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ASPIRIN-INDUCED ULCEROGENIC AND NEPHRITIC DAMAGE IN WISTAR RATS: EFFECT OF ANDROGRAPHIS PANICULATA

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ABSTRACT

The aim of this study was to evaluate the effect of a methanol extract of *Andrographis paniculata* on aspirin-induced ulcerogenic and nephritic damage in Wistar albino rats. 50 rats weighing about 150–250 g were distributed into five equal groups of 10 rats each. Group 1 (normal control) and group 2 (positive control) received normal saline (10 ml/kg), groups 3 and 4 received 200 mg/kg body weight and 400 mg/kg body weight of the extract, respectively, and group 5 (standard control) received 30 mg/kg body weight Omeprazole orally for 7 days. On the eighth day, groups 2, 3, 4, and 5 were administered 200 mg/kg of aspirin orally. After 24 hours, the animals were euthanized using chloroform anaesthesia, and blood was taken using a heart puncture for kidney function analysis. The kidneys and gastrointestinal tracts were removed and preserved in 10% formalin for histological analysis. Other portions of the organs were homogenised for the purpose of carrying out superoxide dismutase (SOD) and catalase (CAT) assays. The results obtained showed that administration of aspirin increased plasma urea and creatinine ($p \leq 0.05$) compared to normal control. Also, the kidney and gastrointestinal tract tissue SOD and CAT activities were significantly ($p \leq 0.05$) decreased. However,

pretreatment with a methanol extract of *Andrographis paniculata* significantly ($p \leq 0.05$) reversed these biochemical changes when compared to the positive control. Administration of aspirin also caused distortion in the histological architecture of the kidneys compared to normal control. Pretreatment with a methanol extract of *Andrographis paniculata*, however, protected the animals from this damage compared to the positive control. The study revealed that the methanolic extract of *Andrographis paniculata* had nephroprotective and antiulcerogenic properties.

KEYWORDS: *Andrographis paniculata*, Gastrointestinal tract, Ulcerogenic, Nephro-protective, Catalase, Superoxide Dismutase, Omeprazole, Aspirin.

INTRODUCTION

Plant has contributed greatly to the civilization of man, from the beginning of mans existence plant has been a vital essence for sustenance of man's life.[13] Man through time has been able to differentiate plants base on their uses as food purposes, shelter, medications etc.[14] As the civilization of man grew so did his knowledge on plants and its uses; The use of botanicals and botanical extracts for therapeutic purposes.[15] 35 – 70,000 species of plant have at a time been used for medicinal purpose.[16] Medicinal plants are plants that are rich in ingredients which can be used as drugs a few examples may include ginger, green tea, walnuts etc.[17] The knowledge on medicinal plants gathered through trials and error over the years has acted as guide to present day medicine of which 25% of prescriptions are substance from plants[18] Morphine, Digoxin, Quinine, Atropine, Reserpine, Pilocarpine, Vincristine, Vinblastine and Taxol are but a few example of the impact of medicinal plants to us in time past, modern medicine is acceptable to the use of medicinal plants once it's been scientifically validated.[19] Therefore, it is hopeful that research is being conducted to use medicinal plants in the manufacture of synthetic pharmaceuticals for the treatment of many disorders.

Among the 40 species in the andrographis genus, *Andrographis paniculata* holds the highest significance as a medicinal plant. The plant is primarily cultivated in Asian countries annually.[1] Often encountered in isolated groupings. It can be found in various habitats, such as plains, hillsides, beaches, and areas that have been disturbed or cultivated, such as roadsides and farms. The indigenous populations of Andrographis paniculata are found in southern India and Sri Lanka, which is probably the main area where it originated and where its genetic diversity is most likely to be found. The herb is an introduced species in the northern portions of India, Java, Malaysia, Indonesia, the West Indies, and other parts of the

Americas. The species is distributed throughout the Philippines, Hong Kong, Thailand, Brunei, Singapore, and other regions of Asia, however its original status is disputed. The plant is extensively cultivated in several places. In a damp and shaded habitat, this plant reaches a vertical height ranging from 30 to 110cm. Its leaves are smooth and measure 8.0cm in length and 2.5cm in width. The flowers of this plant display rose-purple dots on their petals. It is sometimes referred to as the "king of bitters" due to its highly bitter taste throughout its entire body. The plant features a stem that is dark green in colour and has a height ranging from 0.3 to 1.0 metres and a diameter of 2 to 6 millimetres. The stem has a quadrangular shape and has longitudinal furrows. In addition, the younger sections of the stem have little protrusions on their edges and nodes.[2] Disregarding its bitter nature, *Andrographis paniculata* is a widely used medicinal herb worldwide, mostly employed for treating cold, diarrhoea, fever, jaundice, as a liver and heart tonic, and for its antioxidant properties.[3][22] The leaves of this plant exhibit anti-inflammatory, analgesic, antimicrobial, antihyperglycemic, and hypoglycemic qualities. They also possess antioxidant effects, antihypertensive effects, reduce plasma angiotensin converting enzyme (ACE) activity, and decrease lipid peroxidation in the kidneys. It is used medicinally to treat ailments such as cancer, diabetes, hypertension, ulcer, leprosy, bronchitis, skin disorders, flatulence, colic, influenza, dysentery, dyspepsia, and malaria in many regions of Asia, America, and Africa. The extracts also possess a hepatoprotective action, which helps in the treatment of liver damage induced by numerous substances.[1,4,5] The extract of *Andrographis paniculata* is recognized to have a wide range of pharmacological properties. All thanks to its metabolites, in particular Andrographolide which has been linked to its pharmacological effects.[6]

MATERIALS AND METHODS

A total of fifty (50) male Wistar albino rats, with a weight range of 150g to 250g, were obtained from the animal facility at the University of Benin, located in Benin City, Nigeria. Rats were housed in conventional cages for a week, provided with standard pellets and purified water for consumption.

Collection of Extract/Extraction procedure

After collecting the fresh plants of *Andrographis paniculata*, they were placed in a clean and dry tray allowed to shade dry for 3 weeks. The leaves were pulverised into a coarse powder using a dry grinder and then stored in an airtight container for extraction.

The coarse powder of *Andrographis paniculata* leaves was weighed (600g) and was subjected to extraction with (2.6L) of methanol for 72hours (3 days). The methanol extract was collected, filtered and concentrated under reduced pressure at 45°c and 40rpm with rotary vacuums evaporator, the residue was stored for further use.

Experimental Design and Procedures

50 Wistar rats were divided into 5 groups and treated as follows for 7 days.

1. Normal Control: Feed + Distilled Water.
2. Positive Control: Feed + Distilled Water.
3. Test Group: Feed + Distilled Water + *Andrographis paniculata* (200 mg/kg body weight).
4. Test Group: Feed + Distilled Water + *Andrographis paniculata* (400 mg/kg body weight).
5. Standard Control: Feed + Distilled Water + Omeprazole (30mg/kg body weight).

On the 8th day, group 2, 3, 4, and 5 were fasted for a 24hours period and administered with Aspirin (200mg/kg body weight) to induce ulcer.

Animal Sacrifices and Samples collection

On the 9th day, animals were sacrificed after anesthesia using chloroform. Blood from animals was collected through heart puncture, transferred to tubes, and centrifuged for 10 minutes at a speed of 3000 rpm. Supernatant was used for biochemical analysis. The renal and gastrointestinal tracts were removed from the abdomen, weighed, and placed in a 10% neutral buffered formalin solution for antioxidant and histological examinations.

Biochemical assay

Urea

Serum urea shall be estimated using the spectrophotometer according the instruction on the Randox biochemical kit manual.

Principle

Urea is hydrolysed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

Urease

$$NH3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (Blue compound)}$$

Determination of Serum Creatine Kinase (CK) Activity

The activity of Serum LDH amino transferase was estimated spectrophotometrically according the instruction on the Randox biochemical kit manual.

Principle

Creatine Kinase catalyzes the production of ATP by combining Creatine Phosphate with ADP. Hexokinase facilitates the transformation of glucose into glucose-6-phosphate by utilising ATP as a phosphate (PO4) group donor. G-6PDH catalyzes the process of converting Glucose-6-Phosphate to 6-phosphogluconate, which leads to the reduction of NADP to NADPH. The post-lag phase reaction can be detected by quantifying the increase in absorbance at a wavelength of 340nm, which is directly proportional to the level of creatine kinase activity. The production of NADPH is equal in amount to the creation of creatine.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{G -6- P} + \text{ADP}$$

$$\text{G -6- P} + \text{NADP} \xrightarrow{\text{G-6PDH}} \text{6-Phosphogluconate} + \text{NADH} + \text{H}$$

Determination of antioxidant enzymes

Tissues was rapidly excised, washed with cold normal saline, blotted dry and weighed. A 10% (w/v) homogenate shall be prepared by using motar and pestle in 10mM potassium phosphate buffer pH 7.4 containing 30mM KCI and centrifuged at 1000 x g for 10 minutes at 4°C using a refrigerated centrifuge to remove cell debris and nuclei. The supernatants were used for the determination of antioxidant activities.

Superoxide Dismutase (Superoxide oxidoreductase, EC 1.15.1.1)

The method of[20] with some modification, which is an assay based on the ability of SOD to inhibit autoxidation of pyrogallol was used for the determination of the activity of SOD.

Principle

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical ($O_2^{\cdot}$) to yield hydrogen peroxide and oxygen.

Inhibition of autoxidation by SOD

$$\text{SOD Activity} = \text{Pyrogallol} + \text{O} \longleftrightarrow O_2^{\cdot-} + \text{oxidation product}$$

Preparation homogenate

Homogenate of the hearts were treated with the non-ionic detergent Triton X-IOO (1% final concentration) and was allowed to stand on ice for 30 minutes to completely release the enzyme (Cu-Zn SOD).

Determination of catalase activity

The assay method described by[21] relies on the measurement of the rate of decomposition of H_2O_2 following its reaction with the oxidizing agent potassium permanganate ($KMnO_2$). Catalase catalyzes the rapid decomposition of H_2O_2 to water and molecular oxygen. The rate of this reaction is proportional to the activity of the catalase in the sample under investigation.

$$2H_2O_2 \text{----------} 2H_2O + O_2$$

The residual H_2O_2 reacts with excess KMnO4. The concentration of residual permanganate measured spectrophotometrically at 480nm is an indication of the activity of catalase.

Statistical analysis

Results obtained were subjected to statistical analysis. All values were expressed as the mean ± S.D and data were analyzed using one-way ANOVA. Values were considered statistically significant at $P < 0.05$.

RESULTS

Effect of ethanol extract of *andrographis paniculata* leaf on body weight

Decrease in mean body weight, in *Andrographis paniculata*treated rat, at the end of the study. Extract of andrographis paniculata at 200 and 400 mg/kg, ($p< 0.05$). (Table 1).

Ulcer index for each of the Group and The protection percentage

Administration of aspirin 200mg/kg cased ulceration of the gastrointestinal tract which was used as an index to evaluate the percentage of inhibition by the extract. Result obtained showed that administration of the extract (200 and 400mg/kg) gave a percentage inhibition of 13.85% and 22.29 % respectively while the omeprazole group (standard) gave a percentage inhibition of 26.20%. (Table 2).

The protective role of *Andrographis paniculata* on aspirin-induced ulcerogenic and nephritic cell damage

The results in table (3.3) shows that the administration of aspirin resulted in an increase ($p \leq 0.05$) of serum Urea (65.73 ± 1.24) and Creatinine (1.61 ± 0.29) in positive control group, in comparison to normal control group. However, treatment with *Andrographis paniculata* at 200mg/kg and 400mg/kg significantly ($p \leq 0.05$) reduced Urea (48.11 ± 2.15 and 48.31 ± 2.51). It also significantly reduced serum Creatinine (1.27 ± 0.29 and 0.64 ± 0.31) in comparison to positive control group. Treatment with omeprazole (30mg/kg) also resulted to significant ($p \leq 0.05$) decrease in Urea (49.59 ± 1.72) and Creatinine (0.70 ± 0.26) compared to the positive control. The administration of aspirin caused a decrease ($p \leq 0.05$) in the activities of SOD in the kidney and the gastrointestinal tract (2.80 ± 0.07 and 2.83 ± 0.06 respectively). It also significantly ($p \leq 0.05$) decreased kidney CAT activity (0.72 ± 0.01) in comparison with normal control group. However, treatment with *Andrographis paniculata* (200mg/kg and 400mg/kg) caused an increase ($p \leq 0.05$) in the activities of SOD in kidney (4.86 ± 0.02 and 4.89 ± 0.07 respectively) and gastrointestinal tract (4.83 ± 0.07 and 4.87 ± 0.06 respectively). It also increased kidney CAT activity (1.01 ± 0.01 and1.03 ± 0.06) in comparison to normal control. (Table).

Table 1: Mean values of body weights of rats administered ethanol extract of *Andrographis paniculata*.

	Weight (mean ± standard deviation)	
	Before pretreatment	**After pretreatment**
Group 1 Normal control	236.77 ± 21.302[a]	223.44 ± 22.979[a]
Group 2 Positive control	155.185 ± 19.183[a]	151.616 ± 17.406[a]
Group 3 Test group 1 with 200mg/kgbody weight extract	223.239 ± 34.209[b]	204.404 ± 30.366[b]
Group 4 Test group 2 with 400mg/kgbody weight extract	199.511 ± 28.281[c]	182.846 ± 27.330[c]
Group 5 Standard group with 30mg/kg body weight of Omeprazole	206.056 ± 33.681[d]	185.113 ± 31.966[d]

Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at $p<0.05$.

Table 2: Ulcer Index and Protection percentage.

Group	Percentage protection
Group 1 Normal control	100%
Group 2 Positive control	0%
Group 3 Test group 1 with 200mg/kg body weight extract	13.85%
Group 4 Test group 2 with 400mg/kgbody weight extract	22.29%
Group 5 Standard group with 30mg/kgbody weight of Omeprazole	26.20%

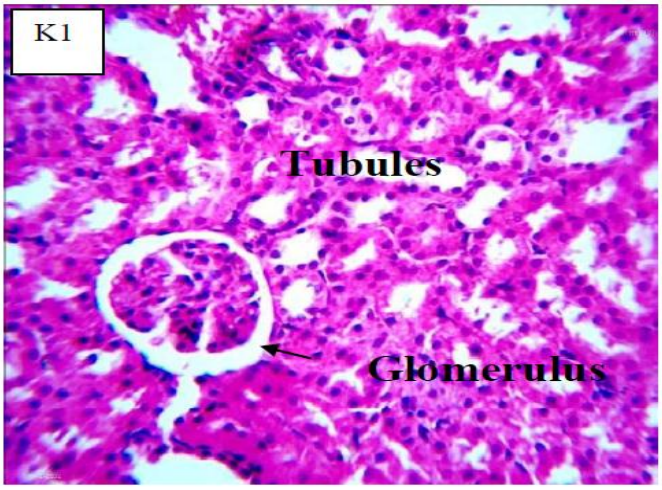
Table 3: Mean values of antioxidants concentration of kidney homogenate, creatinine and urea values of rats administered ethanol extract of *Andrographis paniculata.*

	Urea (mg/dl)	Creatinine (mg/dl)	SOD(kidney) (U/mg protein)	CAT(kidney) (U/mg) protein)	SOD (GIT) (U/mg protein)
Group 1 Normal control	38.32 ± 4.55^{a}	0.59 ± 0.45^{a}	4.68 ± 0.32^{a}	1.00 ± 0.92^{a}	4.81 ± 0.04^{a}
Group 2 Positive control	65.73 ± 1.24^{b}	1.61 ± 0.29^{b}	2.80 ± 0.07^{b}	0.72 ± 0.01^{b}	2.83 ± 0.06^{b}
Group 3 Test group 1 with 200mg/ kgbody weight extract	48.11 ± 2.15^{c}	1.27 ± 0.29^{c}	4.86 ± 0.02^{a}	1.03 ± 0.01^{a}	4.83 ± 0.07^{a}
Group 4 Test group 2 with 400mg/ kgbody weight extract	48.31 ± 2.51^{c}	0.64 ± 0.31^{b}	4.89 ± 0.07^{a}	1.01 ± 0.01^{a}	4.87 ± 0.06^{a}
Group 5 Standard group with 30mg/ kg body weight of Omeprazole	49.59 ± 1.72^{c}	0.7 ± 0.26^{d}	4.91 ± 0.004^{a}	1.02 ± 0.01^{a}	4.84 ± 0.04^{a}

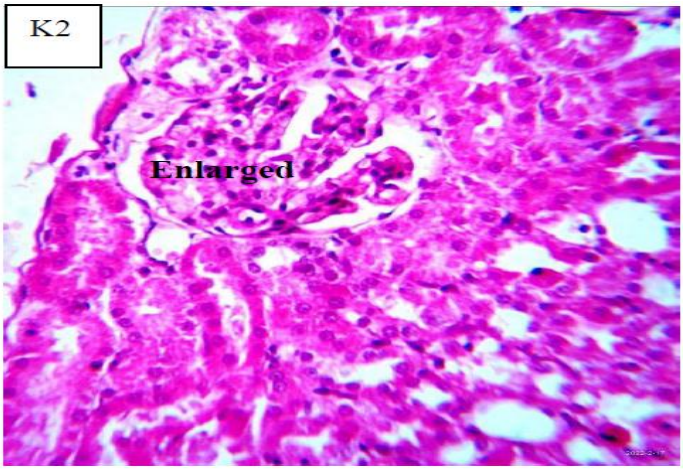
Values are represented as Mean ± SD. Value with different superscripts from control are statistically different at $p<0.05$.

Histopathology of the kidney

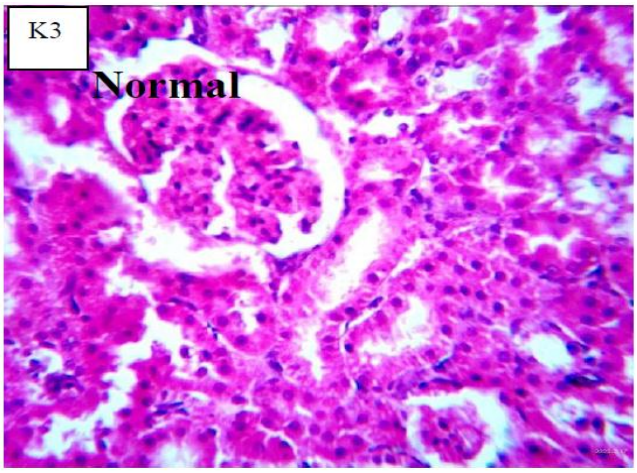
Plate 3.1: Photomicrographs of kidney tissues of an adult wistar rat stained with haematoxylin and Eosin technique.



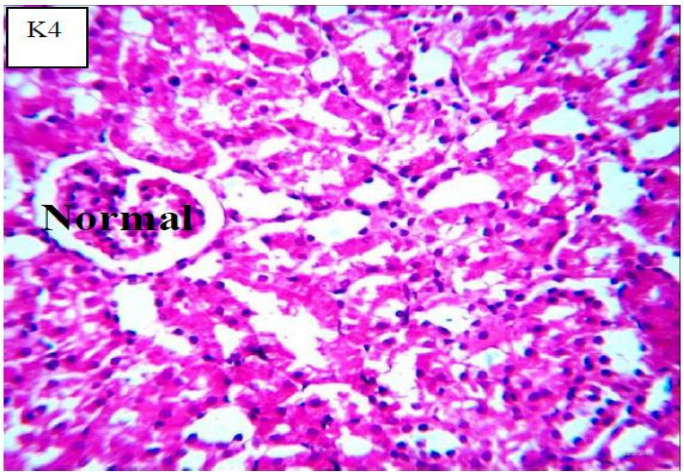
K1: (Normal Control) Transverse section of the kidney stained with haematoxylin and eosin x 400 magnification. Section shows normal histology of the kidney.



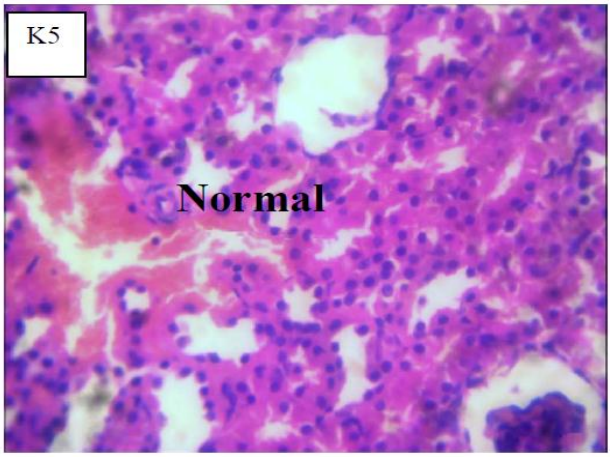
K2: (Positive control) Transverse section of the kidney stained with haematoxylin and eosin x 400 magnification. Section show mild enlargement of the Glomerulus suggestive of membrane proliferative disorder and also marked intimal proliferation and fibrosis with narrowing of the lumen of the blood vessel.



K3: (Test group1) Transverse sections of the kidney stained with Haematoxylin and Eosin x 400 magnification. Sections show normal histology of the kidney.



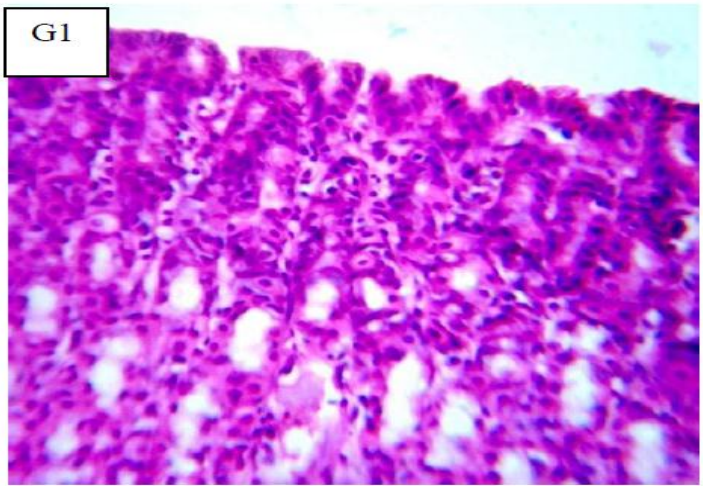
K4: (Test Group II) Transverse sections of the kidney stained with haematoxylin and eosin x 400 magnification. Sections show normal histology of the kidney.



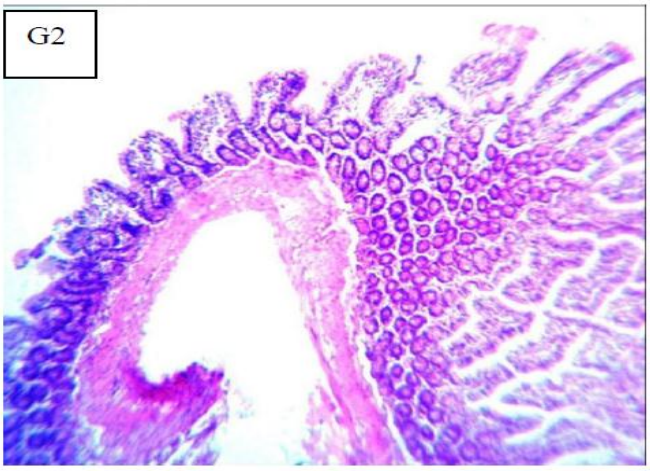
K5: (Standard Control) Transverse sections of the kidney stained with haematoxylin and eosin x 400 magnification. Sections show normal histology of the kidney.

Histopathology of the gastrointestinal tract

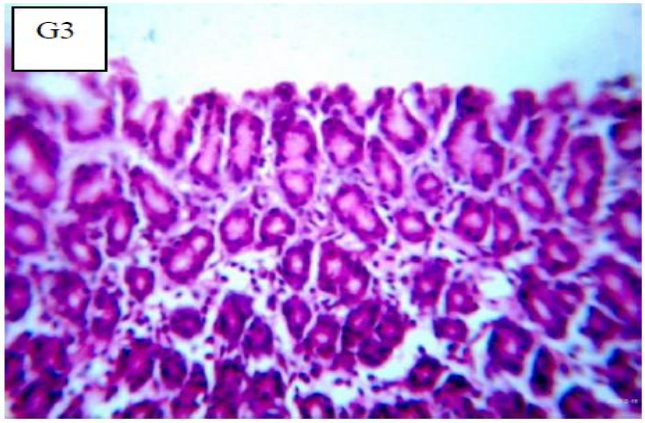
Plate 3.2: Photomicrograph of stomach of an adult Wistar rat stained with haematoxylin and Eosin technique.



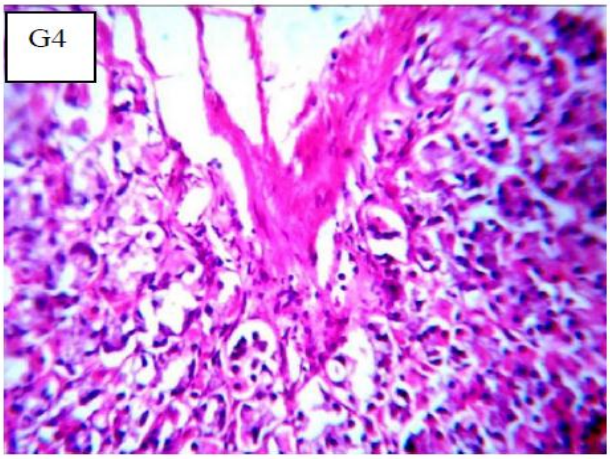
G1: (Normal Control): shows a transverse section of the stomach with normal gastric pits and mucosa and abundant gastric glands consistent with histology of the stomach. X400.



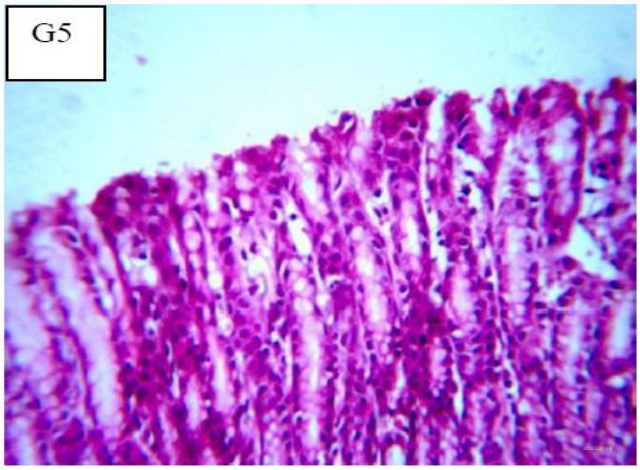
G2: (Positive Control): Transverse section of Haematoxylin and Eosin-stained slides x 400 magnification. Sections areas of ulcerated epithelium with atrophic gastric glands.



G3: (Test Group I): Transverse section of haematoxylin and eosin-stained slides x 400magnification. The section shows gastric mucosa with the disappearance of the original gastric glands and other epithelium replacements.



G4: (Test Group II) Transverse section of haematoxylin and eosin-stained slides x 400magnification. The section shows healing by fibrous.



G5: (Standard Control) Transverse section of haematoxylin and eosin-stained slides x 400magnification. The section shows regenerating epithelium with tall gastric glands displaying normal columnar epithelium.

DISCUSSION

Nephrotoxicity, which is one of the most widespread kidney diseases, is the chronic effect of substances on the renal system and can occur when the body is exposed to toxins. There are numerous therapeutic agents responsible for occurrence of nephrotoxicity like anti-cancer drugs, anti-biotics, some NSAIDs, etc. These agents reduce the kidney's function which results in renal failure.[7,8] The accumulation of excess body waste such as Urea and Creatinine in the blood can be as a result of renal failure.[9]

Results obtained from this study also indicated that there was a significant increase in serum urea and creatinine concentration following the administration of 200mg/kgbody weight aspirin in positive control group in comparison to normal control group. This is indicative of renal damage. However, pretreatment with methanolic extract of *Andrographis paniculata* at 200mg/kg body weight and 400mg/kgbody weight caused a significant and dose dependent decrease of serum Urea and Creatinine concentration compared to positive control. Treatment with Omeprazole at a dose of 30mg/kgbody weight also caused a significant decrease in Urea and Creatinine blood concentration in comparison to positive control.

The ulcer index of the gastrointestinal tract showed that Administration of aspirin 200mg/kg body weight caused ulceration of the gastrointestinal tracts of the rats when compared with the normal control. Administration of the extract (200mg/kg body weight and 400mg/kg body weight) significantly protected the rats from aspirin induced ulceration of the stomach. It gave a percentage inhibition of 13.85% and 22.29 % respectively while the omeprazole group (Standard) gave a percentage inhibition of 26.20% compared to positive control.

The kidney sections obtained from animals pretreated with extract of *Andrographis paniculata* at 200mg/kg body weight and 400 mg/kgbody weight indicated no change in kidney architecture and glomerulus. Also omeprazole treated animals indicated no change in the kidney architecture and glomerulus. Animals without any treatment show mild enlargement of the Glomerulus suggestive of membrane proliferative disorder and also marked intimal proliferation and fibrosis with narrowing of the lumen of the blood vessel.

Oxidative stress results from the imbalance between oxidants also known as reactive oxygen species (ROS) and antioxidants.[10] Oxidative stress is unavoidable in an environment filled with oxygen due to the production of ROS. The body reduces the effect of ROS by the production of cellular antioxidants, the intake of herbal medication, fruits and vegetables

which are rich in antioxidants reduces oxidative stress.[11,12] Results gotten from this study showed that administration of aspirin 200 mg/kg body weight caused a significant decrease in both the kidney and stomach activities superoxide dismutase (SOD) and kidney catalase (CAT) in comparison to normal control. This is indicative of a rise in the production of ROS. However, pretreatment with *Andrographis paniculata* (200mg/kg body weight and 400 mg/kg body weight) resulted to an increase in the kidney and stomach activities of SOD and kidney CAT compared to positive control. Treatment with omeprazole at 30mg/kg body weight also caused an increase in SOD and CAT activities, compared to positive control group. This result is in agreement with previous works [3] that revealed that *Andrographis paniculata* possesses antioxidants properties. Results obtained from this work indicate that *Andrographis paniculata* may possess the potential to provide protection from ulcerogenic and nephritic damage.

Compliance with ethical standards

ACKNOWLEDGMENTS

The authors are grateful to the Technical laboratory staff of the Department of Biochemistry Niger Delta University, Amassoma.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The study protocol was approved by the Ethical and Research Committee of Niger Delta University, Bayelsa State, Nigeria. The ethical principles for medical research involving animal subjects as outlined in the Helsinki declaration in 1975 and subsequent revisions were strictly followed in the course of this study

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