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RESEARCH ARTICLE

Protective Effects of Methanol and Aqueous Leaf Extracts of *Annona muricata* Linn on Sodium Selenite-Induced Oxidative Stress on the Liver in Selenite Cataract Rat Model

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Abstract

Background and objective: Oxidative stress induces lens opacity. *Annona muricata* extracts have demonstrated potential anti-oxidant and medicinal efficacy. This study evaluated the protective effects of methanol and aqueous leaf extracts of *Annona muricata* Linn on sodium selenite-induced oxidative stress on the liver in a selenite cataract rat model

Methods and study design: The study involved 55 Wistar suckling pups randomly split into 11 groups. Sodium selenite (30 µmol/kg, S.C) was administered on the 10th day of life, with the exception of the normal control group. Group C (positive control) was treated with the reference drugs (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 (negative control).

Results: Compared to test control, the malondialdehyde levels of the other groups, and the catalase activity of the aqueous extract treated groups were significantly ($p<0.05$) lower, and the glutathione peroxidase activity of the aqueous extract treated groups were significantly ($p<0.05$) higher. The aqueous extract treatment also significantly lowered plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities ($p<0.05$). Histopathology revealed mild inflammatory cells in groups treated with Methanol extract but showed normal hepatocytes in groups treated with aqueous extract.

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Conclusion: These findings show that the methanol and aqueous extracts of *Annona muricata* have dose-dependent hepatoprotective properties and may be a viable plant for the development of an anti-hepatotoxic drugs.

Keywords: *Annona muricata,* Hepatotoxicity, Oxidative Stress, Anti-oxidation, Anti-hepatotoxicity.

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 cataract 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.

The redox state plays a significant role in the development of many liver diseases. The redox status influences the development of inflammatory, metabolic, and proliferative liver disorders. Hepatocytes generate reactive oxygen species (ROS) largely through cytochrome P450 enzymes in the mitochondria and endoplasmic reticulum. Under appropriate conditions, cells have particular molecular methods to regulate oxidative stress and continue to maintain a balance of oxidant and antioxidant particles. ROS and reactive nitrogen species largely impact hepatic proteins, lipids, and DNA. The process causes structural and functional problems in the liver. 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[6, 7] compared to cataract surgery or treatment of liver diseases.

According to World Health Organization, over 80% of the world's population relies on the use of herbal medicine as a remedy.[8-10] This could explain why traditional medicine is gaining popularity all around the world right now.[11] 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.[12-14] 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.[15] *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.[16]

The *Annonaceae* family, which includes over 130 genera and 2300 species, includes *Annona muricata Lin.*, also known as soursop.[17,18] *A. muricata L.* has a variety of pharmacologically active chemicals. In tropical and subtropical regions like Southeast Asia, South America, and the African rainforests, this plant is commonly farmed. The year-round edible fruit production of the plant makes it a popular traditional remedy for bacterial infections, skin conditions, respiratory disorders, fever, diabetes, high blood pressure, and cancer [19]. The plant has also been studied for its antioxidant properties.[20-23] Nam *et al.*[22], reported that there was a strong positive correlation between the antioxidant activity and total phenol content in *Annona muricata*. *Annona muricata* extracts were rich in various phytochemicals including rutin, epicatechin, ferulic acid, and *p*-coumaric acid. Their findings indicate that *Annona muricata* is a potentially useful source of substances with antioxidant effects.[22] The finding from the study of Muthu and Durairaj[23] reveals that hydroalcoholic extract of *Annona muricata* leaves could be considered as nutraceutical with potent source of antioxidants and radical scavenging activity suitable for prevention of human diseases caused by oxidative stress.

Therefore, there is a need to synergize phyto medicine with conventional medicine in the treatment of oxidative stress-induced cataracts so as to promote affordability of treatment. This study is aimed to

evaluate the anti-oxidative and antihepatotoxic effects of methanol and aqueous leaf extracts of *Annona muricata* on sodium selenite-induced cataract in Wistar suckling pups. The aim of the study is to evaluate the protective effects of methanol and aqueous leaf extracts of *Annona muricata* Linn on sodium selenite-induced oxidative stress on the liver in a selenite cataract rat model.

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). The research was carried out in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE).

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

Test for Alkaloids

This was carried out according to the method of Sofowora.[24] 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.

Test for Tannins

This was carried out by the ferric chloride test as described by Trease and Evans.[25] 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.

Test for Flavonoids

This was carried out by the Shinoda test, as described by Trease and Evans.[25] A piece of magnesium metal was added to 5 g of leaf powder, and then 2-4 drops of 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.

Test for Saponins

The presence of saponin was determined by the Frothing test.[25] 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.

Test for Cardiac-Glycosides

The presence of cardiac glycosides was determined by the Keller-Kiliani test.[25] 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 solutions and precipitates were noted.

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

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 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 water bath at 60°C and filled into beaker and the residue was discarded. Conc. NaOH was added into 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.

Calculation: $$\% \text{ alkaloid} = \frac{\text{Weight residue}}{\text{Weight of sample analysed}} \times 100$$ ..... (i)

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

Calculation:

.....(ii)

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.

Calculation: $$\% \text{ saponin content} = \frac{\text{weight of residue}}{\text{weight of sample analysed}} \times 100$$

.....(iii)

2.3.3 Preparation of the Leaf Extracts

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 was allowed to evaporate at 600 mmHg reduced pressure using a vacuum rotary evaporator, 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.

Aqueous Extract

Another portion (1000 g) of powdered leaves was mashed in 2.5 L of distilled water at room temperature for 48 h (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.

Determination of LD_{50}

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

2.4 Evaluation of Anti-Cataract Activity via Ocular MDA Assay

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).

2.5 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.

Table 1. *Experimental design for the assay*

Group	Treatment group	Details
Group A	Normal control	Distilled water + no treatment (distilled water)
Group B	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).

2.6 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 Biochemical Assays

Superoxide dismutase activity was determined using the spectrophotometric method according to Misra and Fridovich.[26] Catalase activity was determined using the spectrophotometric method according to the method of Beers and Sizer.[27] For the assay of plasma ascorbic acid concentration, the method was adopted from Robitaille and Hoffer[28] and Kim and Kim.[29] Glutathione peroxidase activity was determined using the spectrophotometric method according to Rotruck *et al.*[30] For the assay of ocular malondialdehyde (MDA) concentration, the method adopted was that of Hunter *et al.*[31], as modified by Gutteridge and Wilkins[32]. The colorimetric approach described by Reitman and Frankel[33] was used to assess serum ALT and AST activity for liver function tests. The colorimetric method for assessing ALP activity

was developed by Kind and King[34]. Total bilirubin was determined using the spectrophotometric method published by Malloy and Evelyn[35]. The assay of plasma gamma-glutamyl transferase (GGT) activity and plasma total protein concentration was carried out with the Randox test kit (Randox Laboratories, Crumlin, England, UK). The dye binding method (bromocresol green) of Bartholomew and Delaney[36] was used to assay albumin.

2.8 Histopathologic Evaluation

The liver was harvested, fixed in a solution of 10% formaldehyde and prepared for histological evaluation. The tissues were fixed, sectioned, read and interpreted at the Anatomy Laboratory, Department of Anatomy, Faculty of Basic Medical Sciences, University of Port Harcourt, River State, Nigeria. The tissues were embedded in paraffin wax, and sections of 4-6 µm were obtained.

2.9 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[37] 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.

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 Figure 1. From this regression plot the equation was derived as:

$$\text{Probit} = -39.8579869786464 + 14.7293289928821 \times \log(\text{dose}) \quad \text{..... (iv)}$$

From the above equation, the indices of lethality of the extract were calculated (Table 4). The oral LD_{50} of the 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 2. *Result of the qualitative phytochemical tests on the leaf powder*

S/N	Constituent	Test	Observation	Inference
1	Alkaloids	a. Dragendroff's test a. Meyer's test b. Picric acid	Pink colouration Yellow precipitate Yellow precipitate	Positive Positive 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

Table 3. *Result of the quantitative phytochemical tests on the leaf powde*

Component	Composition (mg/g)
Alkaloids	17.4 ±0.3
Saponins	29.1±0.2
Phenolic compounds	32.5±1.5

Table 4. *The lethality indices of the extract*

Index	Value (mg/kg)
LD_{50}	1110.42 ±21.3
LD_{01}	771.44 ±15.8
Highest non-lethal Dose	696.85±12.3

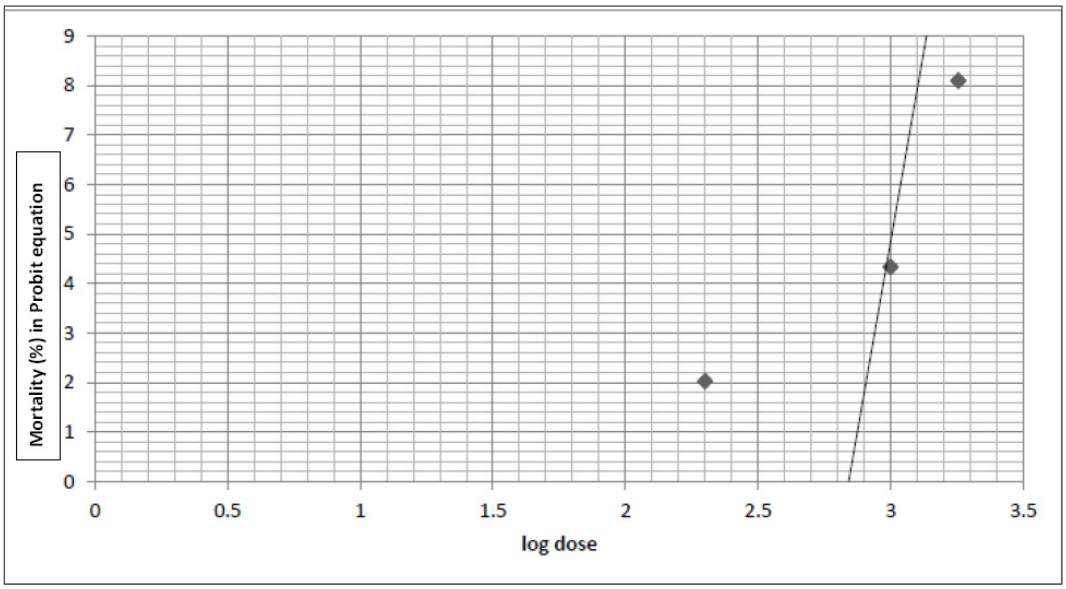

Figure 1. *Probit plot of the frequency of death against the doses of administration (***Note:** *the grids were left to enable easy understanding of the extrapolations)*

3.3 Anti-Oxidative Stress Activity in Blood Serum

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 Figure 2. The plasma glutathione peroxidase activity of the test control (5.93±0.81 x10^{-2} U/L), normal control (6.38±0.50 x10^{-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±0.60x10^{-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 x10^{-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 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 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 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 mg/kg and aqueous extract 150 mg/kg, but were significantly (p<0.05) lower than Test control.

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

Groups	Treatment	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)
A	Normal control	3.30 ± 0.29^{a}	6.38 ± 0.50^{a}	10.20 ± 6.39^{a}	4.30 ± 1.52^{a}
B	Negative or test control	1.83 ± 0.60^{c}	5.93 ± 0.81^{a}	4.43 ± 2.44^{a}	$2.29\pm0.45^{b,c}$
C	Reference treatment	$2.53\pm0.85^{b,c,d,e}$	6.28 ± 0.54^{a}	10.10 ± 11.29^{a}	$4.20\pm0.89^{a,b}$
D	Methanol extract 100 mg/kg	$2.25\pm0.57^{b,c,e}$	5.38 ± 0.84^{a}	5.15 ± 3.41^{a}	$4.06\pm2.81^{a,b}$
E	Methanol extract 150 mg/kg	$2.13\pm0.41^{b,c}$	5.83±0.15a	6.43 ± 3.55^{a}	$2.83\pm0.80^{a,b,c}$
F	Methanol extract 200 mg/kg	$3.20\pm0.28^{a,d}$	5.85 ± 1.14^{a}	10.10 ± 9.98^{a}	$3.28\pm1.41^{a,b,c}$
G	Methanol extract 250 mg/kg	3.45 ± 0.90^{a}	6.43 ± 0.92^{a}	6.80 ± 1.92^{a}	$3.45\pm0.64^{a,b,c}$
H	Aqueous extract 100 mg/kg	$2.95\pm0.13^{a,d,e}$	11.95 ± 1.51^{b}	2.98 ± 0.90^{a}	$2.45\pm0.34^{b,c}$
I	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}
J	Aqueous extract 200 mg/kg	$3.00\pm0.30^{a,d}$	12.33 ± 0.58^{b}	3.67 ± 1.05^{a}	$2.73\pm0.91^{a,b,c}$
K	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.

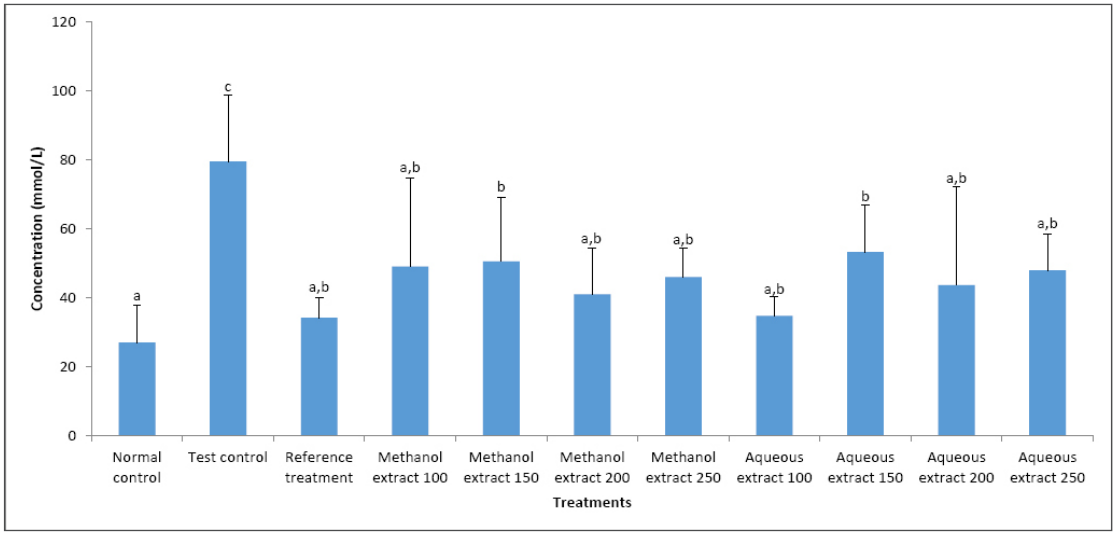

Figure 2. *Effect of methanol and aqueous leaf-extracts of Annona muricata on lens malondialdehyde concentration. Each bar represents the Mean ± SD for each group of rats, n =4. Bars with the different letters (a,b,c) are significantly different at p < 0.05.*

3.4 Hepatoprotective Activity

Table 6 Effect of methanol and aqueous leaf-extracts of *Annona muricata* Linn on markers of liver function of selenite treated rats. The plasma AST activity of the Test control and Reference treatment groups were significantly (p<0.05) higher than those of Methanol extract 150 mg/kg, Methanol extract 250 mg/kg, 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 norm control group was significantly (p<0.05) higher than those of Methanol extract 150 mg/kg, 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 other groups. The plasma ALT activity of the Test control group was significantly (p<0.05) higher than those of all the other groups except Normal control. That of the Normal control group was significantly (p<0.05) higher than those of Reference treatment, Methanol extract 150 mg/kg, Aqueous extract 100, Aqueous extract 150 mg/kg, Aqueous extract 200 mg/kg and Aqueous extract 250 mg/kg, but not significantly higher than the others. The Reference treatment groups was significantly (p<0.05) lower than Normal control, Test control, Methanol extract 200 mg/kg and Aqueous extract 100 mg/kg, but not significantly different from the others. The plasma ALP activities of the Normal control and Test control

groups were significantly (p<0.05) lower than those of all the other groups, but not significantly different from each other. That of the Reference treatment was significantly (p<0.05) lower than those of Methanol extract 200 mg/kg, Aqueous extract 100 mg/kg, Aqueous extract 150 mg/kg, Aqueous extract 200 mg/kg and Aqueous extract 250 mg/kg; significantly (p<0.05) higher than those of Normal control and Test control, but not significantly different from the others. The plasma total protein level of the Test control and Reference treatment groups were significantly (p<0.05) lower than those of Aqueous extract 100 mg/kg, Aqueous extract 150 mg/kg mg/kg, Aqueous extract 200 mg/kg and Aqueous extract 250 mg/kg; significantly (p<0.05) higher than that of Methanol extract 250 mg/kg, but not significantly different from each other, and the remaining groups. That of the Normal control group and Methanol extract 100 mg/kg were significantly (p<0.05) higher than those of Methanol extract 150 mg/kg, Methanol extract 200 mg/kg, Methanol extract 250 mg/kg, Aqueous extract 100 mg/kg, Aqueous extract 150 mg/kg and Aqueous extract 250 mg/kg, but not significantly different from one another, and the remaining groups. The plasma albumin levels of the Test control and Methanol extract 150 mg/kg groups were significantly (p<0.05) higher than those of Reference treatment, Methanol extract 200 mg/kg, 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 one another and the remaining groups. That of the Normal control and Methanol extract 100 mg/kg were significantly (p<0.05) higher than those of Reference treatment, Methanol extract 200 mg/kg, Methanol extract 250 mg/kg, 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 one another, and the remaining groups. That of the Reference treatment group, Methanol extract 200 mg/kg, Aqueous extract 100 mg/kg and Aqueous extract 250 mg/kg, were significantly (p<0.05) lower than those of Normal control, Test control, Methanol extract 100 mg/kg, Methanol extract 150 mg/kg and Aqueous extract 150 mg/kg, but not significantly different from one another, and the remainder of the groups.

The plasma GGT activity of the Test control, Normal control, Methanol extract 100 mg/kg, Aqueous extract 150 mg/kg and Aqueous extract 200 mg/kg, were not significantly different from each other as well as Reference treatment, Methanol extract 150 mg/kg, Methanol extract 250 mg/kg, Aqueous extract 100 mg/kg and Aqueous extract 250 mg/kg, but were significantly (p<0.05) higher than that of Methanol extract 200 mg/kg. That of the Methanol extract 200 mg/kg was significantly (p<0.05) lower than those of Normal control, Test control, Methanol extract 150 mg/kg, Aqueous extract 100 mg/kg, Aqueous extract 150 mg/kg, Aqueous extract 200 mg/kg and Aqueous extract 250 mg/kg, but not significantly lower than Reference treatment and Methanol extract 250 mg/kg. The GGT activity of the Aqueous extract 250 mg/kg group was significantly (p<0.05) higher than those of Reference treatment, Methanol extract 200 mg/kg, Methanol extract 250 mg/kg and Aqueous extract 100 mg/kg, but not significantly different form the others. That of the Reference treatment group and Methanol extract 250 mg/kg were not significantly different from one another, as well as from Normal control, Test control, Methanol extract 100 mg/kg, Methanol extract 200 mg/kg, Aqueous extract 100 mg/kg, Aqueous extract 150 mg/kg and Aqueous extract 250 mg/kg, but significantly (p<0.05) lower than those of Methanol extract 150 mg/kg and Aqueous extract 250 mg/kg. The plasma total bilirubin levels of the Test control, Normal control, Reference treatment, Methanol extract 150 mg/kg, Methanol extract 200 mg/kg, Methanol extract 250 mg/kg, Aqueous extract 100 mg/kg and Aqueous extract 150 mg/kg were not significantly different from one another, as well as from Methanol extract 100 mg/kg and Aqueous extract 250 mg/kg, but were significantly (p<0.05) lower than that of Aqueous extract 200.

Table 6. *Effect of methanol and aqueous leaf-extracts of Annona muricata Linnon plasma markers of liver function in selenite treated rats*

Normal control	Aspartate transaminase activity (AST) (U/L)	Alanine transaminase activity (ALT) (U/L)	Alkaline phosphatase activity (ALP) (U/L)	Gamma glutamyl transferase activity(GGT) (U/L)	Total bilirubin content (TB) (µmol/L)	Total protein (TP) content (g/L)	Albumin content (ALB) (g/L)
Normal control	45.00±8.19^{b}	11.00±5.20^{b,d}	58.00±2.00^{b,e}	28.00±0.00^{c,d}	2.73±0.46^{a}	70.00±2.00^{f}	34.33±3.79^{d,e}

Negative or test control	103.00±11.58^{a,c}	28.25±4.27^{a,c}	32.33±4.55^{a}	25.75±0.96^{a,c,d}	4.18±0.95^{a,c}	54.50±3.51^{a,c}	42.00±1.83^{a}
Reference treatment	108.75±23.46^{c}	29.50±10.63^{c}	33.75±6.24^{a}	25.75±2.63^{a,c,d}	5.58±1.51^{a,c}	50.25±2.22^{c,d}	40.75±2.87^{a,c}
Methanol extract 100 mg/kg	109.00±29.70^{c}	17.00±0.00^{d,f,h}	43.25±6.95^{c}	24.50±0.58^{a,b}	4.65±1.10^{a,c}	50.00±2.45^{c,d}	34.50±1.91^{d,e}
Methanol extract 150 mg/kg	100.25±8.54^{a,c,d}	22.00±3.83^{a,e,f}	49.50±3.87^{c}	26.75±2.63^{a,c,d}	6.03±1.77^{c}	54.50±8.70^{a,c}	43.25±2.06^{a}
Methanol extract 200 mg/kg	73.50±21.08^{d,e}	18.75±2.06^{e,f,g,h}	44.00±9.42^{c}	27.75±0.96^{c}	5.58±1.51^{a,c}	46.50±2.08^{d,e}	40.00±5.48^{a,c}
Methanol extract 250 mg/kg	104.25±11.12^{a,c}	25.00±5.66^{a,e,g}	52.25±3.30^{d,e}	22.00±2.16^{b}	4.18±0.95^{a,c}	44.25±4.86^{d,e}	33.00±2.45^{d,e}
Aqueous extract 100 mg/kg	79.25±32.19^{a,d,e}	21.75±6.50^{a,e,f}	49.50±3.87^{c,d}	24.25±2.22^{a,b}	5.58±1.51^{a,c}	42.75±1.26^{e}	37.00±0.82^{c,e}
Aqueous extract 150 mg/kg	49.00±13.74^{b,e}	9.00±2.00^{b}	57.75±4.57^{b,e}	25.00±2.71^{a}	3.43±0.34^{a,c}	62.00±1.63^{b}	33.00±0.82^{d,e}
Aqueous extract 200 mg/kg	57.25±13.72^{b}	11.25±4.27^{b,d}	61.00±2.16^{b}	27.00±0.00^{a,c,d}	5.53±1.84^{a,c}	62.50±5.51^{b}	24.75±5.56^{b}
Aqueous extract 250 mg/kg	36.00±5.00^{b}	12.33±4.51^{b,d,g,h}	55.33±3.21^{b,d}	26.33±0.58^{a,c,d}	9.20±6.08^{b}	59.00±6.56^{a,b}	32.00±2.00^{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,g,h) are significantly different at p<0.05.

3.5 Histopathological Studies for Liver Injury Assessment

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

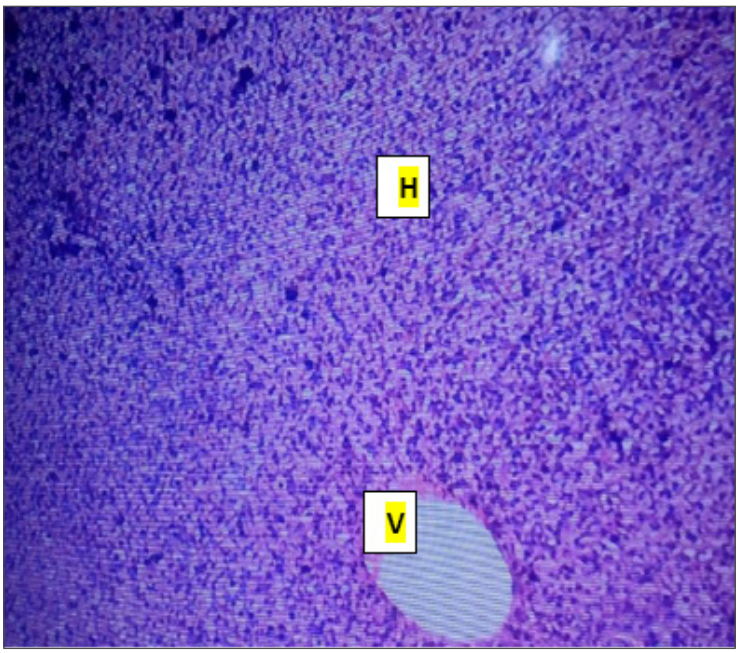

Plate 1. *Section of liver of a Normal control rat, showing portal vein (V) and normal rays of hepatocytes (H).*

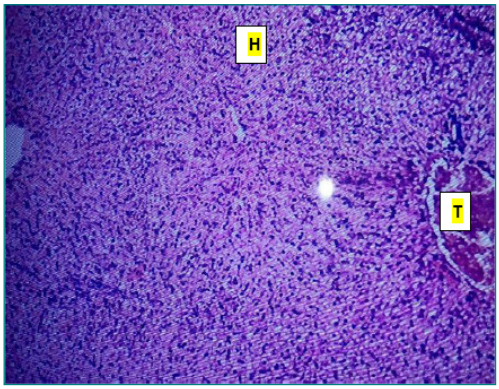

Plate 2. *Section of liver tissue of a Test control rat, showing central vein C. The rays of hepatocytes are seen, and the hepatocytes are filled with fat vacuoles (H). The portal tracts (T) show mild inflammatory cell infiltrates. The animals have mild steatohepatitis (H&E mag X5)*

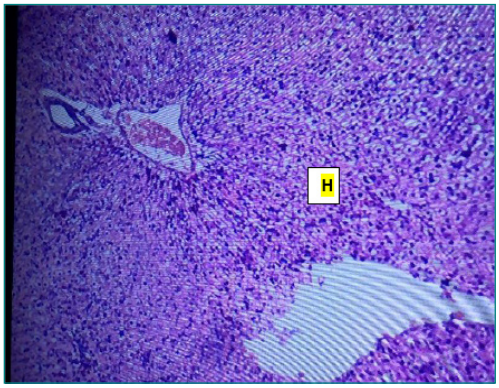

Plate 3. *Section of the liver of an animal treated with Reference drug (ascorbic acid, 100mg/kg), showing central vein. The hepatocytes (H) showed mild steatosis, and few inflammatory cells are seen (H&E mag X5)*

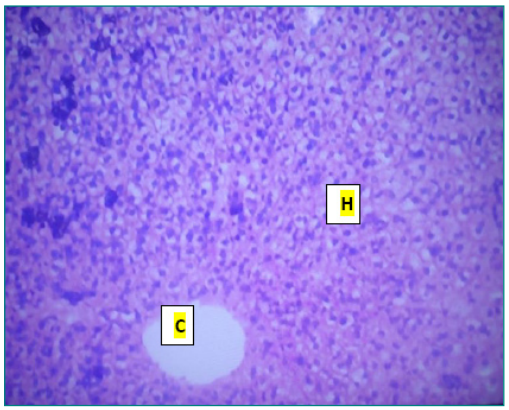

Plate 4. *ection of the liver tissue showing central (C) vein, arrays of hepatocytes (H) are seen, which are filled with fat vacuoles. There is little infiltration by inflammatory cells in animals treated with 100mg/kg of the Methanol extract.*

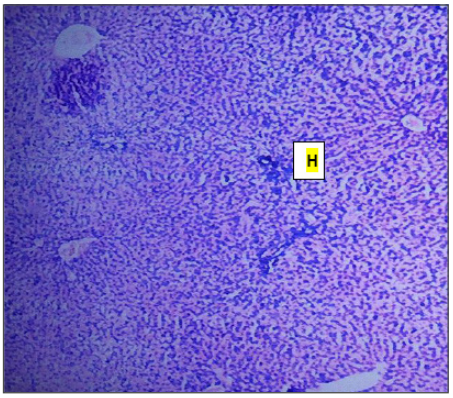

Plate 5. *Section of the liver showed rays of hepatocyte (H) in an animal treated with 150 mg/kg Methanol extract.*

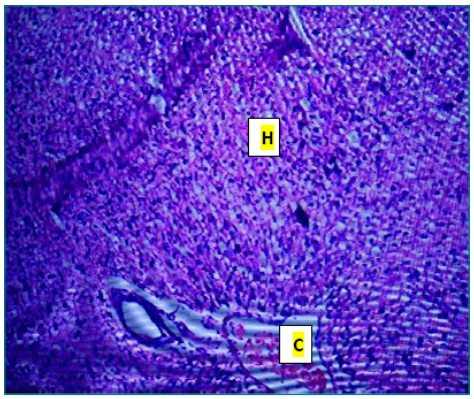

Plate 6. *Section of the liver tissue showed mild steatohepatitis in the rats treated with 200mg/kg Methanol extract. The rays of hepatocytes (H) are filled with fat vacuoles.*

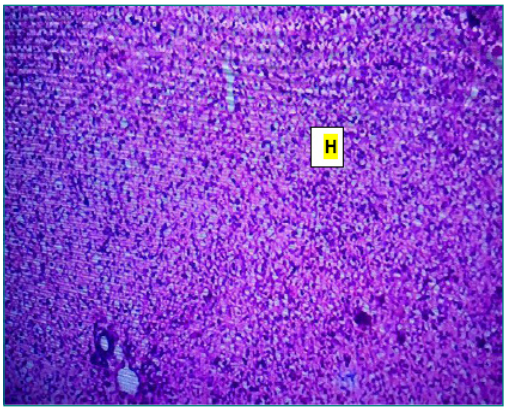

Plate 7. *Section of the liver tissue showed hepatocytes (H) with mild inflammation of the cell in the rats treated with 250mg/kg Methanol extract.*

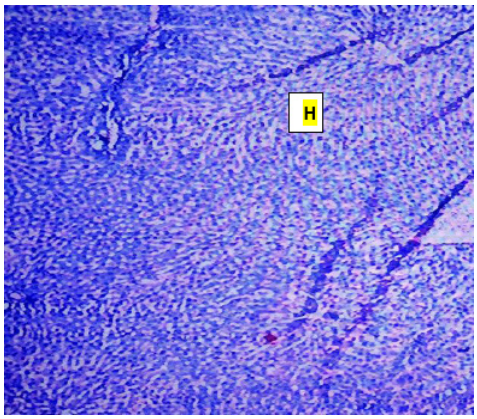

Plate 8. *Photomicrograph of liver tissue of cataractous animals treated with 100mg/kg Aqueous extract, showing rays of hepatocytes (H) with mild inflammation in the liver*

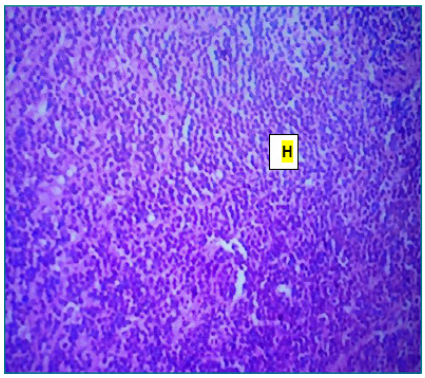

Plate 9. *Section of a liver tissue of a cataractous rat treated with 150 mg/kg of Aqueous extract, showing no inflammation of the cells. Hepatocytes appeared normal.*

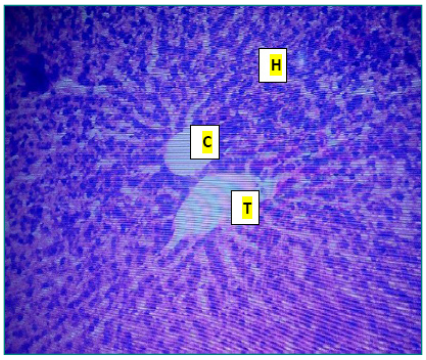

Plate 10. *Section of the liver of a rat treated with 200 mg/kg body weight of Aqueous extract, showing a portal (T) and central (C) vein; there are rays of hepatocytes (H) linking portal tract to the central vein, and the sinusoids appear normal in the rats.*

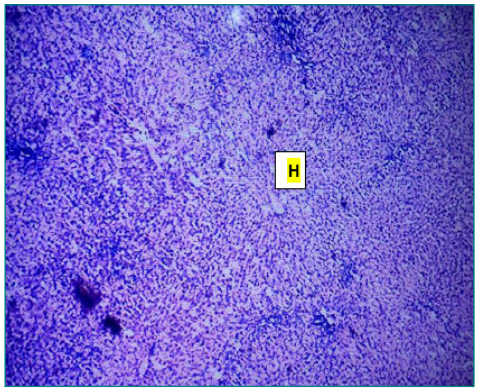

Plate 11. *Section of the liver of a cataractous animals treated with 250 mg/kg Aqueous extract, showing rays of hepatocytes (H); and no inflammatory infiltration of necrosis was seen.*

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 example, some alkaloids, e.g. papaverine, a vasodilator, used to treat spasms of the gastrointestinal tract, bile ducts and urethra[38, 39] and erectile dysfunction.[40] Another alkaloid morphine, is converted to codeine, which is used as a cough suppressant, analgesic, hypnotic and anti-diarrheal agent.[41] Saponins are reported to have a broad range of pharmacological properties, such as expectorant, anti-inflammatory, vaso-protective, hypocholestrolaemic, and hypoglycaemic, anti-parasitic, antioxidant, anticancer and anti-protozoal activities.[42]

Phenolic compounds exert a wide range of pharmacological activities, including antioxidant, anti-carcinogenic, anti-mutagenic, anti-inflammatory, vasodilatory effects and antithrombotic properties.[43] The pharmacological activities of flavonoids include antioxidant, anti-inflammatory, antimicrobial, antitumor, anti-ulcer and hepatoprotective activities.[42] Tannins have been reported to possess anti-cancer, anti-diarrheal, anti-asthmatic, cardioprotective, anti-diabetic, anti-cataractogenic, anti-inflammatory and hepatoprotective properties.42 Alsayed and El-Naga[44], 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 by antioxidants, oxidative stress results.[45, 46] Oxidative stress has been implicated in the pathogenesis of cataracts in lens epithelial cells as reported several studies.[48-50] Increases in tissue and blood levels of thiobarbituric acid-reactive substances (mainly malondialdehyde) are very reliable indicators of oxidative stress and lipid peroxidation.[47] 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.[51-55]

Serum level of hepatic enzymes and metabolites associated with the liver are usually measured as indices to evaluate liver damage. Previous researchers have reputed oxidative stress as one of the major causes of liver damage[56, 57]; and the liver being the major organ site for sodium selenite toxicity.[58] Standard biomarkers of drug-induced liver injury include alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin.[59] Elevations in serum enzyme levels are very reliable indices of liver toxicity whereas increases in both total and conjugated bilirubin levels are indicators of overall liver function. An elevation in transaminase levels in conjunction with a rise in bilirubin level to more than double its normal upper level is considered as an ominous marker for hepatotoxicity.[60]

In this study, the sodium selenite induced liver damage and the hepatotherapeutic effects of the extracts were also monitored. The extracts significantly ($p<0.05$) lowered the plasma AST and ALT activities, and albumin levels of the treated animals compared to the Test control and Normal control. However, they significantly ($p<0.05$) raised the plasma ALP activities and total protein levels of the treated animals, without any significant effect on the plasma GGT activities and total bilirubin levels. The reduction in plasma albumin may be due to the impact of sodium selenite administration which may have caused alteration of intracellular protein synthesis and metabolism.[58] Treatment with *Annona muricata* Linn (aqueous extract) restored the normal liver function. This hepatotherapeutic effect of the extract could be linked to the presence of flavonoids, tannins and alkaloids in them. Flavonoids and tannins have been reported to have hepato-protective activities.[42]

Evaluation of the hepatic tissues treated revealed mild inflammatory cells in groups treated with Methanol extract but showed normal hepatocytes in groups treated with aqueous extract. 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 hepatoprotective effects.

The anti-cataract and hepatoprotective activities of the extracts may be due to their contents of tannins, flavonoids and phenolics, all of which are known to have antioxidant activities.

The limitation of this study is that it is a double outcome study with direct involvement of the eye lens, which is sensitive to Sodium Selenite-induced oxidative stress damage. Because sodium selenite is not a commonly used hepatotoxic substance, the liver was not the main target of the oxidative stress.

5. Conclusion

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 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 liver function and had no deleterious effect on the liver'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 antihepatotoxic drugs.

Author Contributions

NUM.: Investigation, Resources, Supervision, Funding acquisition, NUM —review & editing. NUM.: Methodology, Data curation, Visualization, Formal analysis, Writing—origina, draft. NUM, CRM, MCO, NHN, DCO, WKE, NCK, PCU, UAL, ICA, OSU, AAE, EEM and IKU: Writing—review & editing, Sample collection NUM.: Sample collection. NUM.: Supervision. NUM: Resources. NUM.: Funding acquisition, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

Declared none

Conflicts of Interest

The authors declare no conflicts of interest regarding this manuscript.

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