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RENOPROTECTIVE AND HEPATOPROTECTIVE POTENTIALS OF N-BUTANOL, ETHYL-ACETATE AND CHLOROFORM FRACTIONS OF ETHANOL LEAVES EXTRACT OF PTEROCARPUS SANTALINOIDES ON 5-FLUOROURACIL INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT

Background: The plant *Pterocarpus santallinoides* is endowed with so many phytochemicals with pharmacological potencies hence, it is used as one of the herbal remedies employed for the treatment of diverse kinds of ailments. **Objective/Aim:** This study is aimed at determining the renoprotective and hepatoprotective effects of n-butanol, ethyl-acetate and chloroform fractions of *Pterocarpus santalinoides* ethanol leaf extract on the 5-fluorouracil-induced toxicity potential in albino rats. **Methodology:** A total of thirty albino rats were separated into 5 groups of 6 animals each. The animals were given treatments (distilled water, distilled water, 69.28 mg/kg n-butanol fraction, 69.28 mg/kg ethyl acetate fraction and 69.28 mg/kg chloroform fraction for groups 1, 2, 3, 4 and 5 respectively) orally for 14 days. On the 15th day, groups 2, 3, 4 and 5 were given acute dose of

5-FU (150 mg/kg) via intraperitoneal route to induce cardiotoxicity. The animals were sacrificed after 24 hours, and the sera analyzed for liver enzymes (Alkaline phosphatase, aspartate transaminase, alanine transaminase), electrolytes (potassium(K+) sodium(Na+) and chloride(Cl-)), urea and creatinine; the organs (liver and kidneys) were excised and fixed in formalin solution and thereafter subjected to histopathology. **Results:** Revealed significant ($p < .05$) decrease in ALP, AST and ALT activities across groups compared to group 2 (positive control); significant ($p < 0.05$) decrease in serum urea and creatinine concentrations across the treatment groups compared to positive control; significant ($p < .05$) decrease in serum electrolytes (potassium, sodium, chloride and bicarbonate) across the groups compared to positive control. Results from histopathology revealed severe damage in group 2 kidney and liver tissues compared to group 1 (normal control) and other treatment groups. ***Conclusion*:** This study showed that all the fractions of ethanol leaf extract of *Pterocarpus santalinoides* possessed appreciable renoprotective and hepatoprotective potential against 5-fluorouracil induced toxicity in albino rats.

KEYWORDS: Toxicity, *Pterocarpus santalinoides*, 5-fluorouracil, liver enzymes, haematology, Plant extracts.

INTRODUCTION

Cell deaths and tissue damage resulting from treatment with anticancer drugs such as 5-fluorouracil (5-FU) has become a common phenomenon leading to debilitating side effectssuch as cardiotoxicity, nephrotoxicity, hepatotoxicity, etc. 5-FU is widely applied systematically for the treatment of solid cancers, such as cancers of the gastrointestinal tract, breast, head, neck, pancreas and skin.[1,2] It acts mainly by interfering with DNA replication and transcription by integrating its toxic metabolites and inhibits thymidylate synthase.[3]

Apart from being cardiotoxic, 5-fluorouracil also has hepatotoxic effects, and can cause elevation of aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in tissues.[4] Nephrotoxicity is also another prominent adverse effect of 5-FU treatment in cancer patients. Increased oxidative stress, kidney damage, and apoptosis play an important role in the pathogenesis of nephrotoxicity caused by 5-FU.[5]

The possible mechanism by which 5-FU induce hepatotoxicity and nephrotoxicity could be through inducing oxidative stress; activation of apoptotic pathway by upregulation of bax, caspase-3 and p53 and down regulating Bcl-2.[6] A natural flavonoid called Chrysin, which is

found in many plant extracts (e.g. propolis, blue passion flower) may be a useful modulator in preventing or reducing 5-FU induced renal toxicity.[6] Similarly, naringin, a flavonoid that is usually found in grapefruit, orange and cooked tomato paste,[7] has antioxidant, immunomodulatory and anti-inflammatory properties and has been shown to have a protective effect on 5-FU-induced hepatotoxicity and nephrotoxicity.[8] Administration of L-arginine to 5-FU treated Wistar rats revealed that L-arginine had a very powerful renoprotective effect against the 5-FU induced nephrotoxicity in the albino rats.[9]

Additionally, Treatment of rats with telmisartan (an angiotensin receptor blocker) and quercetin (a flavonoid with antihypertensive potential) attenuated the 5 fluorouracil induced nephrotoxicity in the rats.[10] Even captopril, an angiotensin-converting enzyme (ACE) inhibitor, has shown to have a renoprotective and hepatoprotective effect against 5-FU-induced damage to liver and kidney.[11] High doses of hesperidin and curcumin treatment significantly improved 5-FU–induced oxidative stress/lipid peroxidation, apoptosis/DNA damage, and renal dysfunction.[5] Carvedilol confers hepatoprotective and renoprotective effects from hepatotoxicity and nephrotoxicity induced by doxorubicin or 5-fluorouracil therapy in male Wistar rats.[11]

5-FU is metabolized to 5-fluoro-2-deoxyuridine5- monophosphate (FdUMP), which inhibits thymidylate synthase, a very relevant enzyme involved in the synthesis of thymine. By this, it prevents the production of 2′-deoxythymidine-5′- monophosphate (dTMP) which is verymuch needed for DNA replication and repair consequently, inhibiting cell reproduction, and can cause death of tumor as well as normal body cells.[4,8,12,13]

Many cancer survivors are living with long-term adverse effects of cancer therapy causing non-functional cells due to organ pathology. *Pterocarpus santalinoides* is one of the plant species used for the management and treatment of ailments.[14-16] *Pterocarpus santallinoides* is endowed with so many phytochemicals with pharmacological potencies thus making it one of the herbal remedies employed for the treatment of diverse kinds of ailments. Its leaves are eaten as vegetable and used to treat gastro-intestinal diseases, skin diseases, pain and inflammation of lower abdomen, stomach ache, headache, skin diseases, boils, fevers diabetic syndrome and are known to exhibit antipyretic activity.[17,18] The stem bark extracts were reported to have antibacterial, anti-diabetic and hepatoprotective activities.[19]

It also has hypolipidemic and hypoglycemic potentials.[20] Various parts of *Pterocarpus santalinoides* are employed in traditional medicine across sub Saharan African (sSA) countries, to treat several human diseases. The ethno-medical use of leaves of *Pterocarpus santalinoides* in the treatment of diarrhoea and other gastrointestinal disorders, its triglyceride and glucose lowering properties were previously established experimentally.[16,21] Moreover, the leaves have antimicrobial activity and contain phytochemicals including alkaloids, tannins, saponins, terpenoids, flavanoids and anthraquinones, which may be responsible for these activities.[22] In Nigeria, aged people use *P. santalinoides* leaves for making soup because it is considered effective for relieving age-related cardiovascular diseases including stroke.[23-25] Ethanol leaf extract of *Pterocarpus santalinoids* possess anti-diabetic and antihyperlipidemic potential, which can be useful in the treatment and management of diabetes mellitus.[20] Because of the pharmacological significance of *Pterocarpus santalinoides* and their usage in the folkloric treatment of various diseases, hence, we subjected the ethanol leaf extract of this plant (*Pterocarpus santalinoides)* toexperimental scrutiny, with a view to establish its renoprotective and hepatoprotective potentials against 5-FU induced nephrotoxicity and hepatotoxicity in albino rats.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

All chemicals and reagents used for this research were of analytical grade. Assay kits for the estimation of serum ALP, ALT, AST, urea, creatinine, (Randox Laboratories Ltd.), United Kingdom. Serum electrolytes were assayed using flame photometer.

Plant leaf Collection and Identification

Mature dark green leaves of *Pterocarpus santalinoides* were used for this study. The *Pterocarpus santalinoides* leaf samples were collected from the premises of Abia State Colllege of Health Sciences and Management Technology, Aba, Abia State, Nigeria. The plant sample was identified and authenticated by Prof. (Mrs) Magaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria and assigned with herbarium number - UUPH A32(Ziii)a. The leaves of *Pterocarpus santalinoides* were collected, washed, chopped and air dried under room temperature. These were then pulverized, weighed and macerated with 5 litres of absolute ethanol at room temperature with intermittent agitation for 3 days, it was thereafter filtered, the filtrates were concentrated to

dryness using water bath at 40 oC. The dry extract was weighed and stored in refrigerator (between 2 and 8°C) for further studies.

Extract fractionation

20.00g of the crude ethanol extract of *Pterocarpus santalinoides* leaves was dissolved in 100 ml distilled water, which was then partitioned in increasing order of solvent polarity starting from chloroform, ethyl acetate and then n-butanol. These processes were repeated five times till sufficient fractions were obtained for the experiment. The different fractions obtained (n-butanol, ethyl acetate and chloroform fractions) were concentrated to dryness using water bath with temperature at 40oC.

Animal treatment

Thirty albino rats of Sprague-Dawley strain weighing between 160-180g were used for the experiments. All animals were obtained from animal house of University of Uyo, Uyo, Nigeria. The animals were acclimatized for 14 days to laboratory conditions under 12 hours' light and dark cycle before starting the experiment and fed with standard pellet diet and water. At the end of fourteen days, the animals were divided into 5 groups of 6 albino rats each and were treated orally with distilled water and extract fractions once daily successively for a period of 14 days (2 weeks) as shown in Table 1. On the 15th day, the animals in all thegroups except group 1 were given a single dose (150 mg/kg b.w.) of 5-fluorouracil by intraperitoneal (i.p.) route according to the dosage used by Najim *et al.*[26] to induce tissue damage.

Table 1: Dose regimen for animal treatment.

Group	Treatment	Dosage
1 (Normal control)	Distilled water only	1 ml distilled water
2 (Positive control)	Distilled water + 5-fluorouracil	1 ml distilled water. 5-fluorouracil: 150 mg/kg bw
3 (N-butanol fraction)	N-butanol fraction + 5- fluorouracil	n-butanol fraction: 69.28 mg/kg b.w. 5-fluorouracil: 150 mg/kg bw.
4(Ethylacetate fraction)	Ethylacetate fraction + 5- fluorouracil	ethylacetate fraction: 69.28 5-fluorouracil: 150 mg/kg bw
5 (Chloroform fraction)	Chloroform fraction + 5- fluorouracil	chloroform fraction: 69.28 mg/kg bw 5-fluorouracil: 150 mg/kg bw

Lethal Dose (LD50) Determination

The median lethal dose (LD50) of the extract was estimated with albino mice using Lorke's method.[27] This involved intraperitoneal administration of different doses of the extract (100

- 5000 mg/kg bw) to groups of three mice each. The mice were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD50 was calculated as geometrical means of the maximum dose producing 0% mortality (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

In Phase one, the animals fasted for 18 hours, thereafter they were weighed and grouped into three groups of three mice per group (weight: 20 - 25g). Each group of three received 3,000, 4,000 and 5,000mg/kg bw of crude ethanol extract intraperitoneally (i.p) and was observed for physical signs of toxicity and mortality within 24 hours. 3,000; 4,000 and 5,000mg/kg bw recorded 100% mortality within 24 hours. Based on the value of phase one, phase two studies were conducted.

In Phase two, the animals were equally fasted for 18 hours, thereafter they were weighed and grouped into three groups of three mice per group (weight: 20 - 25g). Each group of three mice received 500, 1,000 and 2000mg/kg bw of crude ethanol extract intraperitoneally (i.p) and was thereafter observed for physical signs of toxicity and mortality within 24 hours. The 500, 1000 and 2000mg/kg bw recorded 100% mortality within 24 hours. Based on the result of phase two, phase three studies were conducted.

In Phase three, the animals were equally fasted for 18 hours, thereafter they were weighed and grouped into four groups of three mice per group (weight: 20 - 25g). Each group of three received 100, 200, 300 and 400mg/kg bw of crude ethanol extract intraperitoneally (i.p) and was thereafter observed for physical signs of toxicity and mortality within 24 hours. The 100, 200 and 300 mg/kg bw recorded 0% mortality while 400mg/kg bw of the crude ethanol extract recorded 100% mortality within 24 hours. Based on these records the median lethal dose (LD50) was calculated as geometrical means of the maximum dose producing 0% mortality (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

Where a = 300 mg/kg

b = 400 mg/kg

$$LD_{50} = \sqrt{300x400}\ mg/kg$$

LD50 = 346.41 mg/kg

Thereafter, 10%, 20% and 30% of the LD50 were calculated for the studies thus:

Low dose (LD): 10% of LD50 = 34.64mg/kg

Medium dose (MD): 20% of LD50 = 69.28mg/kg

High dose (LD): 30% of LD50 = 103.92mg/kg

Animal Sacrifice and Preparation of Sera

On the 15th day, groups 2, 3, 4, 5 and 6 were given equal dose of 5-fluorouracil (150 mg/kg) to induce tissue damage. After 24 hours of inducing tissue damage, the animals were humanely sacrificed immediately after they were anaesthetized in a chloroform vapour saturated desiccator and their blood collected by cardiac puncture into plain tubes for sera preparation. Their kidneys and livers were harvested and fixed in 10% formalin solution for 48 hours before being subjected to histopathological studies. The plain tubes containing blood samples were centrifuged and the serum (supernatant) collected for analysis of the following biochemical parameters using standard kits (analytical grade).

i. Urea
ii. Creatinine
iii. Electrolytes
iv. AST
v. ALT

Estimation of Transferases (ALP, ALT, AST). urea, creatinine

These assays were carried using the kits, as manufactured by Randox Laboratories Ltd., United Kingdom. The processes and procedures are as contained in the manual.

Statistical analysis

One-way Analysis of Variance (ANOVA) was carried out using Statistical Analysis software (SPSS), level of significance was determined using Tukey HSD and mean difference at was considered to be statistically significant. Comparison was made between the extract fractions and normal control and positive control groups to ascertain the effectiveness of the protective potential of the extract fractions, against the positive control.

Histological analysis

The fixed organs were histologically assayed using standard staining techniques for the impact of the treatment on the structural integrity of the liver and kidney tissues.

RESULTS

These experiments were carried out with a view to establish the renoprotective and hepatoprotective potentials of *Pterocarpus santalinoides plant* in an albino rats induced for nephrotoxicity and hepatotoxicity using 5-FU. Below are the results

Table 3: The effects of fractions of *P. santalinoides* leaf extract on serum ALT and AST activity.

Groups	ALT (IU/L)	AST(IU/L)
1 (Normal control)	49.72 ± 0.67 b, c, d, e, f	12.86 ± 0.76 b, c, d, e, f
2 (Positive control)	127.72 ± 3.35 a, c, d, e, f	30.67 ± 1.99 a, c, d, e, f
3 (N-butanol fraction)	60.19 ± 1.35 a, b	20.24 ± 1.50 a, b
4 (Ethyl-acetate fraction)	57.31 ± 1.45 a, b	18.78 ± 0.97 a, b
5 (Chloroform fraction)	63.56 ± 0.89 a, b	20.63 ± 1.17 a, b

Values are represented as mean ± SEM of six determinations. Values with different alphabets differ significantly at $p < 0.05$. Superscript a, b, c, d, e, and f is statistically significant compared to groups 1, 2, 3, 4, 5 and 6 respectively.

Table 4: The effects of fractions of ethanol leaf extract of *P. santalinoides* on the serum electrolytes concentration in albino rats.

Group	Na+(mmol/L)	K+(mmol/L)	HCO3-(mmol/L)	Cl- (mmol/L)
1 (Normal control)	132.83 ± 0.74 b, d, e, f	3.61 ± 0.04 b, c, d, f	23.50 ± 0.71 b	96.86 ± 1.24b, c, d, e, f
2 (Positive control)	146.17 ± 0.30a, c, d, e, f	4.71 ± 0.03 a, c, d, e, f	25.67 ± 0.49 a, c, e, f	104.60 ± 0.24a, c, d, e, f
3 (N-butanol fraction)	141.50 ± 0.56 a, b, c, e, f	4.36 ± 0.02 a, b, c, e, f	24.00 ± 0.25 b	101.07 ± 0.17a, b, c
4 (Ethyl-acetate fraction)	138.17 ± 0.30 a, b, c, d	3.56 ± 0.02 b, c, d, f	21.83 ± 0.47 a, b, d	102.01 ± 0.23 a, b, c
5 (Chloroform fraction)	138.60 ± 0.24 a, b, c, d	4.16 ± 0.02 a, b, c, d, e	23.20 ± 0.37b	101.19 ± 0.01a, b, c

Values are represented as mean ± SEM of six determinations. Values with different alphabets differ significantly at $p < 0.05$. Superscript a, b, c, d, e, and f is statistically significant compared to groups 1, 2, 3, 4, 5 and 6 respectively

The Effects of Fractions of *P. santalinoides* Leaf Extract on the Serum Urea and Creatinine Concentration in Wistar Rats

Group	Urea (mmol/L)	Creatinine (mg/dL)
1 (Normal control)	19.83 ± 0.65 b, c, d, e, f	0.45 ± 0.02 b, d, e
2 (Positive control)	28.67 ± 0.66 a, c, e	0.80 ± 0.03 a, c, d, e, f
3 (N-butanol fraction)	28.83 ± 0.47 a, c, e	0.65 ± 0.02 a, b, f
4 (Ethyl-acetate fraction)	25.17 ± 0.30 a, b, d, f	0.63 ± 0.03 a, b, f
5 (Chloroform fraction)	27.60 ± 0.24 a, c, e	0.50 ± 0.03 b, d, e

Values are represented as mean ± SEM of six determinations. Values with different alphabets differ significantly at $p < 0.05$. Superscript a, b, c, d, e, and f is statistically significant compared to groups 1, 2, 3, 4, 5 and 6 respectively

Histopathological Results

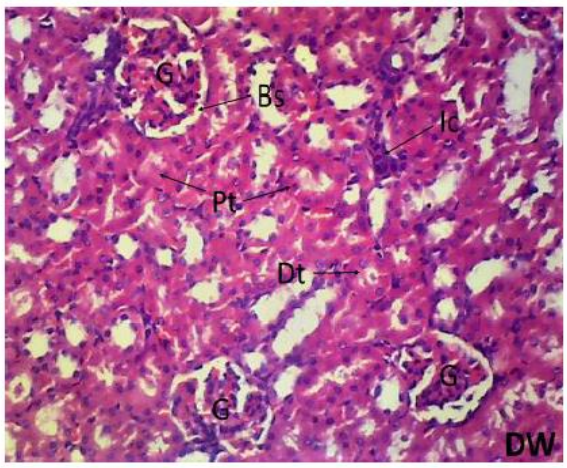

Plate 1: Photomicrograph of the longitudinal section of the kidney tissue of the distilled water treated rats (normal control). Normal histoarchitechture of the renal tissue with glomeruli (G), normal bowman's space (Bs) well presented proximal convulated tubules (Pt) and distal convulated tubules (Dt) and interstitial tissue cells.

Stain: H & E

Magnification: x 100

Inference: No histopathological changes

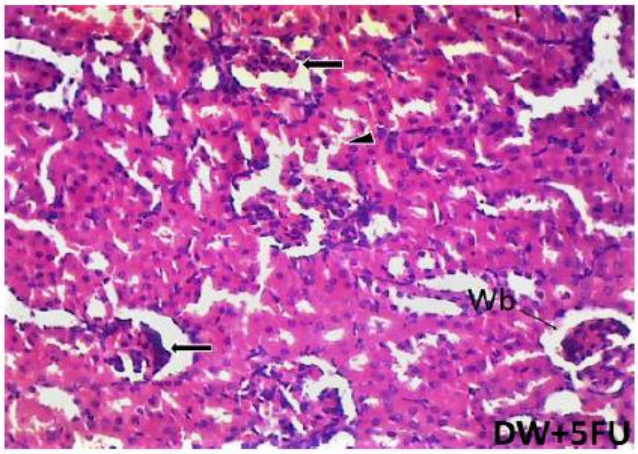

Plate 2: Photomicrograph of the longitudinal section of the kidney tissue of the distilled water + 150 mg/kg bw treated rats (positive control). Abnormal renal tissues with glomeruli having widened bowman's space (Wb), degenerating glomerular tuft (black arrow), degenerating ductal and connective tissue cells (arrow head) and hyperemia (H) within the renal tissue. (x 100).

Stain: H & E

Magnification: x 100

Inference: Severe histopathological changes.

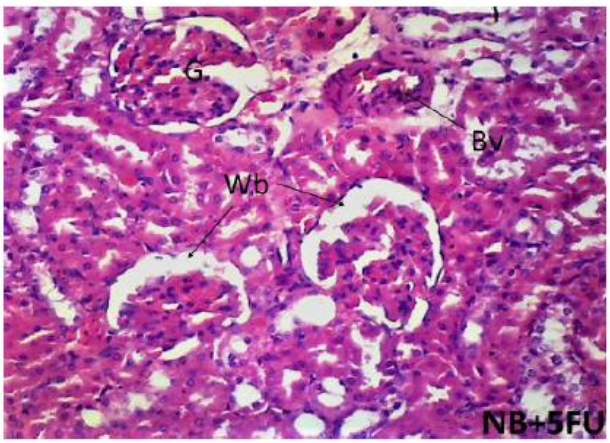

Plate 3: Photomicrograph of the longitudinal section of the kidney tissue of the n-butanol fraction + 150 mg/kg bw treated rats (group 4). Abnormal renal tissues with glomeruli having widened bowman's space (Wb), with well-presented ductal cells, connective tissues cells and blood vessels (Bv) within the renal tissue.

Stain: H & E

Magnification: x 100

Inference: Mild histopathological changes

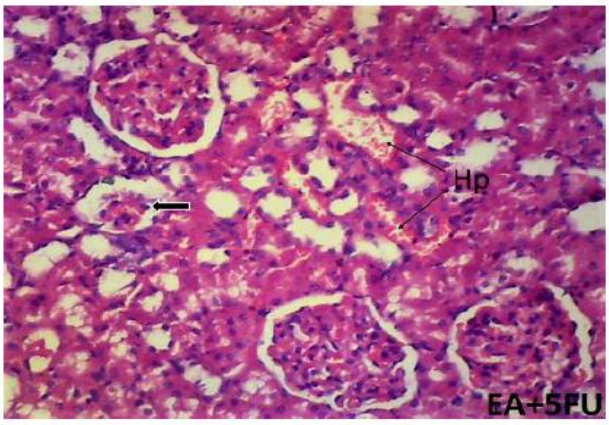

Plate 4: Photomicrograph of the longitudinal section of the kidney tissue of the ethyl-acetate fraction + 150 mg/kg bw treated rats (group 5). Abnormal renal tissues with glomeruli, Degenerating glomerular tuft (black arrow), and hyperemia (H) within the renal tissue.

Stain: H & E

Magnification: x 100

Inference: Moderate histopathological changes

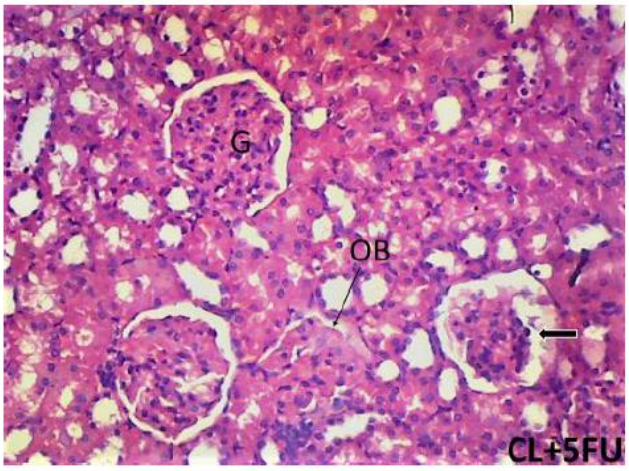

Plate 5: Photomicrograph of the longitudinal section of the kidney tissue of the chloroform fraction + 150 mg/kg bw treated rats (group 6). Abnormal renal tissues with Degenerating glomerular tuft (black arrow), glomerulus with occluding Bowman's space (OB) and well-presented ductal cells and connective tissue cells within the renal tissue.

Stain: H & E

Magnification: x 100

Inference: Moderate histopathological changes.

Result suggestion: Assessment of the histopathology of the Kidney tissue showed protective properties of the extract fractions as compared to the Distilled water treated renal tissue (Normal control), as against the Distilled Water + 5 Fluorouracil treated renal tissue (Positive control) and the aspirin + 5-fluorouracil treated renal tissue (Standard control), although this protection was more significant in the N-butanol fraction treated renal tissue.

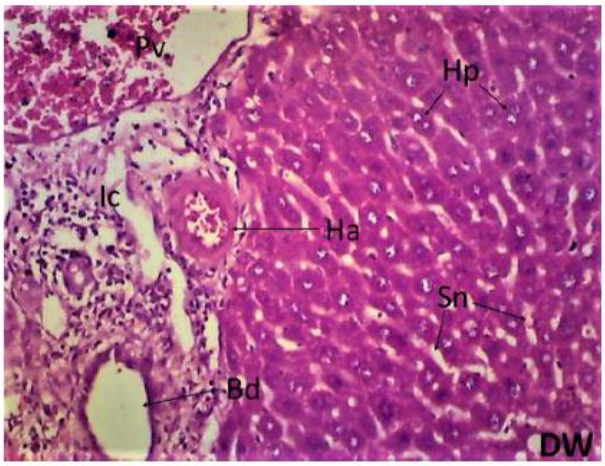

Plate 6: Photomicrograph of the transverse section of the liver tissue of the distilled water treated rats (normal control). Normal hepato-architecture with well-presented portal vein (Pv), bile duct (Bd) and hepatic artery (Ha), interstitial connective tissue cells (Ic) within the portal area, well populated hepatocytes (Hp) with well oriented sinusoids (Sn) within the hepatic lobule.

Stain: H & E

Magnification: x 100

Inference: No histopathological changes.

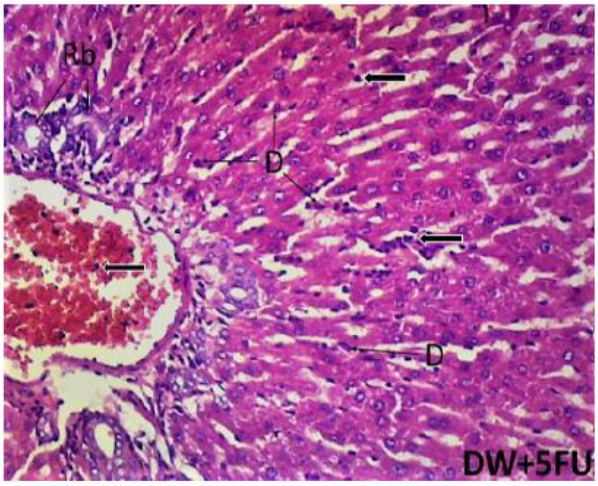

Plate 7: Photomicrograph of the transverse section of the liver tissue of the distilled water + 150 mg/kg bw 5-fluorouracil treated rats (positive control). Abnormal hepatic

tissue with the portal area presenting ruptured bile duct (Rb), widespread presence of organic deposits within the portal vein and the hepatic lobules (black arrow) and degenerating hepatocytes (D) within the hepatic lobules.

Stain: H & E

Magnification: x 100

Inference: Severe histopathological changes.

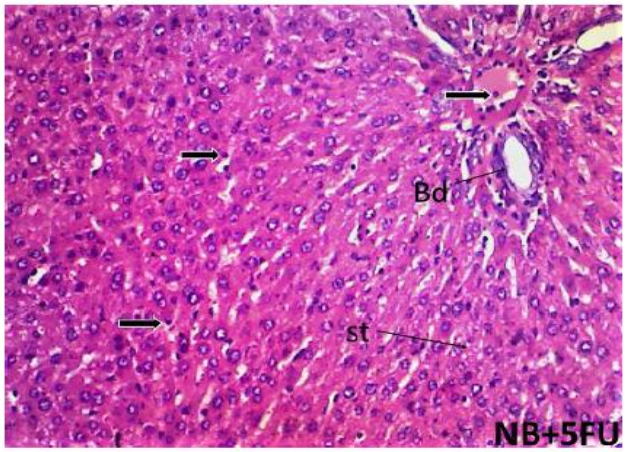

Plate 8: Photomicrograph of the transverse section of the liver tissue of the n-butanol fraction + 150 mg/kg bw 5-fluorouracil treated rats (group 4). Abnormal hepatic tissue with the portal area presenting the bile duct (Bd), scanty presence of organic deposits within the portal vein and the hepatic lobules (black arrow) and micro-vesicular steatosis (st) within the hepatic lobules.

Stain: H & E

Magnification: x 100

Inference: Mild histopathological changes.

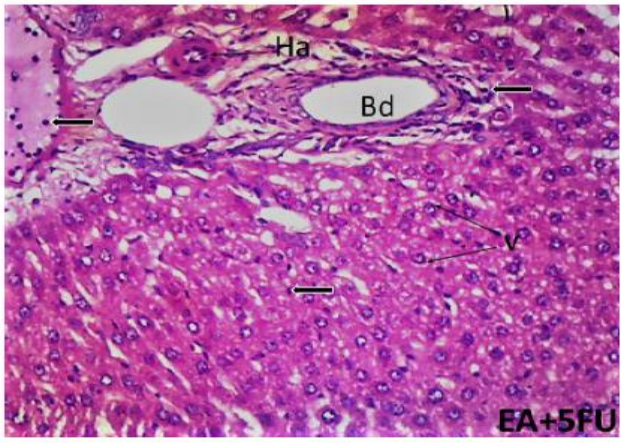

Plate 9: Photomicrograph of the transverse section of the liver tissue of the ethyl-acetate fraction + 150 mg/kg bw 5-fluorouracil treated rats (group 5). Abnormal hepatic tissue

with the portal area presenting the bile duct (Bd), hepatic artery (Ha), scanty presence of organic deposits within the portal vein and the hepatic lobules (Black arrow) and hepatic vacoulations (v) within the hepatic lobules.

Stain: H & E

Magnification: x 100

Inference: Mild histopathological changes

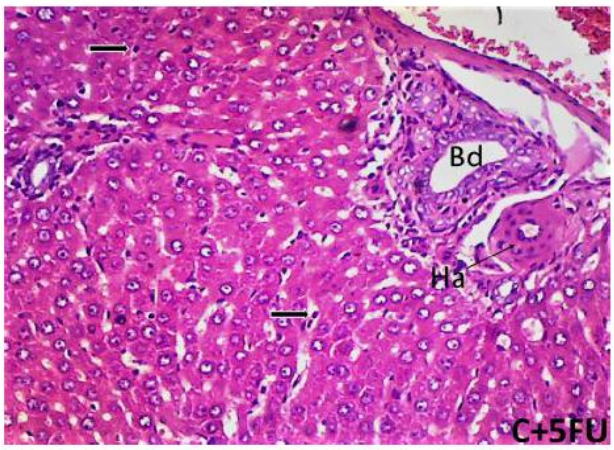

Plate 10: Photomicrograph of the transverse section of the liver tissue of the chloroform fraction + 150 mg/kg bw 5-fluorouracil treated rats (group 6). Abnormal hepatic tissue with the portal area presenting the bile duct (Bd), hepatic artery (Ha), and scanty presence of organic deposits within the hepatic lobules (Black arrow).

Stain: H & E

Magnification: x 100

Inference: No histopathological changes.

Result suggestion: Histopathological assessment of the Liver tissue with the various treatments showed a significant hepatoprotective potency of chloroform fraction of the extract as compared to the control (DW), against the distilled water + 5-fluorouracil treated hepatic tissue.

DISCUSSION

5-fluorouracil (5-FU) is an antimetabolite fluoropyrimidine analogue of the nucleoside pyrimidine that inhibits tumor/cancer formation and progression.[28] 5-FU is widely used systematically for the treatment of solid cancers, including cancers of the gastrointestinal tract, breast, head, neck, pancreas and skin.[1,2] It acts mainly by interfering with DNA synthesis and mRNA synthesis.[3] 5-fluorouracil is metabolized into 5-fluoro-2-deoxyuridine which happens to be an irreversible inhibitor of thymidylate synthase and consequently

thymine synthesis, hindering DNA repair with concomitant cellular toxicityleading to senescence and death.[12] Cardiotoxicity is a well-documented side effect of many drugs for treating cancer including, 5-fluorouracil. Some of the dangerous side effects of 5-FU include; myocardial ischaemia, hypertension, hypotension, left ventricular dysfunction, cardiac arrest, leading to sudden death.[29] The effects of 5-FU cardiovascular toxicity are thought to be due to some mechanism involving the diminished ability of red blood cells to transfer oxygen due to the activation of Rock/Rho pathway.[30,31] The compound 5-fluorouracil, also causes nephrotoxicity and hepatotoxicity.[6] The damage to the kidney or liver directly affects the function of the heart and vascular system.

The aminotransferases are a known marker for liver injury and they are composed of ALT and AST. ALT is localized in the liver, whilst AST is present in both the liver and myocardial tissue. During cardiac malfunction or disease, there is an elevated serum transaminase activity due to anoxic necrosis of the centrilobular liver cells.[32,33] Elevated serum AST/ALT ratio has been shown to be an independent predictor of cardiovascular disease and mortality, this is directly proportional to serum levels of brain natriuretic peptide (BNP). Thus, an elevated AST/ALT ratio is a good indicator for cardiac load or damage, an indicative of AST leakage from the myocardial tissue due to myocardial damage.[34-36] The significant $(p < 0.05)$ increase recorded in serum ALT levels in the positive control (group 2) as compared to the normal control (group 1) in this study is attributable to the 5-FU treatment, confirming the hepatotoxicity effect of the compound. Similarly, the significant $(p < 0.05)$ decrease in serum ALT activity recorded in groups 3, 4 and 5 as compared to the positive control (group 2) is indicative of the effectiveness of the extract fractions treatments in inhibiting the 5-FU induced elevation of serum ALT activity. The group 4(ethyl-acetate fraction) recorded the least serum ALT activity compared to the other extract fractions (N-butanol and chloroform fractions). The significant $(p < 0.05)$ increase in serum AST activity observed in the positive control (group 2) as compared to the normal control (group 1) is attributable to the harmful effect of the 5-FU treatment they received. Furthermore, the significant $(p < 0.05)$ decrease in serum AST activity recorded in groups 3, 4 and 5 suggests that these treatments conferred some degree of protection against the 5-FU-induced toxicity, group 4, producing a stronger effect compared to groups 3 (n-butanol fraction) and 5 (chloroform fraction) in this regard. These results is suggestive of the needs for further molecular studies, establishing the mechanism of action of this plant extract.

The results presented in Table 3 revealed significant $(p < 0.05)$ (increase in serum sodium ion levels in group 2 as against group 1, which is attributable to the 5-FU treatment. The significant (decrease in serum sodium ion concentration recorded in groups 3, 4 and 5 as against group 2 is attributable to the effectiveness of the extract fractions in lowering or resisting any 5-FU induced hypernatremia. Our results suggest that the elevated serum sodium ions observed must have been induced by the 5-fluorouracil treatment and the decreased concentrations observed in the extract fractions treated groups indicated that these treatments effectively resisted the impact of 5-FU on the animals which could have resulted from increased sodium leakages by the kidneys of these experimental animals as compared to the positive control. Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of sodium (Na) (natriuresis) and an accompanying anion, usually chorine (Cl-).[37]

The kidneys are responsible for the maintenance of the body's total potassium content by balancing potassium intake with potassium removal, using the principle of selective reabsorption and tubular secretion.[38] If potassium intake becomes much higher than the kidneys' ability to remove it, or if kidney function declines, hyperkalemia can develop with life threatening consequences. A high potassium level in the bloodstream can also interfere with proper signaling in the heart resulting in fatal complications, which can lead to a heart attack or death if it's not diagnosed and treated.[3] Our results for the serum potassium levels (see Table 4) showed significant $(p < 0.05)$ (increase in serum potassium level in group 2 as against group 1 which is attributable to their exposure to acute dose of 5-FU. Similarly, a significant $(p < 0.05)$ (decline in serum potassium levels was recorded in groups 3, 4 and 5 as against group 2, with group 4 exerting higher potassium lowering potential compared to other fractions treated groups. Diabetes mellitus, heart disease, kidney disease, are associated risks factors for hyperkalemia. Thus, excessive usage ofpotassium-sparing diuretics, *β*-blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, acidosis and high-dietary potassium intake,[39,40] may act to aggravate hyperkalemia mainly due to decreased renal removal of potassium. Hughes-Austin *et al.*[41] in their study revealed that high serum potassium concentration is significantly associated with an elevated risk for all-cause mortality irrespective of the kidney function or other CVD risk factors. Alderman *et al.*[42] reported that low blood potassium (hypokalemia) was linked with increased mortality risk while hyperkalemia was associated with increased risk for cardiovascular events among

those enrolled in the Antihypertensive and Lipid Lowering Treatment Study. Tekin and Celebi[43] in their study observed that a 5-FU-induced elevation in serum nephrotoxicity biomarkers and histopathological changes, was significantly prevented in the groups treated with rutin when compared with rutin untreated group. Results revealed significant increase in serum bicarbonate levels in group 2 as against group 1 (normal control), which further confirms the nephrotoxicity effect of 5-FU treatment in albino rats. Significant $(p < 0.05)$ decrease in serum bicarbonate levels was recorded in groups 3, 4 and 5 as compared to group 2 (positive control) which is attributable to the extract fractions treatment they received.

The significant $(p < 0.05)$ decrease in serum creatinine concentration recorded in the positive control as against the normal control (group 1) suggests that the 5-fluorouracil treatment must have been responsible for this difference due to their nephrotoxicity potential. Furthermore, the significant $(p < 0.05)$ decrease in serum creatinine concentration recordedin the standard control (group 3) and the fractions treated groups (groups 3, 4 and 5) as compared to the positive control (group 2) is indicative of the reno-protective potential of these treatments. The chloroform fraction treatment group (group 5) however, had the least serum creatinine concentrations compared to groups 2, 3, 4 and 5. Besides myocardial infarction, renal dysfunction is another reliable predictor of cardiovascular outcomes and mortality in the general population[44] and heart failure.[45] In fact, chronic kidney disease can be effectively managed by the prevention and treatment of cardiovascular disease.[46]

Increased serum creatinine concentration is associated with increased mortality in hypertensive patients, the elderly, and patients with myocardial infarction or stroke.[47,48] A high serum creatinine concentration outside the normal range is a marker for increased risk of cerebrovascular disease in both normotensive and hypertensive subjects suggesting that impairment of renal function can enhance the chances of suffering from these diseases.[49] A cohort study conducted by Smith *et al*[50] on hospitalized heart failure (HF) patients over a period of 30 months lead more credence to this observation that elevated levels of serum creatinine during heart failure hospitalization were associated with worsened outcomes following hospital discharge bearing, patients whose serum creatinine concentrations were ≥ 0.3 mg/Dl had 60% increased mortality risk. Worsening renal function (WRF) (i.e. small increases in creatinine over a specified period) has been evaluated as an independent prognostic marker in heart failure patients.[47,51] In patients who are hospitalized for

acuteheart failure, WRF not only has been shown to confer additional cardiovascular risk but also, a stronger predictor for death in patients with heart failure, which were due to a high level of creatinine.[50] Many physiologic disturbances such as volume retention are associated with renal dysfunction. Moreover, adverse drug events are more common in persons with renal dysfunction, most especially drugs like renin angiotensin aldosterone (RAA) antagonists, spironolactone and digoxin.[52] Thus, the lower values of creatinine observed in groups 3, 4 and 5 as compared to group 2 in our experiments showed the effectiveness of the fractions of the ethanol leaf extract of *Pterocarpus santalinoides* in protecting the tissues from the cytotoxicity potential of 5-FU.

The result also revealed that the serum urea level of the positive control group (group 2) increased significantly (as against the normal control (group 1), which also indicates that the treatment of the group 2 animals with 150 mg/kg 5-fluorouracil must have been responsible for the observed difference. The urea concentration however decreased significantly in the standard control (group 3) compared to the fractions treatedgroups showing that aspirin possesses the ability to lower blood urea concentration induced by exposure to 5-FU. The ethyl-acetate fraction was more effective in lowering the levels of urea in the blood of the treated animals more than the n-butanol and chloroform fractions treated animals. Urea is a waste product of protein metabolism and blood urea nitrogen (BUN) measures the amount of urea nitrogen in the blood. Urea is formed by the liver and carried by the blood to the kidneys for removal,[53] measuring the amount of urea nitrogen in the blood can be useful test for renal function. However, many factors apart from renal disease can cause BUN alterations, including protein breakdown, hydration status, and liver failure. Persistent high BUN level is associated with increased cardiovascular mortality and bring back to normal levels during hospitalization may improve long-term clinical outcomes.[54] A cohort study conducted by Lan *et al.*[55] on older people also revealed the association between elevated BUN and the incidence of cardiovascular diseases as this study linked a higher BUN concentration with increased occurrence of heart failure. Thus, test forthe concentration of BUN in patient sera is a reliable biomarker of CVD, this was as previously reported by Lan *et al.*[55] where BUN level higher (>13.51 mg/dl) was associated with increased occurrence of heart failure in older females in a Chinese community.

Evaluation of the histopathology of the liver and kidney tissue (plate 1-10), also showed a cardio-protective potential of the extract fractions, especially by the chloroform fraction as

compared to other groups; there was no histopathological changes in the liver and renaltissues of the normal control group. The positive control liver and kidney tissues had severe alterations in their histo-architecture. But the histo-architecture of the animals treated with fractions of ethanol leaf extract of *Pterocarpus santalllinoides* only showed very mild changes in the tissue architecture of their liver and kidneys which confirms their renoprotective and hepatoprotective potential against 5-FU-induced nephrotoxicity and hepatotoxicity in albino rats. The histopathological result also revealed that the chloroform fraction possessed higher hepatoprotective potential compared to the n-butanol and ethyl-acetate fractions.

CONCLUSION

From the results of the serological tests and histopathological evaluations, this study has revealed the renoprotective and hepato-protective potential of n-butanol, ethyl-acetate and chloroform fractions of ethanol leaf extract of *Pterocarpus santalinoides* against 5-FU-induced hepatotoxicity and nephrotoxicity.

Conflict of interests

Authors declare that there are no conflicts of interests associated with this publication.

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