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The Ameliorating Potential of *Annona muricata* on Sodium Fluoride-induced Toxicity on Liver and Kidney of Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BMOO and BAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BAA and VOE managed the analyses of the study. Author VOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Annona muricata* is a plant common to areas of South and North America as well as in West Africa, especially in Western Nigeria. It is one of the tropical plants that demonstrate antioxidant properties. The effect of combined oral administration of sodium fluoride (NaF) and fruit juice, ethanol stem bark and leaf extracts of *A. muricata* on biochemical parameters of mature male wistar rats was investigated.
Methods: Eighty-five (85) adult male wistar rats were divided into 17 groups of 5 rats each. Sodium fluoride (10 mg/kg) and fruit juice, ethanol stem bark and leaf extracts of *A. muricata* at five different doses of 500, 1000, 1500, 2000 and 2500 mg/kg body weight were administered to the rats for 6 weeks. Blood samples were taken after 6 weeks of administration of the extracts, through the ocular puncture and the sera were used for biochemical analyses; creatinine, urea, alanine

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aminotransferase (ALT), aspartate aminotransferase (AST) and thyroid function tests were carried out.

Results: Combined administration of NaF + stem bark and NaF + leaf extracts showed non-significant ($p>0.05$) decrease in the activities of ALT, AST and urea level, while NaF + fruit juice had varied effects on creatinine level and AST activity. Photomicrograph of the liver and the kidney sections of group administered with NaF only showed damaged hepatic tissue with focal aggregate of inflammatory cell, portal aggregate of inflammatory cells and severely damaged renal tissue with coagulative necrosis of the glomeruli that leads to closure of malpighian body, apoptosis of tubular cells, focal area of intrarenal haemorrhage and tubular necrosis. However, administration of (NaF + fruit juice), (NaF + stem bark extract) and (NaF + leaf extract) resulted in mild/moderate ameliorative and regeneration of damaged hepatic and renal tissues.

Conclusion: Concomitant treatment of NaF and fruit juice extract of *A. muricata* was found to have an ameliorative effect on the liver and kidney of sodium fluoride-induced toxicity.

Keywords: Annona muricata; sodium fluoride; photomicrograph; liver; kidney.

1. INTRODUCTION

Metal-ions play important roles in biochemical reactions. Some ions are involved in neurotransmission (Na^+ and K^+), messaging systems (calcium ions) while other ions often find themselves at the active sites of enzymes and other proteins (Cu, Zn, Fe, Mn, and Mg). Ca and Mg ions often help to give shape to macromolecules which is critical because the biochemical function is often exquisitely tied to the shape of molecules [1]. Fluoride (F^-), an anion, is also one of the ions important to human health. Fluoride is not a normal constituent of the mammalian bloodstream [2]. It has no nutritive value [3], or physiologic function but has been believed by some to be useful for teeth based on an initial correlation with natural calcium fluoride in drinking water [4]. It is essential for normal mineralization of bones and function of dental enamel when present in small quantity [5].

At the normal level of ingestion, almost all of the absorbed fluoride is excreted [6] but when it crosses the permissible limit [7], it becomes toxic and create metabolic disturbances in animals and human beings such as dental and skeletal fluorosis [8]. However, following prolonged excessive fluoride intake, fluoride levels in the plasma increases and consequently, the soft tissues are loaded. If the fluoride levels in the soft tissues increase beyond a particular limit, the physiological functioning of the affected organs are impaired [9]. Among the soft tissues, kidneys have the highest fluoride content on both excretion and retention [10]. It has also been shown that elevated concentrations of fluoride can occur in the kidney as it has a major route for removal of fluoride in the body [11,12]. Fluoride nephrotoxicity causes pathological changes in the glomeruli and in the proximal, distal, and collecting tubules of experimental animals [13]. It brought about degenerative and inflammatory damages to the kidney [14]. Thus the kidney is more prone to fluoride toxicity than other soft tissues [15]. The most commonly used medium for identifying fluoride exposure is urine [16,17]. Acute exposure to high doses of fluoride damages renal tissue and causes renal dysfunction.

Various changes also occur after chronic administration of fluoride in the blood, brain and liver of animals. These include abnormal behaviour patterns, altered neuronal and cerebrovascular integrity and metabolism lesions. Generation of free radicals, lipid peroxidation, and altered antioxidant defence systems are considered to play an important role in the toxic effects of fluoride [17,18]. Studies have demonstrated that elevated levels of serum hepatic enzymes have been found following fluoride intoxication [14]. Other histopathological findings include accumulation of amorphous and crystalline bodies in the hepatocytes around the hepatic vein [19]. Some of these harmful effects observed due to fluoride toxicity can be ameliorated by intake of bio-organic molecules present in some plants. Example of such plant is *Annona muricata.*

A. muricata has a long history of usage in herbal medicine in the tropical areas of South and North America as well as in West Africa, especially in Western Nigeria. Phytochemical screening of ethanol leaf extract of *A. muricata* showed the

presence of saponins, triterpenoids, flavonoids, tannins, alkaloids, and cardiac glycosides [20]. This plant contains chemical compounds which display antitumor, pesticidal, antiviral and antimicrobial effects, thus suggesting many potentially useful applications [21]. Considering that fluoride toxicity is a public health issue and that fluoride exposure has a definite effect on the liver and kidney, this study was planned to observe the phytochemical composition of *A. muricata* plant and the ameliorative potential of its stem bark, leaf extract and fruit juice on sodium fluoride-induced toxicity of the liver and kidney of mature male wistar rats.

2. MATERIALS AND METHODS

2.1 Reagents

Aluminium chloride (Qualikems), Ammonia (Qualikems), Ammonium hydroxide (Qualikems), Chloroform (Qualikems), Concentrated sulphuric acid (Qualikems), Dragendorff's reagent, Ethanol (Qualikems), Fehling's solution-I (Loba Chemie India), Fehling's solution-II (Loba Chemie India), Ferric chloride (Kermel), Iodine (Kermel), Lead subacetate (Kermel), Mayer's reagent, Saturation of picric acid (Qualikems), Wagner's reagent, α-naphthol (Qualikems). All other reagents used were of analytical grades.

2.2 Animals

Eighty-five (85) adult male wistar rats (150-250 g) were obtained from the animal house of the College of Medicine, University of Nigeria, Enugu Campus. The animals were housed in steel cages within the Laboratory Animals Facility of Brain-Phosphorylationship Scientific Solution Services, Enugu State, and were given standard feed and clean drinking water *ad libitum*. They were allowed to acclimatize for a period of four weeks before use. All animal experiments were in compliance with the National Institute of Health Guide for care and use of laboratory animals.

2.3 Collection and Extraction of Plant Materials and Fruit Juice

Fresh stem bark, leaves and fruits of *A. muricata* were collected from Abua community in Rivers State, Nigeria in March 2017. The plant materials were identified and authenticated by Mr. Alfred Ozioko of International Centre for Ethnomedicines and Drug Development Nsukka, Nigeria and deposited in herbarium with Voucher Number: Intercedd/16091. The stem bark and the leaves were cut to pieces, dried under room temperature, ground and pulverized to a coarse powder using a Hammer mill (Gallenkamp, U.S.A.). Known quantities (1.851 kg) of the dried stem bark powder and 1.016 kg of the dried leaf powder were extracted with analytical grade ethanol using maceration method for 48 hours. The mixture was vacuum-filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan) to afford 97.352 g (5.257% w/w) for stem bark extract and 126.312 g (12.432% w/w) for leaf extract. The extractive yield was calculated using the relation: Yield (%) = [Weight of extract (g)/Weight of plant material (g)]*100. The fruit juice was used raw without concentrating it. The epicarp and the seeds of the ripen fruits were removed with hand and their mesocarps were sliced with a knife into small sizes and ground with and an electric grinder into paste form. This was further sieved with a muslin cloth to remove the fibres. The filtrate was transferred into a clean glass container, sealed and preserved in a refrigerator at -10°C until use.

2.4 Qualitative Phytochemical Screening

The extract, fractions and pure sample were subjected to both quantitative and qualitative phytochemical screening using standard phytochemical methods as outlined by [22]. Samples were tested for the presence of the following compounds: Alkaloids, Saponins, Terpenoids, Anthraquinones, Coumarins, Flavonoids, Tannins, Carbohydrate, Resin, Reducing Sugars, Starch, Protein and Amino acids.

2.4.1 Test for glycosides

To the solution of the extract in glacial acetic acid, three drops of ferric chloride and concentrated sulphuric acid were added and observed for a reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [23].

2.4.2 Test for oil

About 0.1 g of the extract was pressed between filter paper and filter paper was observed. The translucency of the filter paper indicates the presence of oils.

2.5 *In-vitro* Anti-oxidant Activity Assays

2.5.1 DPPH scavenging assay

The free radical scavenging activity of the fruit juice, stem bark and leaf extracts of *A. muricata* were analysed by the DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay according to the method of [24].

2.5.2 Reducing power

Iron (Fe^{3+}) reducing the power of fruit juice, stem bark and leaf extracts of *A. muricata* were determined according to the method of [25]. The extract, fractions, pure fraction and standard (1ml) of various concentrations (1000, 500, 250, 125, and 62.5 µg mL^{-1}) were mixed with phosphate buffer (pH 6.6, 0.2M, 2.5ml) and potassium ferricyanide (1%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm in a spectrophotometer. The higher absorbance of the reaction mixture indicated the reductive potential of the extract.

2.5.3 Experimental design

Eighty-five (85) sexually matured adult male albino rats were divided into 17 groups of 5 rats each, according to their average weight, and received daily oral dose of the treatments as follows:

Group I: Normal feed and water (Control)
Group II: NaF (10 mg/kg) (Positive control)
Group III: NaF (10 mg/kg) + Fruit Juice (500 mg/kg)
Group IV: NaF (10 mg/kg) + Fruit Juice (1000 mg/kg)
Group V: NaF (10 mg/kg) + Fruit Juice (1500 mg/kg)
Group VI: NaF (10mg/kg) + Fruit Juice (2000 mg/kg)
Group VII: NaF (10 mg/kg) + Fruit Juice (2500 mg/kg)
Group VIII: NaF (10 mg/kg) + Leaf Extract (500 mg/kg)
Group IX: NaF (10 mg/kg) + Leaf Extract (1000 mg/kg)
Group X: NaF (10 mg/kg) + Leaf Extract (1500 mg/kg)
Group XI: NaF (10 mg/kg) + Leaf Extract (2000 mg/kg)
Group XII: NaF (10 mg/kg) + Leaf Extract (2500 mg/kg)
Group XIII: NaF (10 mg/kg) + Stem Bark Extract (500 mg/kg)
Group XIV: NaF (10 mg/kg) + Stem Bark Extract (1000 mg/kg)
Group XV: NaF (10 mg/kg) + Stem Bark Extract (1500 mg/kg)
Group XVI: NaF (10 mg/kg) + Stem Bark Extract (2000 mg/kg)
Group XVII: NaF (10 mg/kg) + Stem Bark Extract (2500 mg/kg)

Blood was taken after the 6th week of administration through ocular puncture. Two ml of the blood samples from each group (n=4)were collected in test tubes and put into centrifuge tubes, spun at 3000 rpm for 10 min and the serum collected for biochemical analyses: creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (ALT) and thyroid tests. The rats were sacrificed under chloroform anaesthesia after collection of blood samples. The liver and kidney were dissected out for histological studies.

2.5.4 Biochemical tests

Determination of Biochemical indices Total bilirubin concentration was determined using the methods of [26], urea and creatinine by the method of [27], the activity of AST and ALT was assayed by the methods of [28] as outlined in Randox kits, UK.

2.6 Statistical Analysis

The data were analysed by (SPSS version 17.5, SPSS Inc.). Significant differences between means were determined by One-way ANOVA and regarded significant at $p < 0.05$. Results were presented as Mean ± Standard Deviation.

3. RESULTS

3.1 Percentage Extractive Yield of the Stem Bark Powder and Fractions

The extraction process yielded 97.35 g (5.26% w/w) for stem bark extract and 126.31 g (12.43% w/w) for leaf extract of ethanol (Table 1).

3.2 Qualitative Phytochemical Screening of Dried Stem Bark, Dried Leaf and Fresh Fruit Juice Extracts

Qualitative phytochemical screening on the dried stem bark, dried leaf and fresh fruit juice extracts of *Annona muricata* is presented in Table 2. The result showed that terpenoid, glycosides, saponins, alkaloids, reducing sugars,

carbohydrates and polysaccharides were present in all, while, phenols, flavonoids and flavones were moderately present in stem bark and leaf extracts only. Relative trace presences of oil were exclusively detected in fruit juice and leaf extract. Steroids, resins and amino acids were not visibly detected. High concentrations of tannins were absent in all except in stem bark extract.

3.3 Percentage DPPH Scavenging Assay

Table 3 shows the results of the evaluation of percentage DPPH scavenging capacity of fruit juice, stem bark and leaf extracts of *A. muricata*. Ascorbic acid exhibited the highest percentage DPPH scavenging activity followed by stem bark extract, leaf extract and fruit juice. At the low concentrations of 1000 and 2500 µg/ml, both extracts and fruit juice exhibited a significant reduction in % DPPH scavenging activity when compared with ascorbic acid. The result further depicts that fruit juice, stem bark and leaf extracts at a highest concentration of 5000 µg/ml exhibited a non-significant difference in comparison with ascorbic acid.

Table 1. The extractive yield of dried stem bark and dried leaf extracts of *A. muricata*

Sample	Weight of plant material (kg)	Weight of extract (g)	Percentage yield (%)
Dried stem bark	1.85	97.35	5.26
Dried leaf	1.02	126.31	12.43

Table 2. Qualitative phytochemical constituents of dried stem bark, dried leaf extracts and fresh fruit juice

Phytochemical constituents	Stem bark extract	Leaf extract	Fresh fruit juice
Alkaloids	++	+	+
Amino acids	ND	ND	ND
Aromatic amino acids	ND	ND	ND
Carbohydrates	+++	+++	+++
Flavones	+	++	ND
Flavonoids	++	+	ND
Glycosides	++	++	+
Phenols	++	+	ND
Reducing Sugars	++	+	+++
Resins	ND	ND	ND
Saponins	+++	++	+
Steroids	ND	ND	ND
Tannins	+++	++	ND
Terpenoids	+++	+	++
Oil	ND	+	++

Key: + = Present in low quantity; ++ = Present in moderate quantity; +++ = Present in large quantity; ND = Not Detected

Table 3. Percentage inhibition of standard, fruit juice, stem bark and leaf extracts of *A. muricata* at various concentrations (µg/ml) in DPPH scavenging model

Concentration (µg/ml)	DPPH radical inhibition (%)			
	Ascorbic acid	Stem bark extract	Leaf extract	Fruit juice
1000	92.62±1.26	64.75±4.94 $^{\alpha}$	55.78±3.61$^{\alpha}$	63.14±14.25 $^{\alpha}$
2500	93.08±3.25	86.08±4.07 $^{\alpha}$	81.07±3.12 $^{\alpha}$	75.27±7.12 $^{\alpha}$
5000	91.44±1.32	98.15±1.34	97.60±1.29	95.46±2.68

Results are expressed as Mean±SD; n=4; The mean values with α as superscript across the row compared with ascorbic acid are considered significant (p<0.05)

3.4 Reducing Power Assay

Fruit juice, stem bark and leaf extracts of *A. muricata* exhibited a varied reduction of potassium ferricyanide to potassium ferrocyanide (Table 4). The formation of ferric ferrous complex by fruit juice, stem bark and leaf extract and ascorbic acid increased steadily with increasing concentration in the following order: Ascorbic acid > Fruit juice > Stem bark extract > Leaf extract.

3.5 Serum ALT and AST Activities

Results obtained for ALT and AST activities (Table 5) showed that sodium fluoride at a dose of 10 mg/kg caused a non-significant ($p>0.05$) increase in ALT activity when compared with normal control group fed with water and feed only. The result further revealed a concentration-dependent reduction that exhibited significance ($p<0.05$) only at 2500 mg/kg in the group treated concomitantly with NaF and *A. muricata* fruit juice, when compared with the group treated with

Table 4. Absorbance of standard, fruit juice, stem bark and leaf extracts of *A. muricata* at various concentrations (µg/ml) in ferric reducing power determination model

Concentration (µg/ml)	Absorbance			
	Ascorbic acid	Stem bark extract	Leaf extract	Fruit juice
400	0.3472±0.330	0.1095±0.007 $^{\alpha}$	0.0575±0.036 $^{\alpha}$	0.1610±0.021 $^{\alpha}$
1000	0.6244±0.139	0.2010±0.035 $^{\alpha}$	0.0788±0.022 $^{\alpha}$	0.1740±0.045 $^{\alpha}$
2500	1.0216±0.175	0.2918±0.082 $^{\alpha}$	0.1847±0.021 $^{\alpha}$	0.3319±0.059 $^{\alpha}$
5000	1.1680±0.171	0.5057±0.040 $^{\alpha}$	0.5461±0.055 $^{\alpha}$	0.5769±0.111 $^{\alpha}$

Results are expressed as Mean±SD; n=4

The mean values with α as superscript across the row compared with ascorbic acid are considered significant ($p<0.05$)

Table 5. Serum alanine aminotransferase and aspartate aminotransferase level of NaF induced rats after 6 weeks of treatment

ALT (IU)		AST (IU)
Group	**6 weeks**	
Control	49.668±30.73	183.91±13.02$^{\beta}$
NaF	75.398±32.06	463.52±324.51 $^{\alpha}$
10 mg/kg + mg/kg Stem bark extract		
NaF + 500	44.554±0.03	232.99±147.97
NaF + 1000	36.745±11.10	182.40±0.39 $^{\beta}$
NaF + 1500	60.874±15.82	434.36±233.29 $^{\alpha}$
NaF + 2000	61.253±11.88	267.99±59.29
NaF + 2500	71.258±36.38	394.65±116.17
10 mg/kg + mg/kg Leaf extract		
NaF + 500	60.680±17.48	244.34±56.09
NaF + 1000	69.570±60.54	287.89±132.41
NaF + 1500	61.533±8.48	346.65±155.47
NaF + 2000	55.343±17.18	362.97±186.27
NaF + 2500	39.990±0.58	493.96±110.43 $^{\alpha}$
10 mg/kg + mg/kg Fruit Juice		
NaF + 500	39.117±13.31	403.74±75.82
NaF + 1000	37.995±0.27	205.77±6.93$^{\beta}$
NaF + 1500	34.848±10.30	430.78±126.84$^{\alpha}$
NaF + 2000	33.980±4.73	556.06±275.49$^{\alpha}$
NaF + 2500	25.320±0.31$^{\beta}$	358.05±124.35

Results are expressed as Mean±SD; n=4

The mean values with β as superscript across the column compared with group treated with NGF alone are considered significant ($p<0.05$). The mean values with α as superscript across the column compared with control group fed with water and feed only are considered significant ($p<0.05$)

sodium fluoride alone. However, ALT activity of groups treated with NaF + Stem bark extract exhibited a non-significant ($p>0.05$) decrease in an inverse concentration dependent manner when compared with the group treated with sodium fluoride alone. On the other hand, AST activity in group given only NaF showed statistically significant ($p<0.05$) increase in comparison with a control group fed water and feed only. Also, groups treated with NaF + 1500 mg/kg stem bark extract, NaF + 2500 mg/kg leaf extract and NaF + 1500 and 2000 mg/kg fruit juice showed a significant ($p<0.05$) increase in AST activity when compared with the normal control group, while group treated with NaF +1000 mg/kg fruit juice and NaF + 1000 /kg stem bark extract exhibited significant ($p<0.05$) decrease in serum AST activity when compared with group treated with NaF alone. The results of our present study showed that the tested extracts and fruit juice when given in combination with NaF did not significantly reduce the hepatic cytotoxic effect induced by fluoride.

3.6 Serum Urea and Creatinine Concentration

From Table 6, it was observed that Sodium fluoride at a dose of 10 mg/kg caused a significant increase ($p<0.05$) in the serum urea level when compared with a normal control group fed with water and feed only. All groups treated with stem bark extract and NaF + 1500, 2000 and 2500 mg/kg fruit juice showed a significant decrease in urea concentrations when compared with group treated with NaF alone. However, in comparison with the control group fed with water and feed only, all the groups treated with leaf extracts and NaF + 500 and 1000 mg/kg fruit juice exhibited significant increase ($p<0.05$) in serum urea concentration. NaF at a concentration of 10 mg/kg caused a non-significant increase in creatinine concentration in group treated with NGF alone when compared with the control. Similarly, the groups treated with NaF and fruit juice concomitantly exhibited non-significant increase at high doses of (1500, 2000 and 2500 mg/kg) but at lower doses of 500 and 1000 mg/kg showed a non-significant decrease in creatinine concentration when compared with group treated with NaF alone. The results of this present study showed that the tested extracts and fruit juice when given in combination with NaF did not significantly reduce the cytotoxic effect induced by fluoride on the kidney.

Table 6. Urea and Creatinine concentration of control and test groups of NaF induced rats after 6 weeks of treatment

Urea (mg/dl)		Creatinine (mg/dl)
Group	**6 weeks**	
Control	$4.05\pm2.70^{\beta}$	0.45±0.15
NaF	$12.00\pm1.31^{\alpha}$	0.59±0.37
10mg/kg + mg/kg Stem bark extract		
NaF + 500	$6.20\pm1.60^{\beta}$	0.52±0.10
NaF + 1000	$6.11\pm2.28^{\beta}$	0.64±0.03
NaF + 1500	$5.84\pm1.60^{\beta}$	0.56±0.34
NaF + 2000	$5.61\pm3.72^{\beta}$	0.54±0.31
NaF + 2500	$4.40\pm2.97^{\beta}$	0.46±0.12
10mg/kg + mg/kg Leaf extract		
NaF + 500	$10.91\pm4.01^{\alpha}$	0.38±0.16
NaF + 1000	$11.19\pm4.56^{\alpha}$	0.41±0.14
NaF + 1500	$10.94\pm1.76^{\alpha}$	0.36±0.17
NaF + 2000	$10.00\pm2.07^{\alpha}$	0.47±0.14
NaF + 2500	$10.48\pm5.70^{\alpha}$	0.47±0.20
10mg/kg + mg/kg Fruit Juice		
NaF + 500	$10.13\pm3.88^{\alpha}$	0.52±0.31
NaF + 1000	$10.06\pm1.69^{\alpha}$	0.44±0.10
NaF + 1500	$7.88\pm1.61^{\beta}$	0.88±0.37
NaF + 2000	$7.11\pm7.77^{\beta}$	0.68±0.23
NaF + 2500	$6.01\pm2.43^{\beta}$	0.85±0.03

Results are expressed as Mean±SD; n=4; The mean values with β as superscript across the column compared with group treated with NGF alone are considered significant ($p<0.05$). The mean values with α as superscript across the column compared with a control group fed with water and feed only are considered significant ($p<0.05$)

Photomicrographs of thin sections (5 µm) of the Liver of experimental rats harvested at the end of 6 Weeks of treatment with fruit extract of *A. muricata* (Plate 1) and stained with H&E (400x).

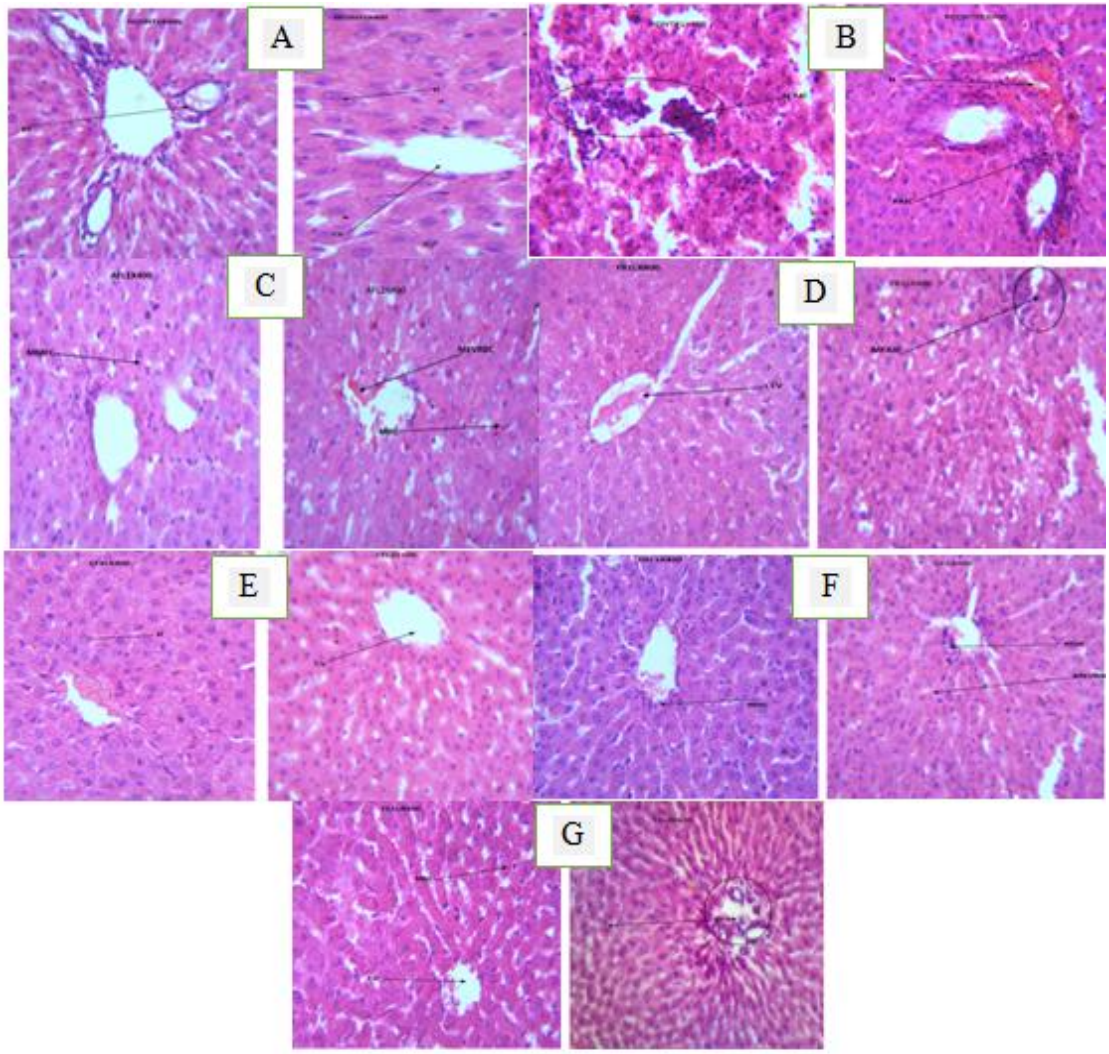

Plate 1. Photomicrographs of a section of the liver of rats treated with fruit extract of *A. muricata*

Plate 1 shows the photomicrographs of a section of the liver of rats in the normal and experimental groups treated with different concentrations of fruit extract of A. muricata. A - Group 1 rats that received feed and water only showed normal hepatic architecture. B - Group 2 rats treated with 10 mg/kg of NaF showed severely damaged hepatic tissue with severe focal aggregate of inflammatory cell, haemorrhage within the portal area and portal aggregate of inflammatory cell. C – group 3 rats treated with 10 mg/kg sodium fluoride + 500 mg/kg of Fruit juice showed moderate healing on the hepatic tissue with mild infiltration of inflammatory cell, mild extravasation of red blood cell and mild fatty changes. D - Group 4 rats treated with 10 mg/kg sodium fluoride + 1000 mg/kg of Fruit juice showed moderate healing on the hepatic tissue with mild focal area inflammatory exudate cell and congestion central vein. E – Group 5 rats treated with 10 mg/kg of sodium fluoride + 1500 mg/kg of Fruit juice showed well regenerated hepatic tissue with hepatocyte and central vein that appears normal. F – Group 6 rats treated with 10 mg/kg sodium fluoride + 2000 mg/kg of Fruit juice showed well regenerated hepatic tissue with mild infiltration of the inflammatory cell around the central vein and mild extravasation red blood otherwise normal. G – Group 7 rats treated with 10 mg/kg sodium fluoride + 2500 mg/kg of Fruit juice showed well regenerated hepatic tissue with mild infiltration of inflammatory cell otherwise normal with portal triad and central vein well represented.

Photomicrographs of thin sections (5 µm) of the Liver of experimental rats harvested at the end of 6 Weeks of treatment with different concentrations of leaf extract of A. muricata (Plate 2) and stained with H&E (400x).

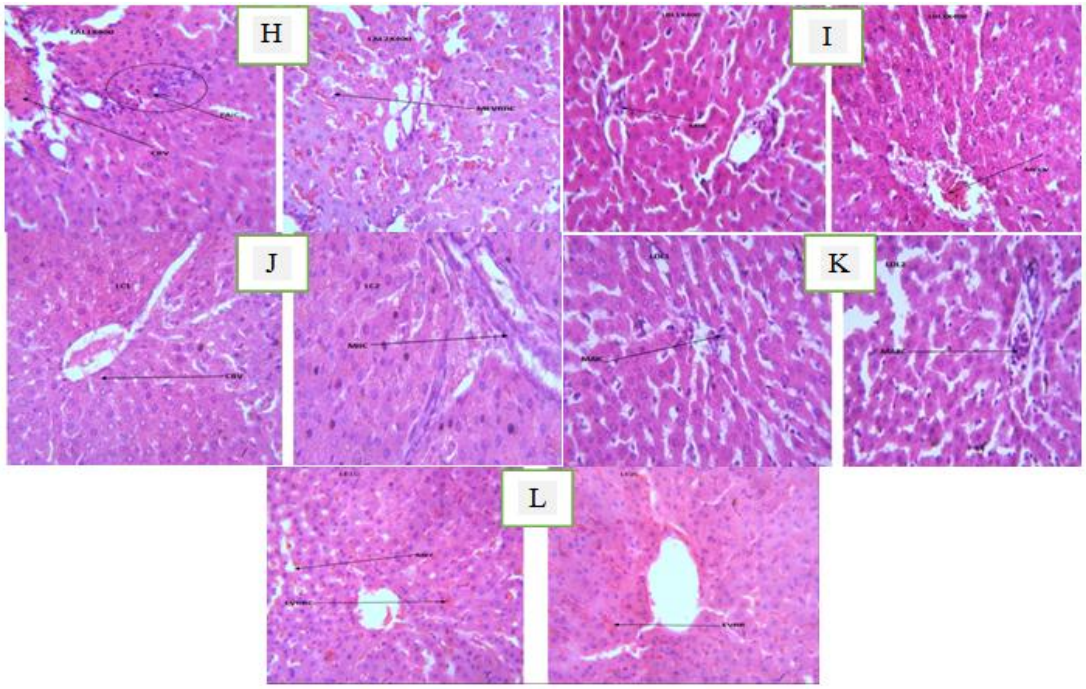

Plate 2. Photomicrographs of a section of the liver of rats treated with leaf extract of *A. muricata*

Plate 2 shows the photomicrographs of a section of the liver of rats in the experimental groups treated with different concentrations of leaf extract of A. muricata. H – Group 8 rats treated concomitantly with 10 mg/kg sodium fluoride and 500 mg/kg of leaf extract showed mild regenerated hepatic tissue with moderate focal aggregate of inflammatory cell, moderate extravasation of red blood cell cytoplasmic ground glass appearance and congestion blood vessel. I – Group 9 rats treated concomitantly with 10 mg/kg sodium fluoride and 1000 mg/kg of leaf extract showed moderate regenerated hepatic tissue with mild infiltration of inflammatory cell and congestion of the central vein. J – Group 10 rats treated concomitantly with 10 mg/kg sodium fluoride and 1500 mg/kg of leaf extract showed moderate regenerated hepatic tissue with mild infiltration of inflammatory cell and congestion blood central vein. K – Group 11 rats treated concomitantly with 10 mg/kg sodium fluoride and 2000 mg/kg of leaf extract showed moderate regenerated hepatic tissue with a moderate focal aggregate of inflammatory cell and distortion of the hepatocyte architecture. L – Group 12 rats treated concomitantly with 10 mg/kg of sodium fluoride and 2500 mg/kg of Leaf extract showed moderate regeneration with moderate extravasation of red blood cell and mild fatty change.

4. DISCUSSION

Studies on phytochemicals are receiving increased attention because of interesting findings regarding their biological activities [29]. This present study of phytochemical screening of *A. muricata* stem bark and leaf extracts and fruit juice confirms the presence of saponins, terpenoids, flavonoids, steroid, and cardiac glycosides in its ethanolic extracts. These phytochemicals were widely reported as scavengers of free radicals in the biological system and amelioration of various diseases associated with free radicals. Flavonoids exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic and antioxidant properties [30], while phenolic compounds are the group of compounds that acts as primary antioxidants because they can react with oxygen free radicals and lipid peroxyl radicals. Odabasoglu et al. [31], also reported a high correlation between antioxidant activity and phenolic compounds.

The extracts of *A. muricata* commonly used traditionally to treat many diseases whose pathogeneses are among other factors are linked

to oxidative stress. However, information on the antioxidant potentials of this plant that could have made it possible for their use in the treatment of such diseases has not been investigated. In this study, we report the antioxidant potentials of ethanolic extracts of both leaf and stem bark and the fruit juice of *A. muricata.* The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. The use of DPPH, a relatively stable radical, is based on the measurement of the scavenging activity of antioxidants towards the stable radical. In this study, DPPH radical scavenging and total reducing power methods were respectively used to determine the radical scavenging and electron donating abilities of all the ethanolic extracts and the fruit juice. It is evident from our results that stem bark, leaf extracts and fruit juice contain promising radical scavenging and reducing power agents which may implicate the therapeutic actions of this plant.

The flavonoids, phenols, and tannins were detected in moderate quantity in the stem bark and in low quantity in leaf extract, but beyond the detectable limit in fruit juice. However, terpenoids were detected in large quantity in stem bark, but in low quantity in both leaf extract and fruit juice. From the result of the phytochemical screening, the stem bark was found to possess higher phenolics content than leaf extract. This data suggests the correlation between total phenol content and DPPH radical scavenging power considering the high DPPH scavenging activity observed with stem bark extract and leaf extract than in the fruit juice.

Photomicrographs of thin sections (5 µm) of the Liver of experimental rats harvested at the end of 6 Weeks of treatment with different concentrations of stem bark extract of A. muricata (Plate 3) and stained with H&E (400x).

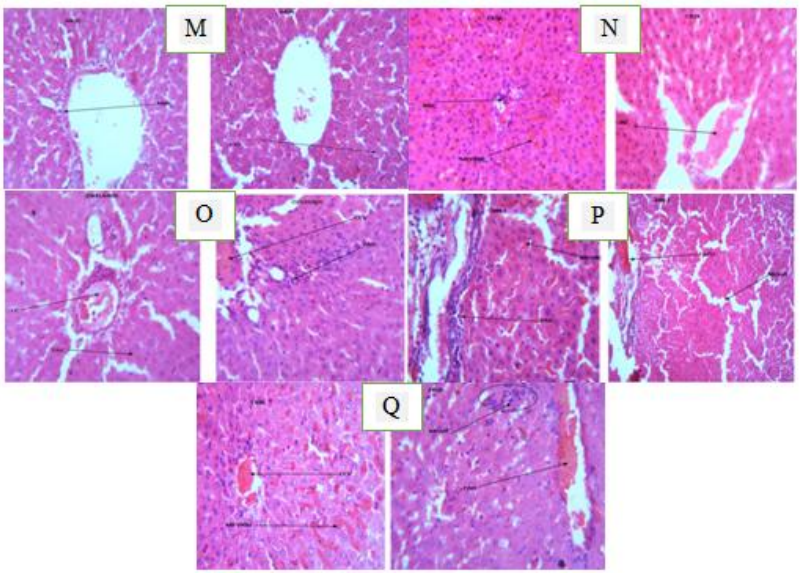

Plate 3. Photomicrographs of a section of the liver of rats treated with stem bark extract of *A. muricata*

Plate 3 shows the photomicrographs of a section of the liver of rats in the experimental groups treated with different concentrations of Stem bark extract of A. muricata. M – Group 13 rats treated with 10 mg/kg sodium fluoride + 500 mg/kg of Stem bark extract showed moderate healing on the hepatic tissue with mild infiltration of inflammatory cell and clumping of the hepatic tissue. N – Group 14 rats treated with 10 mg/kg sodium fluoride + 1000 mg/kg of Stem bark extract showed moderate healing of the hepatic tissue with mild infiltration of inflammatory cell, moderate extravasation of red blood cell and congestion blood vessel. O – Group15 rats treated with 10 mg/kg sodium fluoride + 1500 mg/kg of Stem bark extract showed moderate healing of the hepatic tissue with a focal aggregate of inflammatory cell and congestion of the central vein. P – Group 16 rats treated with 10 mg/kg sodium fluoride + 2000 mg/kg of Stem bark extract showed mild healing of the hepatic tissue with a moderate portal aggregate of inflammatory cell, moderate extravasation of red blood cell, focal area of portal haemorrhage. Q – Group 17 rats treated with 10 mg/kg sodium fluoride + 2500 mg/kg of Stem bark extract showed mild healing of the hepatic tissue with a moderate focal aggregate of inflammatory cell, moderate extravasation of red blood cell, focal area of haemorrhage and congestion of central vein.

Photomicrographs of thin sections (5 µm) of the Kidney of experimental rats harvested at the end of 6 Weeks of treatment with fruit juice of A. muricata (Plate 4) and stained with H&E (400x).

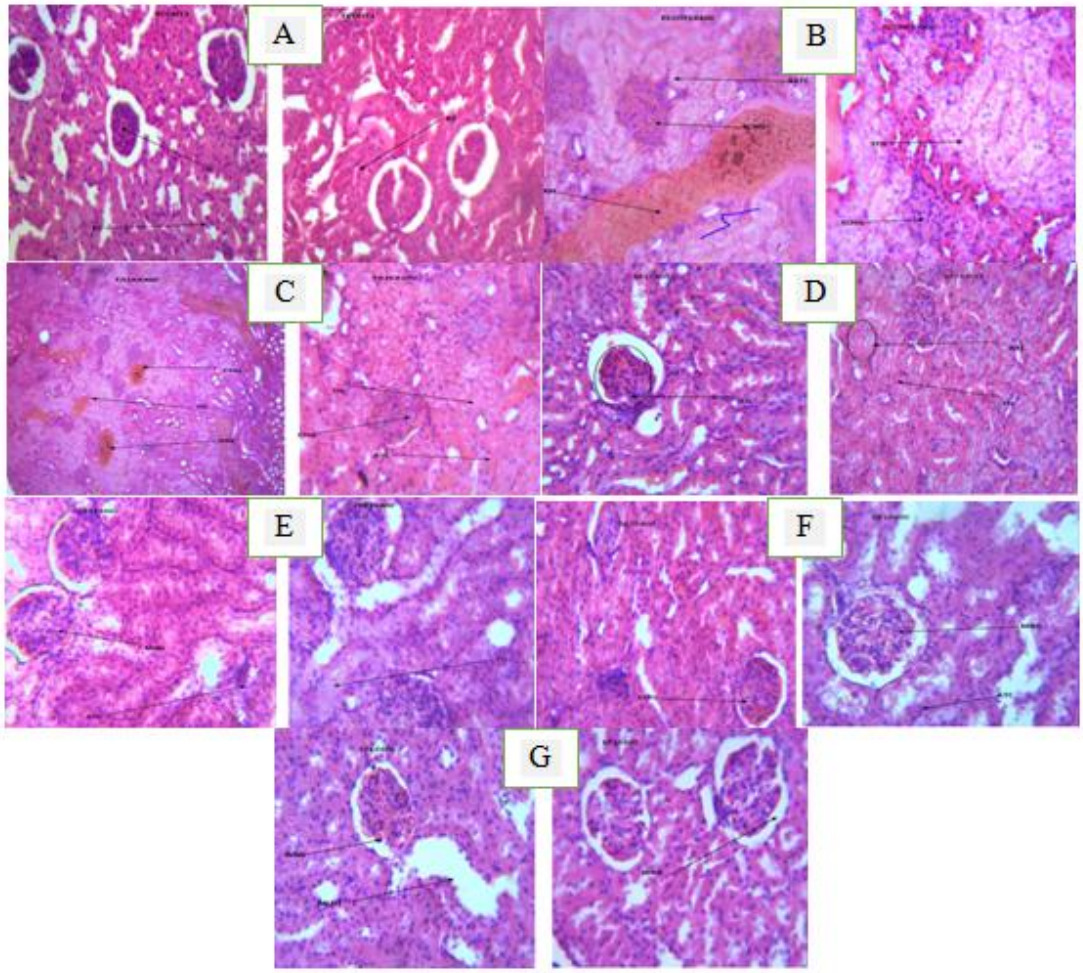

Plate 4. Photomicrographs of a section of the kidney of rats treated with fruit extract of *A. muricata*

Plate 4 shows the photomicrographs of a section of the kidney of rats in the normal and experimental groups treated with different concentrations of fruit extract of A. muricata. A - Group 1 rats that received feed and water only showed normal renal architecture with glomeruli, renal tubules and tubular cell appearing normal. B– Group2 rats induced with 10 mg/kg sodium fluoride showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body and severe apoptosis of tubular cells, focal area of intrarenal haemorrhage and severe tubular necrosis. C - Group 3 rats treated with 10 mg/kg sodium fluoride + 500 mg/kg of Fruit juice showed renal tissue with coagulative necrosis of the glomeruli, tubular necrosis, intra hepatic haemorrhage, apoptosis of tubular cell and mild fatty change. D - Group 4 rats treated with 10 mg/kg sodium fluoride + 1000 mg/kg of Fruit juice showed mild regenerated renal tissue with moderate tubular necrosis, apoptosis of tubular cell and clumping of the glomeruli. E – Group 5 rats treated with 10 mg/kg sodium fluoride + 1500 mg/kg of Fruit juice showed mild regenerated renal tissue with moderate tubular necrosis, apoptosis of the tubular cell. However, there are moderate regenerated glomeruli in some. F – Group 6 rats treated with 10 mg/kg of sodium fluoride + 2000 mg/kg of Fruit juice showed moderate regenerated renal tissue with mild apoptosis of the tubular cell. However, there are moderate regenerated glomeruli in some. G – Group 7 rats treated with 10 mg/kg sodium fluoride + 2500 mg/kg of Fruit juice showed well regenerated renal tissue with mild loss of renal tissue otherwise normal with well-regenerated glomeruli and renal tubules.

Photomicrographs of thin sections (5 µm) of the kidney of experimental rats harvested at the end of 6 Weeks of treatment with different concentrations of leaf extract of A. muricata (Plate 5) and stained with H&E (400x).

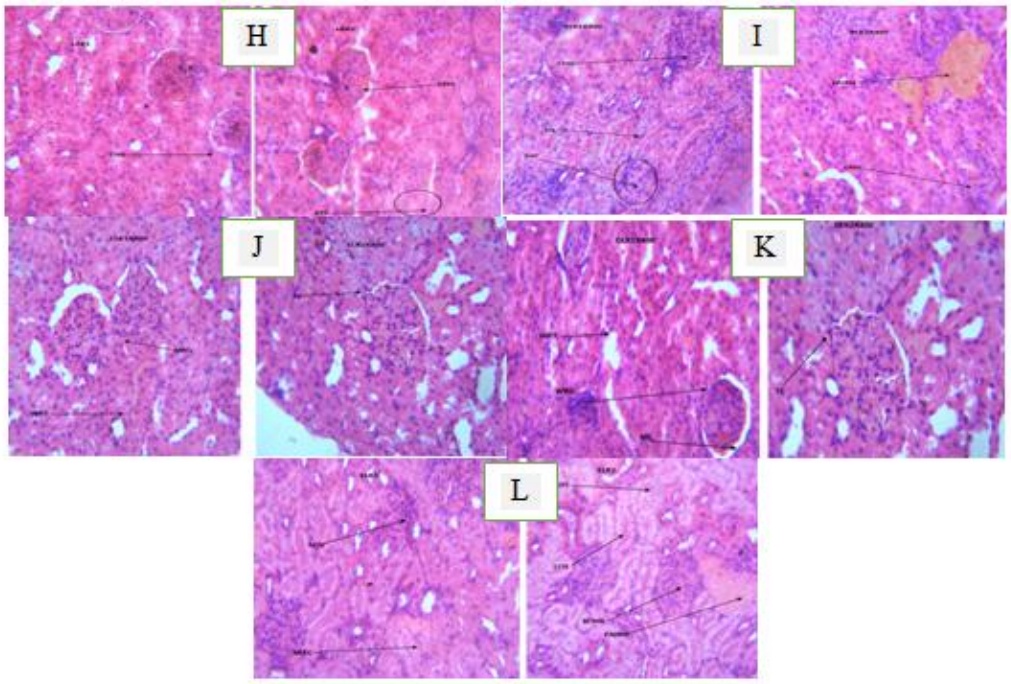

Plate 5. Photomicrographs of a section of the kidney of rats treated with leaf extract of *A. muricata*

Plate 5 shows the photomicrographs of the kidney of rats in the experimental groups treated with different concentrations of leaf extract of A. muricata. H – Group 8 rats treated with 10 mg/kg sodium fluoride + 500 mg/kg of leaf extract showed mild regenerated renal tissue with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body and moderate apoptosis of tubular cells. I – Group 9 rats treated with 10 mg/kg sodium fluoride + 1000 mg/kg of leaf extract showed mild regenerated renal tissue with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body, severe infiltration of inflammatory cell, tubular necrosis and focal area of intrarenal haemorrhage. J – Group 10 rats treated with 10 mg/kg sodium fluoride and 1500 mg/kg of leaf extract showed moderate regeneration with well regenerated renal tubules + tubular cell and moderate regenerated glomeruli. K - Group 11 rats treated with 10 mg/kg sodium fluoride + 2000 mg/kg of leaf extract showed moderate regeneration with well regenerated renal tubules that are lined by tubular cell and well regenerated glomeruli with malpighian body seen in the kidney section. L – Group 12 rats treated with 10 mg/kg sodium fluoride + 2500 mg/kg of leaf extract showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body and severe apoptosis of tubular cells, focal area of intrarenal haemorrhage and severe tubular necrosis.

The liver is a major site for potential fluoride toxicity and detoxification since it is predisposed to the relatively high concentration of exogenous toxic substances (e.g sodium fluoride) ingested through the gut. Ingested fluoride in the liver is associated with both pathomorphological and biochemical changes. Transitory nature of pathomorphological changes in hepatocytes indicates adaptive potentials or defence mechanisms against orally administered sodium fluoride [32]. Biochemical changes may result in alterations in the synthetic, metabolic, detoxification and excretion, homoeostasis and storage functions of the liver, on whose efficiency our health and energy largely depend on.

This present research work on markers of hepatocellular injury showed that fluoride ions altered the concentration of alanine aminotransferase in the serum. Apparently, fluoride ion may have enhanced the permeability of hepatocyte membranes (or the membrane of the extra-hepatic cells) and the escape of alanine aminotransferase to the circulatory system. According to the literature, fluoride is well known for its hepatotoxicity effect on markers of

Photomicrographs of thin sections (5 µm) of the kidney of experimental rats harvested at the end of 6 Weeks of treatment with different concentrations of stem bark extract of A. muricata (Plate 6) and stained with H&E (400x).

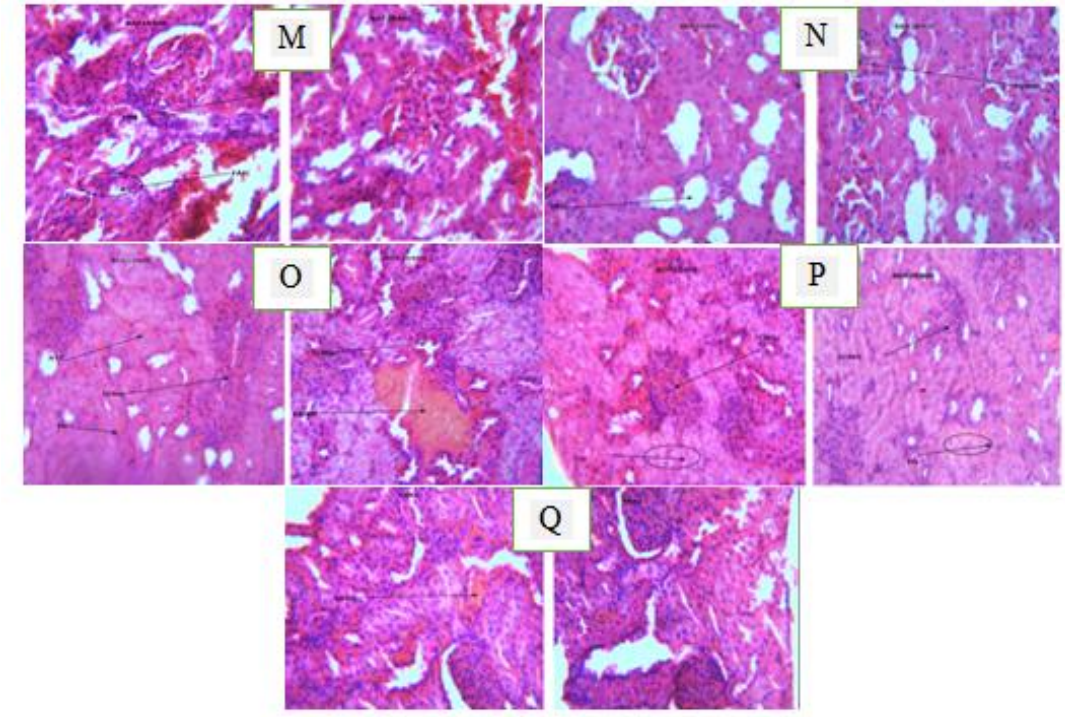

Plate 6: Photomicrographs of a section of the kidney of rats treated with stem bark extract of *A. muricata*

Plate 6 is the photomicrographs of a section of the kidney of rats in the experimental groups treated with different concentrations of Stem bark extract of A. muricata. M – Group 13 rats treated with 10 mg/kg sodium fluoride + 500 mg/kg of Stem bark extract showed mild regenerated renal tissue with moderate focal aggregate of inflammatory cell, glomeruli atrophy and intra hepatic haemorrhage. N – Group 13 rats treated with 10 mg/kg sodium fluoride and 1000 mg/kg of Stem bark extract showed moderate regenerated renal tissue with moderate fatty changes and extravasation of red blood cell within the glomeruli. O – Group15 rats treated with 10 mg/kg sodium fluoride + 1500 mg/kg of Stem bark extract showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body, tubular necrosis, focal area of intrarenal haemorrhage and apoptosis of tubular cell. P –Group 16 rats treated with 10 mg/kg sodium fluoride + 2000 mg/kg of Stem bark extract showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body, tubular necrosis, focal area of intrarenal haemorrhage and apoptosis of the tubular cell. Q – Group 17 rats treated with 10 mg/kg of sodium fluoride + 2500 mg/kg of Stem bark extract showed moderate regeneration with mild intra renal haemorrhage and mild loss.

hepatocellular injury/damage [17,33,34]. Free radicals and lipid peroxidation are known to play an important role in fluorosis [35].

Fluoride induces hepatotoxicity through the formation of free radicals and decreases the activity of the antioxidant systems in hepatocytes of mammals [33,36]. Fluoride exposure also induces histopathological changes in liver involving focal necrosis, infiltration of leucocytes, swelling of kupffer cells, extensive vacuolization, ultrastructural alterations in hepatocytes and increased apotosis [19,37,38,39]. Fluoride treatment caused free radical toxicity by a decrease in the activities of free radical scavenging enzymes, SOD, glutathione peroxidase, catalase [34] by chelating the metal ions at the active sites of these enzymes.

The observed non-significant increase in ALT for NaF treated group in this research work suggested that elevation of ALT is more pronounced in cases of hepatic disease than in hepatic toxicity, since serum ALT poorly

correlates with the degree of liver cell damage. This finding is in total agreement with previous research work by [17], who reported a non-significant increase in ALT for NaF treated group.

However, concomitant administration of NaF and fruit juice exhibited a non-significant decrease in ALT activity at lower doses but a significant decrease at the highest dose. These mitigating effects of fruit juice could be attributed to its rich vitamin content, which agrees with the antioxidant protective role of vitamin-C [40,41]. It could also be attributed to the antioxidant properties of the phytochemical constituents of soursop fruit juice.

Similarly, groups treated respectively with NaF + stem bark extract and NaF + leaf extract showed a non-significant decrease in ALT activity. These observations could be explained from the correlations between their therapeutic inhibition pattern (suppression of ALT activity) and their percentage vitamin-C contents. However, the qualitative phytochemical screening indicates the presence of some possible antioxidant phytochemicals.

Liver plays a central role in detoxifying reactions like hydroxylation, hydrolysis, oxidation, carboxylation, reduction and demethylation. Intake of high concentration of fluoride causes dental and skeletal fluorosis and alteration in makers of hepatocellular damage. The degree of increased levels of AST activity may reflect the extent of hepatocellular necrosis. Apparently, fluoride ion may have enhanced the permeability of hepatocyte membranes (or the membranes of extra-hepatic cells) and the escape of aspartate aminotransferase to the circulatory system. According to literature, fluoride is well known for its hepatotoxic effect on markers of hepatocellular damage [17,33,35]. The generation of ROS and lipid peroxidation was being considered to play an important role in the pathogenesis of chronic fluoride toxicity [42].

Many studies have reported that excessive fluoride exposures can damage the redox balance of the cells in tissues, decrease antioxidant defence capacity in brain [43] and increase the toxic effects on visceral organs mediated by generation of ROS and lipid peroxidation [18]. In the present study, significant decrease in AST observed at 1000 mg/kg for both fruit juice and stem bark extract may not be attributed to vitamin C contents but to the active phytochemical constituents, since most of the attracted groups exhibited non-significant decrease in AST activity, an indication of little or non-significant recovery of hepatocytes from the toxicity. The observed significant increase in AST for NaF treated group in this research may indicate that serum AST correlates with the degree of liver cell damage. However, our observations agreed with [17], who reported a significant increase in NGF treated group when compared with the control group. This research further reveals that fluoride interferes with the structural and (biochemical) functional integrity of the liver.

Results for renal function showed that NaF toxicity is associated with both structural and biochemical changes in the kidney. The kidney reserve is such that about 50% kidney function must be lost before creatinine level in blood is raised. Serum creatinine level usually parallels the severity of the kidney disease. Our results regarding the non-significant increase in serum creatinine level in NaF treated group in relation to the control group is consistent with the previous study by [17]. The observed non-concentration dependent non-significant decrease in serum creatinine level in both groups treated with NaF + Stem bark extract and NaF + Leaf extract may be attributed to the active phytochemical constituents of both extracts, most of which may possess strong antioxidant properties. However, concomitant administration of NaF and fruit juice exhibited a non-significant decrease in serum creatinine level at lower doses but a non-significant increase at higher doses when compared with the group treated with NaF alone. This could be attributed to its poor phytochemical constituents. The observed significant increase in serum urea concentration for NaF treated group in this research will suggest the degree of nephrotoxicity on the proximal convoluted tubule membrane by NaF. Injury to proximal tubular lining cells is manifested by increased excretion of substances normally reabsorbed by these cells, such as glucose, urea, amino acids, phosphate and sodium. Creatinine and urea are indicative of membrane permeability, cell function and tissue damage. These alterations correlated with the histological changes in the kidney and agrees with [44].

The photomicrograph of the liver sections of control rats that received feed and water only showed normal hepatic architecture with central vein, portal triad and hepatocyte that is normal, while that of group treated with sodium fluoride alone showed severely damaged hepatic tissue

with focal aggregate of inflammatory cell, haemorrhage within the portal area and portal aggregate of inflammatory cell. Fruit juice at the lowest dose as well as the leaf and stem bark extracts showed moderate healing on the hepatic tissue with mild infiltration of inflammatory cell, mild extravasation of red blood cell and mild fatty changes. However, at doses of fruit juice of 500 mg/kg and above, there was well regenerated hepatic tissue with hepatocyte and central vein that appears normal.

The photomicrograph of the kidney sections of control rats that received feed and water only showed normal renal architecture with glomeruli, renal tubules and tubular cell appearing normal, while that of group treated with sodium fluoride alone showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian layer and severe apoptosis of tubular cells, focal area of intrarenal haemorrhage and severe tubular necrosis. Fruit juice at the lowest dose depicted the same features as the group treated with sodium fluoride alone but with mild fatty change. However, at medium doses fruit juice showed mild/moderate regenerated renal tissue, mild apoptosis of tubular cell with moderate regenerated glomeruli in some. The highest dose showed well regenerated renal tissue with mild loss of renal tissue otherwise normal with well-regenerated glomeruli and renal tubules. Leaf extract at the lowest dose showed mild regenerated renal tissue with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body, moderate apoptosis of tubular cells and severe infiltration of inflammatory cell, tubular necrosis and focal area of intra renal haemorrhage. The different doses of leaf extracts showed moderate regeneration with well regenerated renal tubules that are lined by tubular cell and well-regenerated glomeruli with malpighian body seen in the kidney section.

Stem bark extract at the lowest dose showed mild regenerated renal tissue with a moderate focal aggregate of inflammatory cell, glomeruli atrophy, intra hepatic haemorrhage with moderate fatty changes and extravasation of red blood cell within the glomeruli. However, at medium doses stem bark extract showed renal tissue that is severely damaged with severe coagulative necrosis of the glomeruli that leads to closure of malpighian body, tubular necrosis, focal area of intrarenal haemorrhage and apoptosis of the tubular cell**.** The highest dose showed moderate regeneration with mild intra renal haemorrhage and mild loss.

5. CONCLUSION

Phytochemical screening of *A. muricata* stem bark, leaf and fruit juice confirms the presence of saponins, terpenoids, flavonoids, steroid, and cardiac glycosides in its ethanol fraction of the extracts. These phytochemicals have been widely reported as scavengers of free radicals in the biological system. *A. muricata* stem bark and leaf extracts and fruit juice from this research have been associated with the amelioration of NaF- induced toxicity on the liver and kidney of wistar rats.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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