactive compounds in them [10].

Despite the wide acceptance that herbal medicine is gaining in recent times, one of the major criticisms associated with its use is the absence of scientific evaluation of their

safety profiles, since some of them have turned out to be toxic [22]. It is therefore pertinent that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated [23].

Median lethal dose, otherwise called LD₅₀ is the amount of a material given at once, which causes the death of one half (50%) of the experimental animals exposed to it and this has remained a useful tool in safety assessment of substances [8]. Absence of death after an oral administration of 1600 mg/kg body weight (table 1a) suggests that the extract may be largely safe at this dose. The LD₅₀ obtained in this study is calculated to be 3,808 mg/kg body weight. Hence, the extract can be considered safe for therapeutic purposes at a dose lower than this value (3,808 mg/kg). Although acute ingestion of quantities as high as 20 g/kg body weight of the leaves have been reported to be toxic to cattle [24], ingestion of 400 mg/kg of the extract to wistar rats did not result in any adverse effect [25].

The present study has shown a significant cardiac dysfunction in the experimental animals as evidenced in the increased mean arterial pressure (MAP) and heart rate (HR) after isoproterenol administration (Table 2). Pre-treatment with *A. muricata* significantly prevented the continued increase in MAP and HR (determinants of myocardial oxygen demand); and hence, cause a decline in workload and also helped the heart to maintain myocardial oxygen balance in ischemic tissues. This aligns with the report of Onwubuya and Oladejo [8] and Ojha *et al.*, [16]. Restoration of altered MAP and HR also increased blood flow through the sub-endocardial region, which bears the maximal burnt of ischemic insult in isoproterenol induced myocardial infarction. The pretreatment with *A. muricata* might have improved the perfusion to sub endo-cardium. Similar assertion was made by Peng *et al.*, [26] and Mohammad *et al.*, [27] in their separate studies.

Electrolytes are minerals in the blood and other body fluids that carry an electric charge, and they could affect the body's functions in many ways as they have been known to help in conducting nerve impulses, contracting muscles, hydrating, and regulating pH levels [28]. Electrolytes, particularly potassium and sodium play a crucial role in the function of the myocardium, the muscular tissue of the heart. Movement of these ions across the semi-permeable myocardial cell membrane causes the voltage across the membrane to exceed a threshold and generate an action potential, resulting in muscle contraction. They also carry electrical charge and are maintained to tight physiological concentrations through various mechanisms to ensure appropriate heart function. Hence, an imbalance of these electrolytes can have detrimental effects on the heart, causing or contributing to arrhythmia and cardiac arrest [29].

As evident in this study (Table 3), serum potassium (K) level of the extract pre-treated groups A-C (100 - 400 mg/kg) falls within the normal potassium level of 3.5 to 5.2 mmol/L compared to the untreated group (7.02 ± 1.75 mmol/L). This is consistent with the findings of Zubaidi *et al.*, [25]. Potassium, the most abundant intracellular cation, is an essential nutrient that is naturally present in many foods and available as a dietary supplement. Potassium is present in all body tissues and is required for normal cell function because of its role in maintaining intracellular fluid volume and transmembrane electrochemical gradients [30]. Since high potassium levels above the normal range has been implicated in the pathogenesis of various ailment especially myocardial infarction, the ability of the plant extract to regulate this electrolyte could therefore help in mitigating the disease state. Several possible mechanisms for this protective effect are evident. The plant extract could have influenced the serum potassium level which could plausibly act as diuretic agent and thereby reduce

extracellular fluid volume, which in turn could result in decreased blood pressure. Another alternative mechanism of action is that potassium may have altered the activity of the renin-angiotensin system and reduce angiotensin influences on vascular, adrenal or renal receptors. Other possible mechanism could be the possibility of potassium to modify central or peripheral neural mechanisms that regulate blood pressure. Several studies have reported the relationship between potassium rich diet and reduced blood pressure [31, 32].

Another electrolyte worthy of note in myocardial functioning is the sodium (Na). Sodium is an essential nutrient involved in the maintenance of normal cellular homeostasis and in the regulation of fluid and electrolyte. It also helps in maintaining normal blood pressure, supports the work of the nerves and muscles, and regulates the body's fluid balance.

As evident in this study (table 3), the serum sodium level of the extract treated group falls within the normal range (135-145mmol/L). Since elevated level of sodium has been implicated in disease condition, particularly hypernatremia which could often lead to increase risk of cardiovascular disease, the ability of the extract to mitigate this effect could help improve the disease condition. Although sodium's role in controlling myocardial infarction seems complex, available evidence suggests a direct relationship between sodium intake and blood pressure [33, 34, 35]. Similarly, Catanozi *et al.*, [30] have reported that sodium restriction results in increased atherosclerosis in animal models. Hence, the mechanism of action of the plant extract in regulating sodium balance in the experimental animals could plausibly be linked to the pressure natriuresis mechanism, where an increase in blood pressure in the renal arteries causes increased salt and water excretion.

Conclusion

Available evidence as contained in this study showed that the plant extract is considerably safe for therapeutic purposes, regulating serum potassium and sodium imbalance during myocardial infarction in experimental animals and consequently may serve as an agent in the treatment of cardiovascular disorders especially myocardial infarction.

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