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Research Article

The ameliorative effects of methanol and aqueous leaf-extracts of *Annona muricata* Linn, against oxidative stress on the kidney in selenite-induced cataract rat model

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Abstract

Oxidative stress negatively impacts renal tissue and causes inflammation, which leads to more tissue destruction, remodelling and the accumulation of defective biomacromolecules. The aim of the study is to evaluate the ameliorative effects of methanol and aqueous leaf-extracts of *Annona muricata Linn*, against oxidative stress on the kidney in selenite-induced cataract rat model. Phytochemical analyses and the acute toxicity test were carried out. The study involved 55 males and female Wistar suckling pups randomly split into 11 groups. A single subcutaneous injection of sodium selenite (30 µmol/kg) was used to induce oxidative stress in pups on the 10th day of life, with the exception of the normal control group. Group C (positive control) was treated with the reference drug (vitamin C) (100 mg/kg, oral) for 21 days. Groups D, E, F and G received methanol extract orally at 100 mg/kg, 150 mg/kg, 200 mg/kg, and 250 mg/kg, respectively for 21 days. Groups H, I, J and K received aqueous extract orally at 100 mg/kg, 150 mg/kg, 200 mg/kg, and 250 mg/kg, respectively, for 21 days. Water was given to group A (normal control) instead of the extract and no treatment was given to group B (the negative or test control). The aqueous extract showed more potent effects than the

Keywords

Annona muricata, nephrotoxicity, oxidative stress, anti-oxidation, anti-nephrotoxicity.

Abstract *(Continued)*

methanol extract. Compared to the test control, after treatment the catalase activity and sodium levels were significantly decreased ($p<0.05$); while the glutathione peroxidase activity and potassium levels were significantly increased ($p<0.05$). The aqueous extract treatment also significantly lowered plasma sodium (Na^+) and chloride (Cl^-). The changes in the plasma levels of urea and creatinine were significantly stabilised. Renal tissue sections of a cataractous rat treated with the graded doses of both the methanol and aqueous extracts showed normal glomeruli and tubules, and no inflammatory cell infiltrate was seen in the interstitium. The aqueous extract of *Annona muricata* possesses a dose-dependent renal-ameliorative property and could be a promising plant for the development of an anti-nephrotoxic drug.

1. Introduction

Cataract remains a major public health problem, and to effectively deal with it, its mechanism of formation must be well understood in order to ascertain the key steps which may be used as targets for therapeutic regimes [1]. This way, appropriate models can be selected for screening potential anti-cataract agents [2]. Sodium selenite-induced cataract is one of the best models for studying the fundamental mechanism of senile/age related cataracts, for identifying potential anti-cataract agents, as well as the effect of different stressors on the lens [3]. This is because a high dose of sodium selenite induces cataracts in suckling pups [4], with an accompanying increase in lenticular lipid peroxidation and the formation of H_2O_2 in the aqueous humour [5]. Oxidative stress remains a major threat to lens transparency and the redox systems. Free radical-induced oxidative damage is one of the main factors that trigger the formation of age-related cataracts [5].

The redox state plays a significant role in the development of many liver diseases. The redox status has an impact on the development of kidney damage, especially in chronic kidney disease (CKD) [6, 7]. This imbalance, which is visible even in the initial phases of CKD, can cause damage to cells and tissues. Under appropriate conditions, cells have particular molecular methods to regulate oxidative stress and continue to maintain a balance of oxidant and antioxidant particles. Reactive oxygen species and reactive nitrogen species largely impact hepatic proteins, lipids, and DNA [7]. The process causes structural and functional problems in the kidney. These free radicals are neutralized in the human lens or body cells by antioxidant enzymes, vitamins and drugs (traditional medicines inclusive). These traditional medicines with strong antioxidants are cheaper [8, 9] compared to cataract surgery or treatment of kidney diseases.

According to the World Health Organization, over 80% of the world's population relies on the use of herbal medicine as a remedy [10-12]. This could explain why traditional medicine is gaining popularity all around the world right now [13]. These products from traditional medicine provide new approaches to modern healthcare. Many modern pharmaceuticals are actually derived from plants and plant related products. Therefore, there is a need to investigate these traditional medicinal plants in order to ensure their proper use, as well as evaluate the possibility of them being sources of new drugs. Such studies are often designed to prove the efficacy of such medicinal formulations, via bioassay, as well as identify their active components through chemical analysis [14-16]. *Annona muricata* (commonly referred to as graviola) extracts are just one of several botanical chemicals that have shown potential medicinal efficacy, and all of the plant's aerial parts are used as natural remedies [17]. The use of medicinal plants for disease management (especially infectious diseases) is due to their content of a wide array of compounds, including alkaloids, flavonoids, tannins and phenolic compounds, all of which exert different physiological actions on the human body [18]. Therefore, there is a need to synergize phytomedicine with conventional medicine in the treatment of cataracts so as to promote affordability of treatment. The aim of the study is to evaluate the protective effects of methanol and aqueous leaf extracts of *Annona muricata Linn,* against

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oxidative stress on the kidney in a selenite-induced cataract rat model in Wistar suckling pups.

2. Materials and methods

2.1. Care and management of animals

Fifty-five (55) healthy Wistar suckling pups weighing between 4.7 g and 12.8 g were used for the experiment. The animals were bred in the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were kept with their mothers to suckle then, and their mothers were provided with water ad libitum. The animals were kept in well-ventilated cages under standard conditions of temperature and humidity. Ethical approval was obtained from the University of Port Harcourt Research Ethics Committee, with Reference Number (UPH/CEREMAD/REC/MM79/085).

2.2. Collection of the leaves of Annona muricata

Fresh leaves of *Annona muricata* (locally known as" Graviola" or "soursop" in English and "shawshopu" in the Igbo language of eastern Nigeria) were gotten from the field of the University of Port Harcourt, River State between February and March 2024. The leaves were identified and authenticated at the Plant Science and Biotechnology Department, University of Port Harcourt, River State, Nigeria, as *Annona muricata* (family: Annonaceae). A voucher specimen (UPH/V/1191) of the plant has been deposited in the Herbarium of Plant Science and Biotechnology, University of Port Harcourt. The leaves were washed, chopped and air dried at room temperature for four weeks. After which, they were ground into powder form using an electric grinder (Sorex SHB-520, Korea) at the Pharmaceutical Sciences Department, University of Port Harcourt. The resultant powder was stored and used for the study.

2.3. Phytochemical screening

2.3.1. Qualitative analysis of the phytochemical contents

2.3.1.1. Test for alkaloids

This was carried out according to the method of Sofowora [19]. Briefly, a portion (0.5 g) of the leaf powder was stirred with 5 mL of 5% HCl in a steam bath and filtered. An aliquot (1 mL) of filtrate was treated with five drops of Mayer's Reagent. Another aliquot (1 mL) of the filtrate was treated with Dragendroff's reagent. A third aliquot (1 mL) was also treated with three drops of picric acid. The nature and colour of the resultant precipitates were observed, noted and recorded.

2.3.1.2. Test for tannins

This was carried out by the ferric chloride test as described by Trease and Evans [20]. Another portion (0.5 g) of the leaf powder was stirred with 10 mL of distilled water and filtered. To the filtrate was added 5% $FeCl_3$ (three drops). The nature and colour of the resultant precipitates were noted.

2.3.1.3. Test for flavonoids

This was carried out by the Shinoda test, as described by Trease and Evans [20]. A piece of magnesium metal was added to 5 g of leaf powder, and then 2-4 drops concentrated hydrochloric acid were added to dissolve the powder and initiate the reaction. The nature and colour of the resultant precipitate and solution were noted.

2.3.1.4. Test for saponins

The presence of saponin was determined by the Frothing test [20]. A portion (0.5 g) of the leaf powder was placed in a test tube and 10 mL of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 10 min and observed.

2.3.1.5. Test for cardiac-glycosides

The presence of cardiac glycosides was determined by the Keller-Kiliani test. A portion (5 g) of the leaf powder was treated with 1 mL of $FeCl_3$ Reagent. Concentrated H_2SO_4 (2-4 drops) was added to this solution. The nature and colour of the resultant solution and precipitate were noted.

2.3.1.6. Test for phenols

The ferric chloride test was adopted. A portion (5 g) of the leaf powder was put in 5 mL of distilled water and stirred in a water bath and filtered. 1-2 drops of 1% neutral $FeCl_3$ solution was added. The nature and colour of the resultant solutions and precipitates were noted. Then sodium carbonate was added, and the observation repeated.

2.3.2. Quantitative analysis of the phytochemical content

2.3.2.1. Determination of alkaloid content

The samples were extracted with methanol, and the resultant extract was treated with H_2SO_4 and NaOH, and then re-extracted with chloroform. Briefly, 20 g of

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the powdered leaves was placed in the macerator jar. Aliquot (200 mL) of absolute methanol was poured into the maceration jar containing the milled leaves. The mixture was macerated for 20 min and agitated at regular intervals. It was later warmed for about 10 min in a water bath at 40°C to quicken extraction. The mixture was filled into a crucible and the residue was discarded. The filtrate was dried in water bath at 50°C and methanol extract was obtained. Aliquot (10 mL) of H_2SO_4 was introduced into the methanol extract and stirred. The mixture was boiled for about 10 min in a water bath at 60°C and filled into a beaker and the residue was discarded. Conc. NaOH was added to the filtrate dropwise and litmus paper was used as to detect when the solution became alkaline. After which the mixture was poured into the separating flask and was partitioned with three times of 10 mL each of the chloroform for exhaustive extraction of crude alkaloids. At each interval, it was agitated for 2 - 5 min. The chloroform fraction was collected into a crucible and the aqueous layer was discarded. The chloroform fraction was dried on the water bath at 4 °C and crude alkaloid was obtained. And the weight of the resultant residue was recorded.

$$\text{Alkaloid (\%)} = \frac{\text{Weight residue}}{\text{Weight of sample analysed}} \times 100$$

2.3.2.2. Determination of phenolic compounds

The phenolic compounds were extracted with distilled water, and filtered through lead acetate, before drying and weighing. Another portion (20 g) of the powdered leaves was macerated with 200 mL of distilled water in 250 mL capacity beaker. The mixture was boiled for 30 min in a water bath at 50°C and filtered while hot. Eighty grams (80 g) of anhydrous lead acetate was packed on the clamped cylindrical glass column to serve as filtration bed and the column was clamped on retort stand. The filtrate was carefully poured into the column via capillary action while the phenolic compounds were complexed with lead acetate. The content of the column was unpacked into a crucible and was placed into the hot oven at 50°C and the dried mixture was obtained and weighed.

$$\text{Phenolic compound (\%)} = \frac{\text{weight of residue}}{\text{weight of sample analysed}} \times 100$$

2.3.2.3. Determination of saponin content

The saponin contents of the samples were determined by the solvent extraction gravimetric method. A third portion (20 g) of the powdered leaves was macerated with 200 mL of 20 % methanol in a macerating jar. The mixture was boiled in a water bath for 20 min with continuous stirring at 50 °C and filtered while hot into a crucible. The filtrate was reduced to 40 mL over water bath at about 70 °C and was transferred into a 250 mL separating flask (funnel) and clamped. Diethylether (20 mL) was added and the mixture was shaken vigorously. The aqueous layer was collected and diethyl ether was filtered off and discarded. Aliquot (60 mL) of n-butanol was added and 10 mL of 5% aqueous NaCl was used to wash the mixture twice and discarded. The remaining solution was allowed to evaporate for 30 min at 50 °C and was placed in a hot oven to dry, and the weight of the crude saponins (the residue) was obtained.

$$\text{Saponin content (\%)} = \frac{\text{weight of residue}}{\text{weight of sample analysed}} \times 100$$

2.4. Preparation of the leaf extracts

2.4.1. Methanol extract

A portion (600 g) of powdered leaves was mashed in 500 mL of 70% methanol for 72 h at room temperature and pressure. The crude methanol extract was filtered and the filtrate (extract containing menthol) was allowed to evaporate at 600 reduced pressure using a vacuum rotary evaporator to remove methanol, yielding 19.43 g of a brown residue (methanol extract). The residue was freeze-dried in a vacuum freezer drier and stored in a dried and tight container until needed for bioassay.

2.4.2. Aqueous extract

Another portion (1000 g) of powdered leaves was mashed in 2.5 L of distilled water at room temperature for 48h (with occasional shaking). This was then filtered, and the filtrate (aqueous extract) was concentrated to dryness using a vacuum rotary evaporator under 600 reduced pressure, providing 24.32 g of a light green residue (aqueous extract). The residue was freeze dried in a vacuum freeze-drier and stored in a dried and tight container until needed for bioassay.

2.5. Determination of LD_{50}

Three groups of four suckling Wistar rat pups were used for the test. The extract was administered

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Table 1. Experimental design for the assay

Group	Treatment group	Details
Group A	Normal control	Distilled water + no treatment (distilled water)
Group B	Negative or test control	Sodium selenite (30 µmol/kg body weight) + no treatment (distilled water)
Group C	Reference treatment	Sodium selenite (30 µmol/kg body weight) + vitamin C (100 mg/kg)
Group D	Methanol extract 100	Sodium selenite (30 µmol/kg body weight) + methanol extract (100 mg/kg)
Group E	Methanol extract 150	Sodium selenite (30 µmol/kg body weight) + methanol extract (150 mg/kg)
Group F	Methanol extract 200	Sodium selenite (30 µmol/kg body weight) + methanol extract (200 mg/kg)
Group G	Methanol extract 250	Sodium selenite (30 µmol/kg body weight) + methanol extract (250 mg/kg)
Group H	Aqueous extract 100	Sodium selenite (30 µmol/kg body weight) + aqueous extract (100 mg/kg)
Group I	Aqueous extract 150	Sodium selenite (30 µmol/kg body weight) + aqueous extract (150 mg/kg)
Group J	Aqueous extract 200	Sodium selenite (30 µmol/kg body weight) + aqueous extract (200 mg/kg)
Group K	Aqueous extract 250	Sodium selenite (30 µmol/kg body weight) + aqueous extract (250 mg/kg).

subcutaneously (S.C.) at doses of 200, 1000 and 1800 $mg{\cdot}kg^{-1}$, respectively, to each group. The animals were observed over a period of 48 h for signs of acute toxicity. The number of deaths caused by the extract in each group within the time was noted and recorded. The LD_{50} was then calculated by a probit plot of the log of dosages against percentage deaths (in probability units).

2.6. Evaluation of the anti-cataract activity

Sodium selenite (Sigma) (procured from a chemical shop) and L-ascorbic acid (procured from a pharmacy in the University of Port Harcourt) were administered orally. The rat pups were divided into 11 groups of five each (Table 1). The first group (normal control) received distilled water, while each rat pup in the other groups received a single subcutaneous (S.C.) injection of sodium selenite (30 µmol/kg body weight) on postpartum day ten. The first (normal control) and second (test control or cataract-untreated) groups received distilled water; the third group (reference treatment) received vitamin C; the fourth to seventh groups (Methanol extract 100-250) received 100, 150, 200, 250 $mg{\cdot}kg^{-1}$ body weight of the methanol extract respectively; while the eighth to eleventh groups (aqueous extract 100 - 250) received 100, 150, 200, 250 $mg{\cdot}kg^{-1}$ body weight of the aqueous extract respectively. The treatment continued for 21 days after the initiation of cataract. Cataract was observed when the pups' first opened their eyes (approximately 16-20 days after birth).

2.6.1. Morphological examination of rat pup lenses

When the rat pups first opened their eyes (approximately 16-20 days after birth), pup lenses were examined by an Ophthalmologist from the Ophthalmology Department, University of Port Harcourt Teaching Hospital (UPTH), to provide a morphological evaluation of any lenticular opacification. Prior to performing the examination, the pupils were dilated using topical ophthalmic solution containing 1.0% tropicamide with phenylephrine. One drop of the solution was instilled every 7 min for 1h into each eye of each rat pup, with the animals being kept in a dark room and viewed. Photographs were taken of the lens after pupil dilation, and graded for the presence and severity of cataract. At the end of the experimental period, the rats were rapidly sacrificed by cervical dislocation; and the lenses were removed intra-capsularly through an incision 2mm posterior to the limbus under surgical microscopic magnification.

2.7. Sample collection

The animals were sacrificed by cervical dislocation under chloroform anaesthesia. Blood samples were collected into heparin sample bottles, and centrifuged at 3500 rpm for 20 min. The plasma was collected and stored for the biochemical assays.

2.7.1. Biochemical assays

Superoxide dismutase activity was determined according to Misra and Fridovich [21]. Catalase activity was determined according to the method of Beers and Sizer [22]. For the assay of plasma ascorbic acid concentration, the method was adopted from Robitaille and Hoffer [23] and Kim and Kim [24]. Glutathione peroxidase activity was assessed according to Rotruck *et al*. [25]. For the assay of ocular malondialdehyde (MDA) concentration, the method

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Table 2. Result of the qualitative phytochemical tests on the leaf powder

S/N	Constituent	Test	Observation	Inference
1	Alkaloids	a. Dragendroff's test	Pink colouration	Positive
		b. Meyer's test	Yellow precipitate	Positive
		c. Picric acid	Yellow precipitate	Positive
2	Tannins	Ferric chloride test	Brown colour	Positive
3	Saponins	Froth test	Slightly foamy	Positive
4	Flavonoids	Shinoda test	Dark orange colour	Positive
5	Cardiac – glycosides	Keller-Killiani test	Greenish blue	Negative
6	Phenols	Ferric chloride test	A pale green colour	Positive

adopted was that of Hunter *et al*. [26], as modified by Gutteridge and Wilkins [27]. The assay of plasma urea and plasma creatinine concentrations was carried out with the Randox test kit (Randox Laboratories, Crumlin, England, UK). The assay of plasma sodium, plasma potassium and plasma chloride concentrations was carried out with the Teco Diagnostics kit (Teco Diagnostics, Lakeview Ave, Anahelm, CA).

2.7.2. Histopathologic evaluation

The kidneys were harvested, fixed in a solution of 10% formaldehyde and prepared for histological evaluation. The tissues were fixed, sectioned, read and interpreted by Mr Moses D.I. of Anatomy Laboratory, Department of Anatomy, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria. The tissues were embedded in paraffin wax, and sections of 4-6 µm were obtained.

2.8. Statistical analysis

Data were analyzed using a statistical package for social sciences (SPSS) version 23.0. A One-way ANOVA test was used to evaluate the differences between means and standard deviations, and LSD test was used to compare the means. P-values of 0.05 or less were considered significant. For LD_{50} determination, probit-log analysis as described by Finney [28] was used. All graphs were prepared with the Excel 2010 package.

3. Results

3.1. Phytochemical content

Tables 2 and 3 show the results of the preliminary analysis of the phytochemical composition of the leaves. We detected alkaloids, flavonoids, saponins, tannins, and phenolics, and quantification revealed moderate contents of these compounds.

Table 3. Result of the quatitative phytochemical tests on the leaf powder

Component	Composition (mg/g)
Alkaloids	17.4
Saponins	29.1
Phenolic compounds	32.5

3.2. Indices of lethality

The aqueous extract at 1000 and 1800 mg/kg produced 25% and 100% mortality, respectively, within 48 hours. The probit plot of the frequency of deaths against the various doses of administration of the extract is shown in Fig. 1.

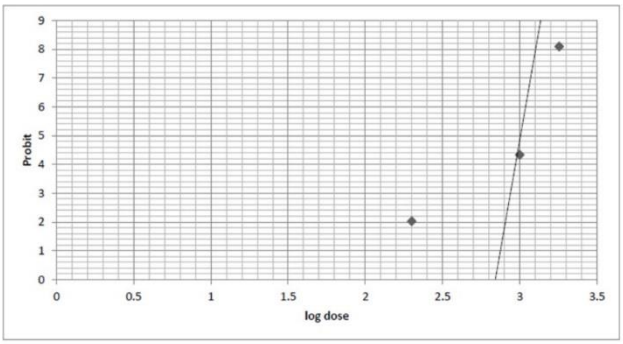

Figure 1. Probit plot of the frequency of death against the doses of administration.
(Note: the grids were left to enable easy understanding of the extrapolations)

From this regression plot, the equation was derived as:

Probit = -39.8579869786464 + 14.7293289928821 x log (dose)

From the above equation, the indices of lethality of the extract were calculated (Table 4). The oral LD_{50} of the

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extract was calculated to be 1110.42 mg/kg, while its LD_{01} was 771.44 mg/kg. The dose of zero mortality was calculated as 696.85 mg/kg.

Table 4. The lethality indices of the extract

Index	Value (mg/kg)
LD_{50}	1110.42
LD_{01}	771.44
Highest non-lethal Dose	696.85

3.3. Effect on indicators of oxidative stress

The effects of methanol and aqueous leaf extracts of *Annona muricata* Linn on plasma and ocular markers of oxidative stress in selenite-treated rats are shown in Table 5 and Fig. 2. The plasma glutathione peroxidase activity of the test control (5.93 ± 0.81 x10^{-2} U/L), normal control (6.38 ± 0.50 x 10^{-2} U/L), reference treatment (6.28±0.54 x10^{-2} U/L), methanol extract 100 mg/kg (5.38 ± 0.84 x10^{-2} U/L), methanol extract 150 mg/kg (5.83±0.15 x10^{-2} U/L), methanol extract 200 mg/kg (5.85 ± 1.14 x10^{-2} U/L), and methanol extract 250 mg/kg (6.43 ± 0.92 x10^{-2} U/L),, were significantly (p<0.05) lower than the Aqueous extract 100 mg/kg (11.95 ±1.51 x10^{-2} U/L), Aqueous extract 150 mg/kg (12.00 ± 0.00 x10^{-2} U/L), Aqueous extract 200 mg/kg (12.33 ± 0.58 x10^{-2} U/L) and Aqueous extract 250 mg/kg (12.33 ± 0.58 x10^{-2} U/L), but not significantly different from one another.

The activity of SOD in the plasma of the test control group (1.83 ± 0.60 x10^{-2}, U/L) was significantly (p < 0.05) lower than those of normal control (3.30 ± 0.29 x10^{-2}, U/L), methanol extract 200 mg/kg (3.20 ± 0.28 x10^{-2}, U/L), methanol extract 250 mg/kg (3.45 ± 0.90 x10^{-2}, U/L), aqueous extract 100 mg/kg (2.95 ± 0.13 x10^{-2}, U/L), aqueous extract 150 mg/kg (2.80 ± 0.28 x10^{-2}, U/L) and aqueous extract 200 mg/kg (3.00 ± 0.30 x10^{-2}, U/L), but was not significantly different from the others. That of normal control and methanol extract 250 mg/kg were significantly (p<0.05) higher than the test control, reference treatment (2.53 ± 0.85 x10^{-2}, U/L), methanol extract 100 mg/kg (2.25 ± 0.57 x10^{-2}, U/L), methanol extract 150 mg/kg (2.13 ± 0.41 x 10^{-2}, U/L) and aqueous extract 250 mg/kg (2.17 ± 0.15 x10^{-2}, U/L), but not significantly different from one another, or the remaining groups. The reference treatment group was significantly (p < 0.05) lower than those of the normal control, test control and methanol extract 250 mg/kg, but not significantly different from the others. There were no significant differences in the plasma catalase activities of all the groups. The plasma ascorbic acid level of the test control group was significantly (p < 0.05) lower than that of the normal control, but not significantly different from those of the others. That of the normal control was significantly (p < 0.05) higher than those of the test control, aqueous extract 100 mg/kg, aqueous extract 150 mg/kg, and aqueous extract 250 mg/kg but not significantly different from the others. That of reference treatment and methanol extract 100 mg/kg, were significantly (p < 0.05) higher than those of aqueous extract 150 mg/kg and aqueous extract 250 mg/kg, but not significantly different from the others. The plasma malondialdehyde levels of the test control group was significantly (p < 0.05) higher than all the other groups. That of the normal control group was significantly (p < 0.05) lower than the test control, methanol extract 150 mg/kg, and aqueous extract 150 mg/kg, but not significantly different from the others. Those of reference treatment, methanol extract 100 mg/kg, methanol extract 200 mg/kg, methanol extract 250 mg/kg, aqueous extract 100 mg/kg, Aqueous extract 200 mg/kg and aqueous extract 250 mg/kg, were not significantly different from each other, as well as normal control, methanol extract 150 and aqueous extract 150 mg/kg, but were significantly (p < 0.05) lower than the test control.

3.4. Effect on markers of kidney function

Table 6 shows the effect of methanol and aqueous leaf extracts of *Annona muricata* Linn on plasma creatinine and urea levels of selenite treated rats. The plasma creatinine level of the test control group was not significantly different from the other groups. However, that of aqueous extract 100 mg/kg was significantly (p<0.05) higher than that of the methanol extract 250 mg/kg, but not significantly different from the others. The plasma urea levels of the test control group was significantly (p < 0.05) lower than those of aqueous extract 100 mg/kg, aqueous extract 150 mg/kg, aqueous extract 200 mg/kg and aqueous extract 250 mg/kg, but not significantly different from those of the other groups. That of the reference treatment group was significantly (p < 0.05) lower than those of normal control, aqueous extracts 100, 150, 200, and 250 mg/kg, but not significantly different from those of the other groups. That of the normal

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Table 5. Effect of methanol and aqueous leaf-extracts of Annona muricata Linn on markers of oxidative stress

Treatment (mg/kg)	Superoxide dismutase activity ($x10^{-2}$, U/L)	Glutathione peroxidase activity ($x10^{-2}$, U/L)	Catalase activity ($x10^{-8}$, U/L)	Ascorbic acid concentration (mmol/L)
Normal control	3.30 ± 0.29^{a}	6.38 ± 0.50^{a}	10.20 ± 6.39^{a}	4.30 ± 1.52^{a}
Negative or test control	1.83 ± 0.60^{c}	5.93 ± 0.81^{a}	4.43 ± 2.44^{a}	$2.29\pm0.45^{b,c}$
Reference treatment	$2.53\pm0.85^{b,c,d,e}$	6.28 ± 0.54^{a}	10.10 ± 11.29^{a}	$4.20\pm0.89^{a,b}$
Methanol extract 100 mg/kg	$2.25\pm0.57^{b,c,e}$	5.38 ± 0.84^{a}	5.15 ± 3.41^{a}	$4.06\pm2.81^{a,b}$
Methanol extract 150 mg/kg	$2.13\pm0.41^{b,c}$	5.83 ± 0.15^{a}	6.43 ± 3.55^{a}	$2.83\pm0.80^{a,b,c}$
Methanol extract 200 mg/kg	$3.20\pm0.28^{a,d}$	5.85 ± 1.14^{a}	10.10 ± 9.98^{a}	$3.28\pm1.41^{a,b,c}$
Methanol extract 250 mg/kg	3.45 ± 0.90^{a}	6.43 ± 0.92^{a}	6.80 ± 1.92^{a}	$3.45\pm0.64^{a,b,c}$
Aqueous extract 100 mg/kg	$2.95\pm0.13^{a,d,e}$	11.95 ± 1.51^{b}	2.98 ± 0.90^{a}	$2.45\pm0.34^{b,c}$
Aqueous extract 150 mg/kg	$2.80\pm0.28^{a,b,d,e}$	12.00 ± 0.00^{b}	8.85 ± 4.79^{a}	2.20 ± 0.36^{c}
Aqueous extract 200 mg/kg	$3.00\pm0.30^{a,d}$	12.33 ± 0.58^{b}	3.67 ± 1.05^{a}	$2.73\pm0.91^{a,b,c}$
Aqueous extract 250 mg/kg	$2.17\pm0.15^{b,c}$	12.33 ± 0.58^{b}	6.47 ± 0.61^{a}	2.13 ± 0.67^{c}

Values are mean ± standard deviation, n=4 number of animals per group. Values on the same column with different letters (a,b,c,d,e) are significantly different at $p<0.05$.

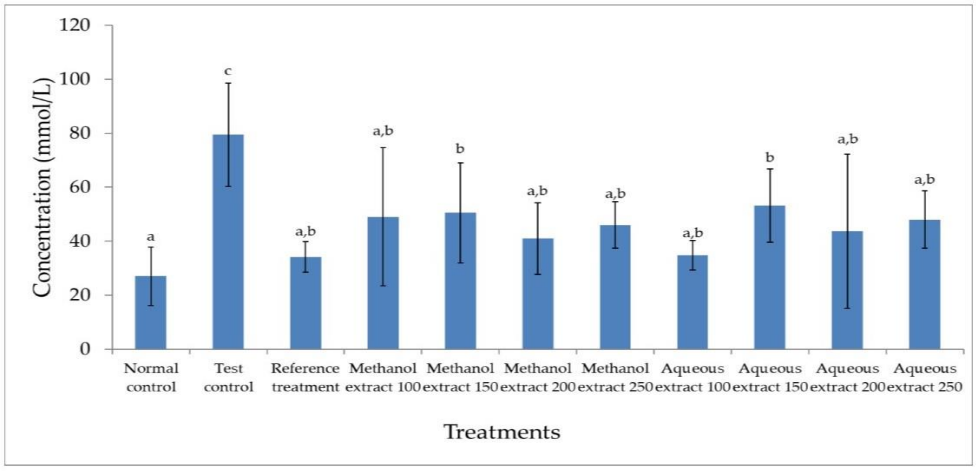

Figure 2. Effect of methanol and aqueous leaf-extracts of *Annona muricata* on lens malondialdehyde concentration.

Each bar represents the Mean ± SD for each group of rats, n =4. Bars with the different letters (a, b, c) are significantly different at $p < 0.05$.

Table 6. Effect of methanol and aqueous leaf-extracts of *Annona muricata* Linn on markers of kidney function

Treatment	Urea (mmol/L)	Creatinine (mmol/L)
Normal control	$6.30\pm0.65^{a,b,c,f}$	$102.00\pm20.66^{a,b}$
Negative or test control	$5.28\pm1.38^{c,d,e}$	$96.25\pm17.56^{a,b}$
Reference treatment	$4.55\pm1.23^{d,e}$	$108.00\pm7.66^{a,b}$
Methanol extract 100 mg/kg	4.30 ± 0.77^{e}	$90.25\pm10.21^{a,b}$
Methanol extract 150 mg/kg	$5.70\pm1.00^{c,d,e,f}$	$96.25\pm9.74^{a,b}$
Methanol extract 200 mg/kg	$6.05\pm1.18^{c,d,f}$	$94.00\pm19.60^{a,b}$
Methanol extract 250 mg/kg	$5.98\pm1.09^{c,d,f}$	86.50 ± 6.56^{a}
Aqueous extract 100 mg/kg	7.68 ± 0.51^{a}	111.50 ± 21.95^{b}
Aqueous extract 150 mg/kg	$6.93\pm1.58^{a,f}$	$105.50\pm14.93^{a,b}$
Aqueous extract 200 mg/kg	7.70 ± 0.69^{a}	$105.67\pm13.20^{a,b}$
Aqueous extract 250 mg/kg	9.43 ± 0.51^{b}	$97.33\pm13.32^{a,b}$

Values are mean ± standard deviation, n=4 number of animals per group. Values on the same column with different letters (a,b,c,d,e,f) are significantly different at $p<0.05$.

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Table 7. Effect of methanol and aqueous leaf-extracts of *Annona muricata* Linnon electrolytes in selenite treated rats

Treatment	Potassium (mEq/L)	Chloride(mEq/L)	Sodium(mEq/L)
Normal control	3.65±0.13[a]	89.00±4.55[a]	110.00±6.78[a]
Test control	3.40±0.34[a]	105.00±5.94[e,f]	118.50±7.55[a,c]
Reference treatment	4.28±0.05[a]	100.75±8.50[d,e,f]	112.25±9.95[a]
Methanol extract 100	3.80±0.22[a]	106.50±6.03[e]	114.00±3.92[a]
Methanol extract 150	4.40±0.18[a]	94.50±6.61[a,f]	127.75±11.44[c]
Methanol extract 200	4.13±0.05[a]	96.50±5.00[a,e]	112.50±13.03[a]
Methanol extract 250	13.55±18.97[b]	106.25±7.14[e]	114.00±9.20[a]
Aqueous extract 100	4.03±0.15[a]	71.00±6.48[b,c]	97.00±5.29[d]
Aqueous extract 150	3.81±0.18[a]	93.00±17.07[a,d]	79.75±4.27[b]
Aqueous extract 200	3.97±0.15[a]	65.33±2.31[c]	86.00±13.08[b,d]
Aqueous extract 250	3.83±0.32[a]	77.33±4.51[b]	88.00±2.00[b,d]

Values are mean ± standard deviation, n=4 number of animals per group. Values on the same column with different letters (a,b,c,d,e,f) are significantly different at p<0.05.

control group was significantly ($p < 0.05$) higher than those of reference treatment, methanol extract 100 mg/kg; significantly ($p < 0.05$) lower than the aqueous extract 250 mg/kg, but not significantly different from the others.

3.5. Moderation of plasma electrolytes

The effect of methanol and aqueous leaf extracts of *Annona muricata* Linnon plasma electrolytes of selenite-treated rats is presented in Table 7. The plasma potassium levels of the test control group was significantly ($p<0.05$) lower than methanol extract 250 mg/kg, but not significantly lower than all the others. There were no significant differences in the plasma potassium levels of all the groups, except that of methanol extract 250 mg/kg, which was significantly ($p < 0.05$) higher than all the other groups. The plasma sodium levels of the test control group was significantly ($p < 0.05$) higher than those of aqueous extract 100 mg/kg, aqueous extract 150 mg/kg, aqueous extract 200 mg/kg and aqueous extract 250 mg/kg, but not significantly different from the others. That of the normal control, reference treatment, methanol extract 100 mg/kg, methanol extract 200 mg/kg and methanol extract 250 mg/kg were significantly ($p < 0.05$) higher than those of aqueous extract 100, 150, 200 and 250 mg/kg; significantly ($p<0.05$) lower than methanol extract 150 mg/kg, but not significantly different from one another, and Test control. The plasma chloride concentration of the Test control group was significantly ($p<0.05$) higher than those of normal control, aqueous extract 150, 200 and 250 mg/kg, but not significantly different from those of the others. That of Normal control group was significantly ($p<0.05$) lower than those of test control, reference treatment, methanol extract 100 and 250 mg/kg; significantly ($p<0.05$) higher than aqueous extract 100, 200 and 250 mg/kg, but not significantly different from the other three groups. That of the reference treatment group was significantly ($p<0.05$) higher than those of the normal control, aqueous extract 100, 150, 200 and 250 mg/kg, but not significantly different from the other groups.

3.6. Histopathological studies

Plates 1 to 11 show the photomicrographs of the kidney of normal and selenite-treated rats.

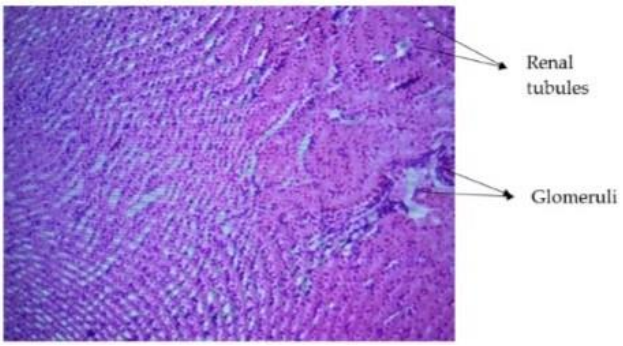

Plate 1. Section of renal tissue of a Normal control rat, showing glomeruli, tubules and blood vessels, with no inflammatory cell infiltrate seen in the interstitium.

4. Discussion

Phytochemical screening of the leaves showed the presence of alkaloids, tannins, flavonoids, saponins and phenolics (Tables 2 and 3). These compounds are known to have pharmacological activities. For

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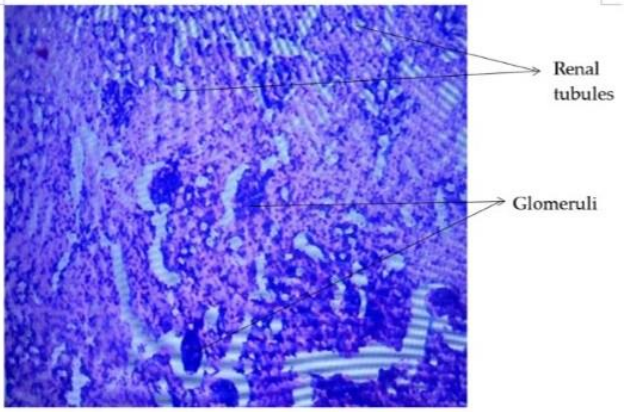

Plate 2: Section of renal tissue of a test control (untreated cataractous) rat showing numerous glomeruli, tubules and blood vessels, with no inflammatory cell infiltrate seen in the interstitium.

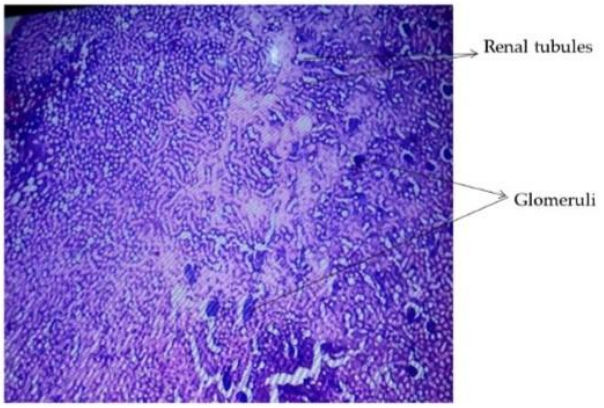

Plate 3. Section of renal tissue of a cataractous animals treated with the Reference drug (ascorbic acid, 100 mg/kg), showing numerous glomeruli, tubules and blood vessels, with no inflammatory cell infiltrate seen in the interstitium.

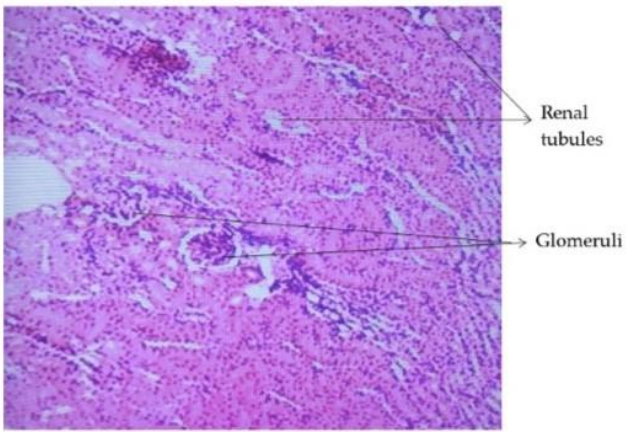

Plate 4: Section of renal tissue of a cataractous rat treated with 100 mg/kg Methanol extract, showing few glomeruli, tubules and blood vessels, with no inflammation cell infiltrate seen in the interstitium.

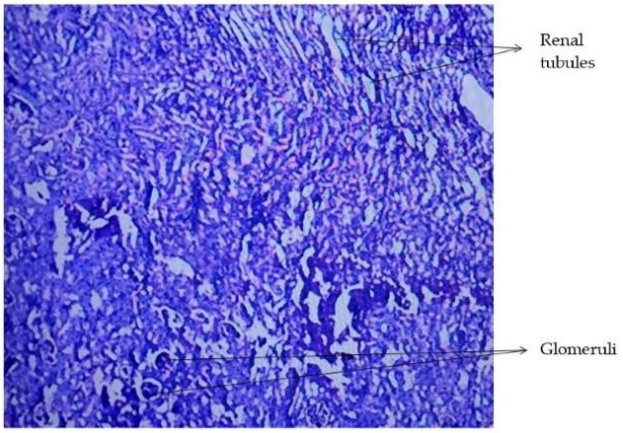

Plate 5: Section of renal tissue of a cataractous rat treated with 150 mg/kg of Methanol extract, showing numerous glomeruli, tubules and blood vessel, with no inflammation cell infiltrate seen in the interstitium.

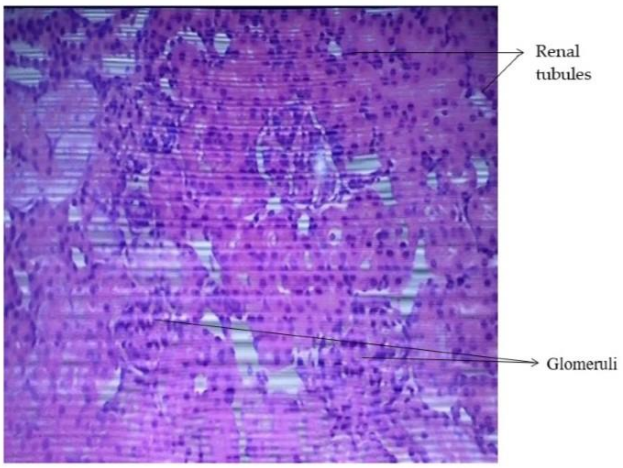

Plate 6. Section of renal tissue of a cataractous rat treated with 200 mg/kg of Methanol extract, showing numerous glomeruli and tubules. The glomeruli and tubules appear unremarkable, and no inflammation cell infiltrate seen in the interstitium.

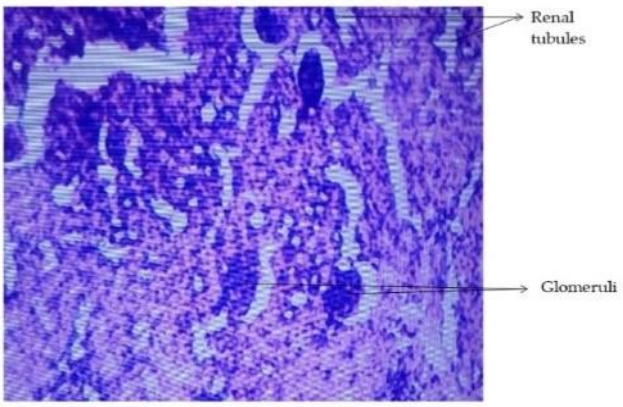

Plate 7. Section of renal tissue of a cataractous rat treated with 250 mg/kg of Methanol extract, showing glomeruli and tubules, with no inflammation cell infiltrate seen in the interstitium.

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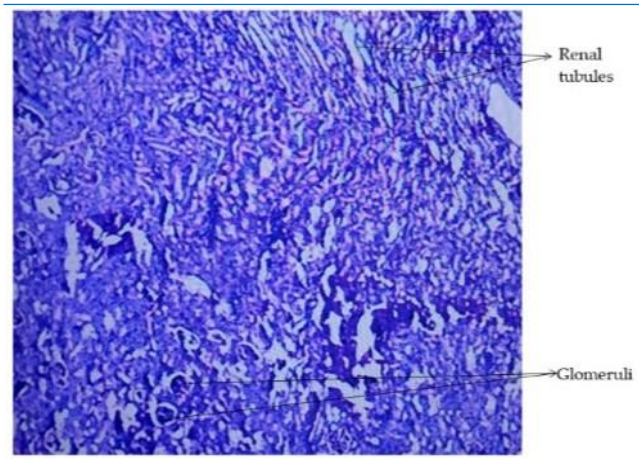

Plate 8. Section of renal tissue of a cataractous rat treated with 100 mg/kg Aqueous extract, showing glomeruli and tubules, with no inflammation cell infiltrate seen in the interstitium.

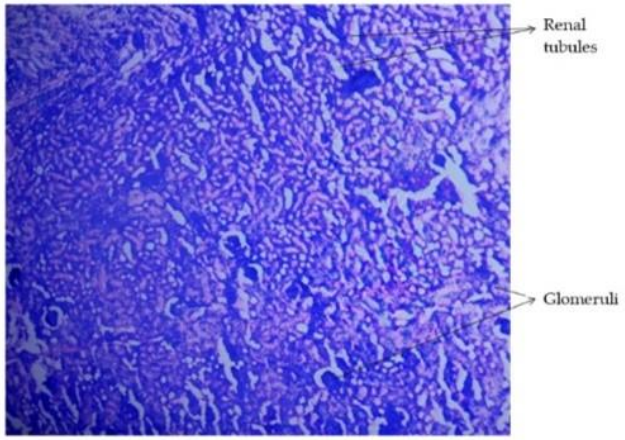

Plate 9: Section of renal tissue of a cataractous rat treated with 150 mg/kg Aqueous extract, showing glomeruli and tubules, with no inflammation cell infiltrate seen in the interstitium.

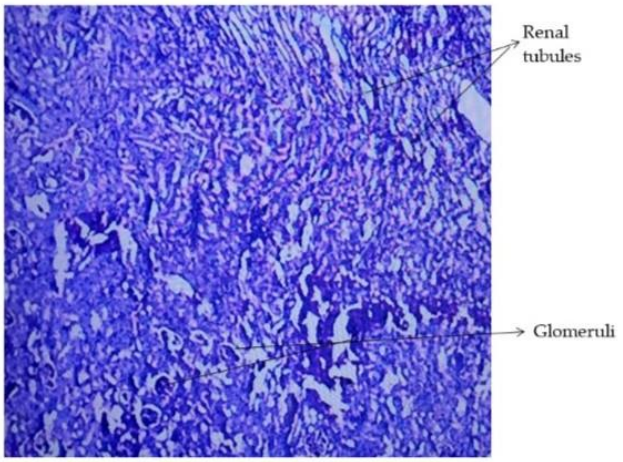

Plate 10: Section of renal tissue of a cataractous rat treated with 200 mg/kg aqueous extract showed glomeruli and tubules, with no inflammation cell infiltrate seen in the interstitium.

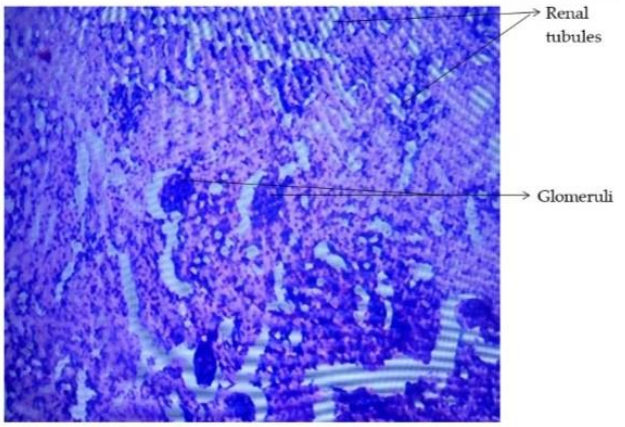

Plate 11. Section of renal tissue of a cataractous rat treated with 250 mg/kg Aqueous extract, showing numerous glomeruli and tubules, with no inflammation cell infiltrate seen in the interstitium.

example, some alkaloids, e.g. papaverine, a vasodilator, used to treat spasms of the gastrointestinal tract, bile ducts and urethra [29, 30] and erectile dysfunction [31] Another alkaloid morphine, is converted to codeine, which is used as a cough suppressant, analgesic, hypnotic and anti-diarrheal agent [32]. Saponins are reported to have a broad range of pharmacological properties, such as expectorant, anti-inflammatory, vaso-protective, hypocholestrolaemic, hypoglycaemic, anti-parasitic, antioxidant, anticancer and anti-protozoal activities [33].

Phenolic compounds exert a wide range of pharmacological activities, including antioxidant, anti-carcinogenic, anti-mutagenic, anti-inflammatory, vasodilatory effects and antithrombotic properties [34]. The pharmacological activities of flavonoids include antioxidant, anti-inflammatory, antimicrobial, antitumor, anti-ulcer and hepatoprotective activities [33]. Tannins have been reported to possess anti-cancer, anti-diarrheal, anti-asthmatic, cardioprotective, anti-diabetic, anti-cataractogenic, anti-inflammatory and hepatoprotective properties [33]. Alsayed and El-Naga [35], reported that a tannin derivative, ellagitannin, has been reported to possess gastroprotective potential. Therefore, these pharmacological activities of the constituents may be responsible for the biological activities of the extracts. Whenever the rate of production of free radicals is higher than the body's ability to counteract or detoxify their harmful effects through neutralization

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by antioxidants, oxidative stress results [36, 37]. Oxidative stress has been implicated in the pathogenesis of cataracts in lens epithelial cells [5]. Increases in tissue and blood levels of thiobarbituric acid-reactive substances (mainly malondialdehyde) are very reliable indicators of oxidative stress and lipid peroxidation [38]. In this study, the extracts improved the glutathione peroxidase (only the aqueous extract) and SOD activities of the treated animals. It also lowered the MDA levels of the treated animals, while having no significant effect on their catalase activities and ascorbic acid levels compared to the test control. Therefore, the levels of malondialdehyde in the treated animals clearly indicate that the extracts protected against oxidative stress. This antioxidant protective effect may have been mediated by the saponin and phenolic compounds (e.g. flavonoids) present in the leaves. These compounds are reputed to possess anti-oxidant properties [39-45].

The measures of renal function assess the normal functioning of kidneys. They signify the glomerular filtration rate, concentration and diluting capacity of kidneys (tubular function). Increase and decrease in the concentration of these markers indicates kidney dysfunction. According to Stevens and Levey [42], glomerular filtration rate (GFR) is widely accepted as the best index of kidney function in health and disease, and can be estimated based on serum creatinine. Creatinine is a waste product made by the muscles in the body, which passes into the bloodstream, and is eventually passed out of the body in urine. A high blood level of creatinine shows kidney dysfunction. There was no significant difference between the plasma creatinine levels of the test control and normal control, and the other groups. This could be because the exposure time of sodium selenite for this study was too short, and there might not have been enough time for renal dysfunction (and thus elevated creatinine) to develop, even if damages were to eventually occur. Urea, a major nitrogenous end product of protein and amino acid catabolism, is produced by the liver and distributed throughout the intracellular and extracellular fluid. In kidneys, it is filtered out of blood by glomeruli and is partially reabsorbed with water [43]. Increased Blood Urea Nitrogen (BUN) is an indicator of severe kidney damage, whereas decreased BUN occurs when there is excessive fluid in the body. In this study, the plasma urea levels produced by the methanol extract were not significantly different from those of the test control and the normal control; however, those of the aqueous extract was significantly higher than those of the test control, but not significantly different from those of the normal control. Taking this result in light of the creatinine level, it means that the observed difference in urea levels may not be due to kidney dysfunction but as a result of increased protein and amino acid metabolism. Thus, the result of this study showed that both extracts had no negative effect on the kidney.

The aqueous extract significantly ($p < 0.05$) lowered the plasma sodium levels of the treated rats, compared to the Test control and normal control; while the methanol extract increased ($p < 0.05$, though not significantly except the 150 mg/kg treatment) the plasma sodium levels of the treated rats, compared to normal control. The treatment with the aqueous extract also significantly lowered the sodium selenite-induced increase in plasma chloride levels of the treated animals, compared to test control and normal control. Earlier, studies have shown that reduction in plasma sodium and chloride concentrations is one of the modes of action of diuretics, which lower plasma levels of these electrolytes by reducing their reabsorption at different sites in the nephrons [46]. This indicates that the aqueous extract may have diuretic properties.

An evaluation of the kidney tissues, treated revealed a normal kidney with normal glomeruli, tubules and blood vessels. All of these are in tandem with the observed level of MDA, thus indicating that a possible amelioration of lipid peroxidation by the extracts could be responsible for their anti-cataract and nephroprotective effects. The anti-cataract and nephroprotective activities of the extracts may be due to their contents of tannins, flavonoids and phenolics, all of which are known to have antioxidant activities [47].

5. Conclusions

The results from this study showed the ability of aqueous extract of the leaves of *Annona muricata* to reduce lenticular oxidative stress by reducing MDA levels and increasing glutathione peroxidase and

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catalase activities. Thus highlighting its potential as an agent for the management and control of cataract-induced oxidative stress. The study also showed that the extracts normalised plasma levels of markers of kidney function and had no deleterious effect on the kidney's histoarchitecture, and so is relatively safe. All of these imply that *Annona muricata* Linn could be a promising plant for the development of an anti-cataract and antinephrotoxic drugs.

Ethical Statement

Ethical approval was obtained from University of Port Harcourt Research Ethics Committee, with Reference Number (UPH/CEREMAD/REC/MM79/085).

Authors' contributions

Conceptualization, methodology, formal analysis, investigation, project administration, resources, software, supervision, validation, visualization, funding acquisition, writing—review and editing, N.U.M.; Data curation, N.U.M., K.V.S, O.J.C., C.C.E., C.A.A., N.M.N., O.J.O, U.N.O, N.K.O, N.O.D, O.G.C, K.I.E., C.N.O., E.O.N., C.L.U, O.J.C, I.K.U.; N.U.M.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of interest

The authors declare no conflicts of interest regarding this manuscript.

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