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EVALUATION OF NEPHRO-PROTECTIVE ACTIVITIES OF BETAGLUCAN ENRICHED EXTRACT OF PLEUROTUS TUBER-REGIUM SCLEROTIA (PLEUROTECEAE)

C. B. Ugwu*[1], E. S. Iloh[2], M. C. Ugwu[3] and C. S. Nworu[4]

[1]Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria.

[2]Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria.

[3]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

[4]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria.

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***Corresponding Author**
C. B. Ugwu
Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria.

ABSTRACT

Renal diseases are increasing worldwide with attendant high morbidity and debility. There is no known cure for kidney damage as there is still no licensed drugs that satisfactorily restore lost kidney functions. Nephroprotection was assessed by determining the serum concentrations of creatinine, urea, potassium, chloride and sodium in kidney tissue homogenates after a repeated high dose of gentamicin and paraquat. The protective effects of the extract on the hepatic and nephrotic architecture were evaluated through histopathogical studies. Preliminary phytochemical studies showed that extract is rich in saponins and carbohydrates. Beta glucan enriched fraction of *Pleurotus tuberreguim* sclerotia administration (100-250 mg/kg, p.o) significantly reduced the levels of serum creatinine, blood urea, chloride and sodium in gentamicin treated rats when compared to the animals treated with gentamicin alone. Rats in control group showed normal glomerular and tubular histology whereas gentamicin treated group was found to cause distorted tubular shape, cellular infiltration of the tubules (tubulitis), glomerular and blood vessel congestion and also resulted in the presence of inflammatory cells in kidney sections. Similar result was also found in the paraquat treated rats. The study suggest that, in addition to its high nutritive value, *P.*

tuberregium has nephroprotective effects and its consumption could protect the kidneys from oxidative damage caused by drugs and other environmental toxicants.

KEYWORDS: Gentamicin, Paraquat, Pleurotus tuberreguim, Beta glucan.

INTRODUCTION

Pleurotus tuberregium (Osu) is a tropical edible mushroom that produces the edible sclerotium or underground tuber as well as mushroom (Oranusi et al., 2014). Pleurotus tuber-regium is a tropical sclerotial mushroom which has been gaining interest in Nigeria. Being sclerotial, the mushroom produces a sclerotium, or underground tuber, as well as a mushroom (Adedokun & Thomas 2014). Both the sclerotium and the mushroom are edible. In Nigeria P. tuber-regium is used as both food and medicine. Beta glucan comprises a group of β-D-glucose polysaccharides naturally occurring in the cell walls of cereals, yeast, bacteria and fungi, with variable physicochemical properties dependent on source. Kidney failure describes a medical condition in which the kidneys fail to adequately filter toxins and waste products from the blood. Kidney damage is said to occur when glomerular filtration rate (GFR) is <60 ml/min/L (Okwuonu, *et al*., 2017). The two forms are acute and chronic; a number of other diseases or health problems may cause either form of renal failure to occur. Gentamicin is one of the leading causes of drug-induced nephrotoxicity. This has led to the use of gentamicin-nephrotoxicity induction in animal models (Nworu *et al*., 2014).

Paraquat with a chemical name of 1, 1'- dimethyl-4, 4'-bipyridium and chemical formula $C_{12}H_{14}N_2Cl_2$ is a toxic chemical that is widely used as a herbicide (Raghu *et al*., 2013). It is eliminated mainly by the kidney and acute renal failure has been recognized as a complication of paraquat poisoning, with reports of both oliguric and non-oliguric cases. Distortion in renal tubules occurs when exposed to paraquat and paraquat can induce congestion of kidney blood vessels and degeneration of glomeruli (Gu et al., 2016).

MATERIALS AND METHODS

Study area

This study was conducted in Nnamdi Azikiwe University, Department of pharmacology and toxicology Awka. Awka is located in south eastern Nigeria.

Study materials

Pleurotus tuberregium (Pleuroteceae) was sourced from Eke Awka market. The identification and authentication was done by a Taxonomist, Mr Alfred Ozioko of International Centre for Ethno medicine and drug development, Nsukka.

Wistar rats and mice were used for this study. The animals were obtained from the animal house of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka. The animals were housed in groups of 4, in standard cages, at room temperature (25±3°C), with 12 h dark/12 h light cycles, food and water *adlibitum*. Twelve hours prior to the experiments they were transferred to the laboratory and given only water *ad libitum*.

3.2.2. Extraction of beta glucan enriched extracts

The β-glucan rich polysaccharide fraction of *Pleurotus tuberregium*(PTR)was extracted traditionally from 1 kg portion of the powdered sclerotia of *Pleurotus tuberregium*. The mushroom (1kg) was extracted with 96 % ethanol (11.25 L) at 80^{o}C for 3 h to remove low molecular mass compounds. The air dried residue was extracted with 2.5 L of hot distilled water (98^{o}C) for 4 h. Beta glucan was precipitated from PTR with the addition of 1.5 volumes of absolute ethanol overnight at 4^{o}C. The precipitate was centrifuged and the supernatant recovered after centrifugation freeze dried to obtain a beta glucan rich fraction.

Determination of the median lethal dose (LD_{50}) of the beta glucan enriched extract of *pleurotus tuberregium* sclerotia

Acute toxic effect of the extract was determined by estimating the median lethal dose (LD_{50}) using the method of Lorke (1983).

NEPHRO-PROTECTIVE ASSAYS

Induction of nephrotoxicity using gentamicin

The gentamicin-induced nephrotoxicity in rats was performed according to the method of Bag & Mumtaz (2013) with some modification**.** Twenty five Wistar albino rats of either sex were assigned to five groups (n= 5) and were given the following treatments:

Group I: Rats in thisgroup were injected with normal saline,intraperitoneally and served as control. **Group II**: Rats in this group were injected with gentamicin (100 mg/kg, i.p) for seven consecutive days. **Group III**: Rats in this group were injected with gentamicin (100 mg/kg,i.p) and administered beta glucan (50 mg/kg p.o) for seven consecutive days**. Group IV**: Rats in this group were injected with gentamicin (100 mg/kg, i.p) and administered beta

glucan (100 mg/ kg, p.o) for seven consecutive days. Group **V**: Rats in this group received gentamicin (100 mg/kg, i.p) and administered beta glucan (250 mg/ kg, p.o) for seven consecutive days.

Induction of nephrotoxicity using paraquat

The effect of the extract on nephrotoxicity induced by paraquat was performed by Sharifi-Rigi, A., Heidarian, E. (2019).Twenty five Wistar albino rats of either sex were assigned to five groups (n= 5).**Group I**: Rats in this group received normal saline,intraperitoneally and served as control. **Group II**: Rats in this group were injected with paraquat (25mg/kg, i.p) for 14 days. **Group III**: Rats inthis group were injected with paraquat (25mg/kg, i.p) and administered beta glucan (50 mg/kg, p.o) for 14 consecutive days. **Group IV**: Rats inthis group were injected with paraquat (25mg/kg, i.p) and administered beta glucan (100 mg/ kg, p.o) for 14 consecutive days. **Group V**: Rats in this group were injected with paraquat (25mg/kg) and administered beta glucan (250mg/kg) for 14 days.

After 24 hours of the last dosing of the nephrotoxic agents, they were anaesthetised with diethyl ether and 2-3 ml of blood samples was collected via the retro-orbital plexus. Serum was separated by centrifugation and the concentrations of urea, creatinine and electrolytes such and (potassium, sodium and chloride) in the sera samples were measured using standard methods. The Rats were sacrificed; kidneys excised, rinsed clean in saline and preserved in 10 % formalin for histopathological study conducted in Department of Veterinary Medicine, University of Nigeria, Nsukka.

3.2.7. Statistical Analysis

To demonstrate statistical significance of data, One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test was performed using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). Generally, differences between test and control treatments are considered significant at $P < 0.05$.

RESULTS

Extraction

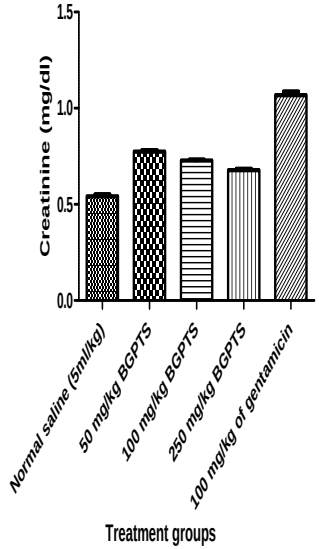
The extraction of the mushroom plant, *Pleurotus tuberregium* (PTR) 1000g yielded 17.12 g (1.71 % w/w) of the β-D glucan rich polysaccharide fraction.

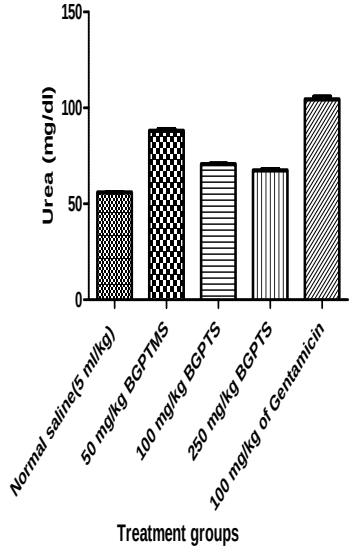
Result of Phytochemical Analysis of Extract

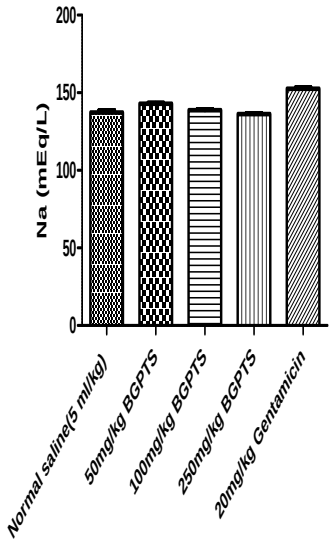
The extracts were found to be abundantly rich in saponins and carbohydrates. Protein and alkaloids moderately present while tannins, terpenoids and cardiac glycosides were absent.

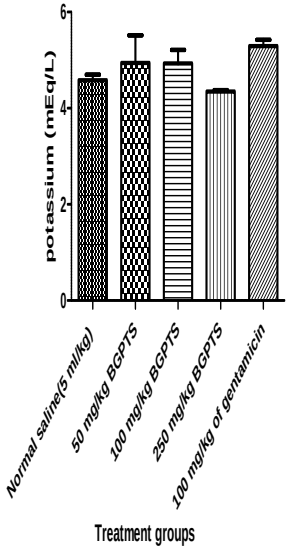
Acute Toxicity Test

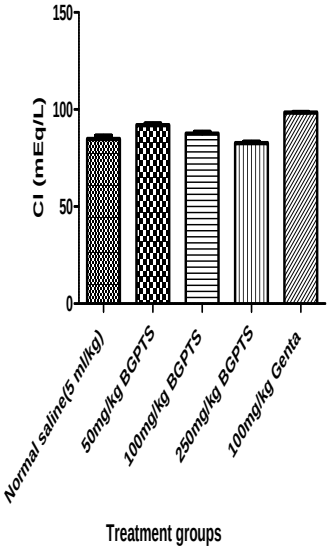
In the acute toxicity tests in mice, beta-glucan enriched extract of*Pleurotus tuberregium* sclerotia (βGPTS) administered orally at doses up to 5 g/kg body weight did not cause deaths in the mice after 24 h observation period in the two phases of the tests. There was generally the absence of symptoms of toxicity. The LD_{50} is therefore deemed to be greater than 5000mg/kg and it's safe when administered orally for all practical purposes.

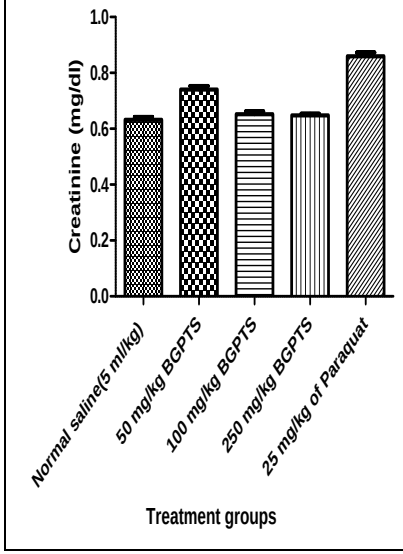

Creatinine (mg/dl)
1.5
1.0
0.5
0.0
Normal saline (5ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
100 mg/kg of gentamicin
Treatment groups

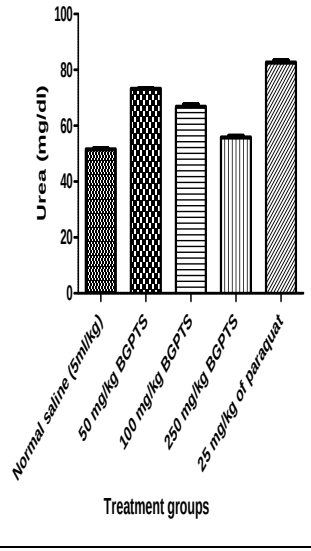

Urea (mg/dl)
150
100
50
0
Normal saline(5 ml/kg)
50 mg/kg BGPTMS
100 mg/kg BGPTS
250 mg/kg BGPTS
100 mg/kg of Gentamicin
Treatment groups

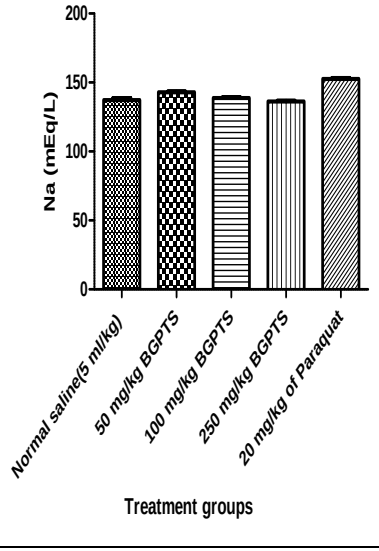

Na (mEq/L)
200
150
100
50
0
Normal saline(5 ml/kg)
50mg/kg BGPTS
100mg/kg BGPTS
250mg/kg BGPTS
20mg/kg Gentamicin
Treatment groups

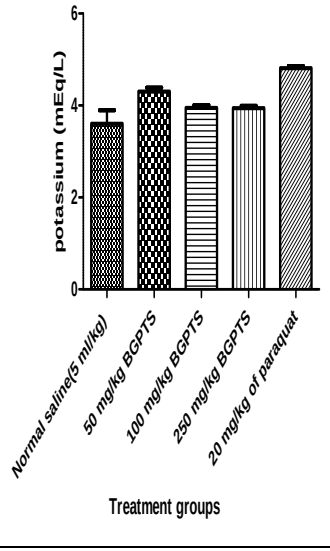

potassium (mEq/L)
6
4
2
0
Normal saline(5 ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
100 mg/kg of gentamicin
Treatment groups

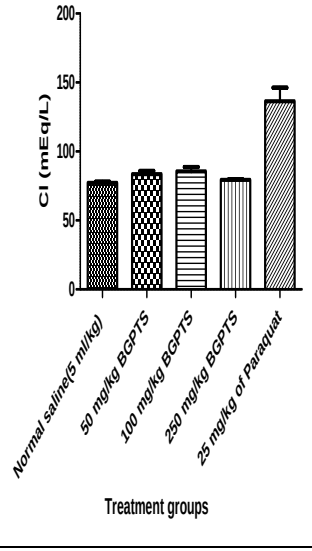

Cl (mEq/L)
150
100
50
0
Normal saline(5 ml/kg)
50mg/kg BGPTS
100mg/kg BGPTS
250mg/kg BGPTS
100mg/kg Genta
Treatment groups


Creatinine (mg/dl)
1.0
0.8
0.6
0.4
0.2
0.0
Normal saline(5 ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
25 mg/kg of Paraquat
Treatment groups


Urea (mg/dl)
100
80
60
40
20
0
Normal saline (5ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
25 mg/kg of paraquat
Treatment groups


Na (mEq/L)
200
150
100
50
0
Normal saline(5 ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
20 mg/kg of Paraquat
Treatment groups


potassium (mEq/L)
6
4
2
0
Normal saline(5 ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
20 mg/kg of paraquat
Treatment groups


Cl (mEq/L)
200
150
100
50
0
Normal saline(5 ml/kg)
50 mg/kg BGPTS
100 mg/kg BGPTS
250 mg/kg BGPTS
25 mg/kg of Paraquat
Treatment groups

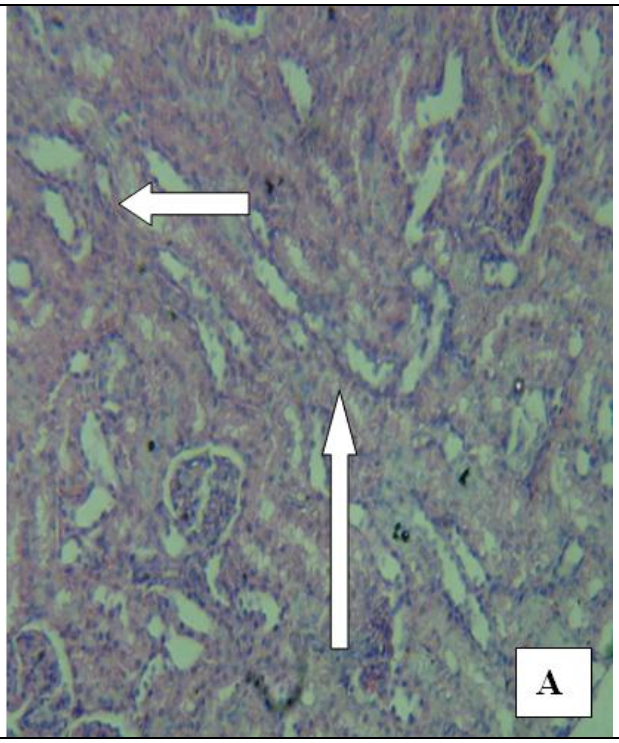


Plate A: (Group 1-genta) section of kidney tissue shows normal kidney histology Bowman's capsules (Short arrow) and tubules (Long arrow) are normal (x100, H&E)

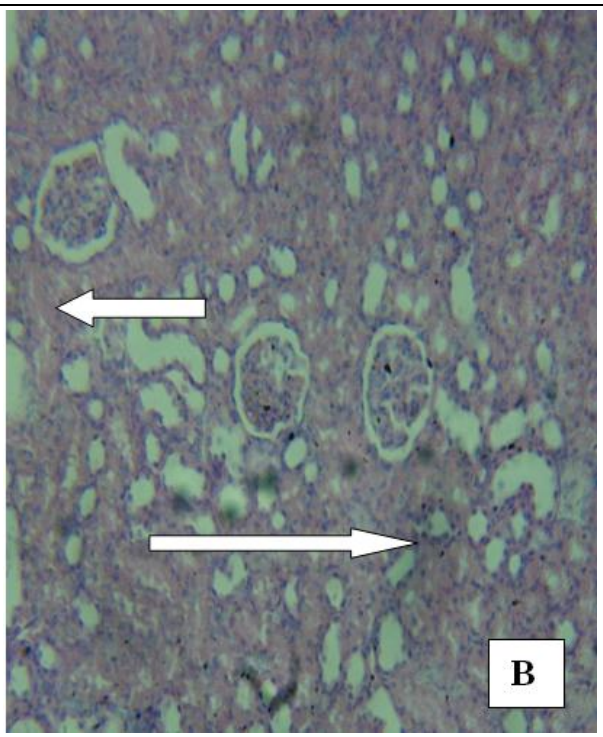


Plate A:Plate B: (Group 2-genta) section of kidney tissue shows normal histology of Bowman's (Short arrow) capsules and tubules but with diffuse areas of necrosis (Long arrow) (x100, H&E).

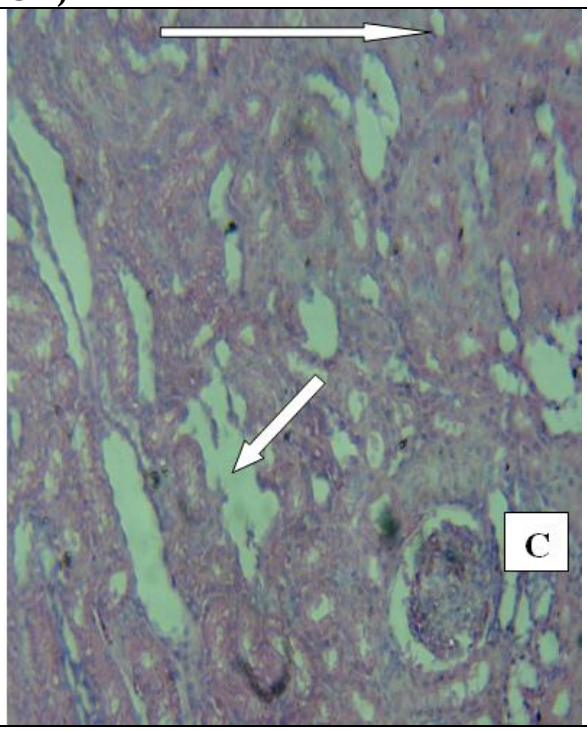


Plate C: (Group 3-genta) section of kidney tissue shows normal Bowman's capsules (Short arrow) and tubules architecture with early sign of fibrosis (Long arrow) (x100, H&E).

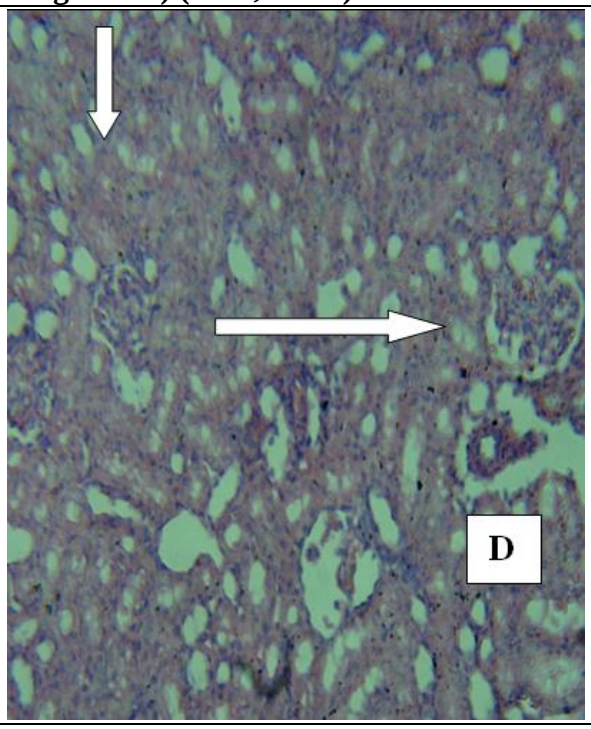


Plate D: (Group 4-genta) Section of kidney tissue shows distortion of glomerular (Long arrow head) and tubular (Short arrow head) architecture with mild presence of inflammatory exudates (I) (x100, H&E).

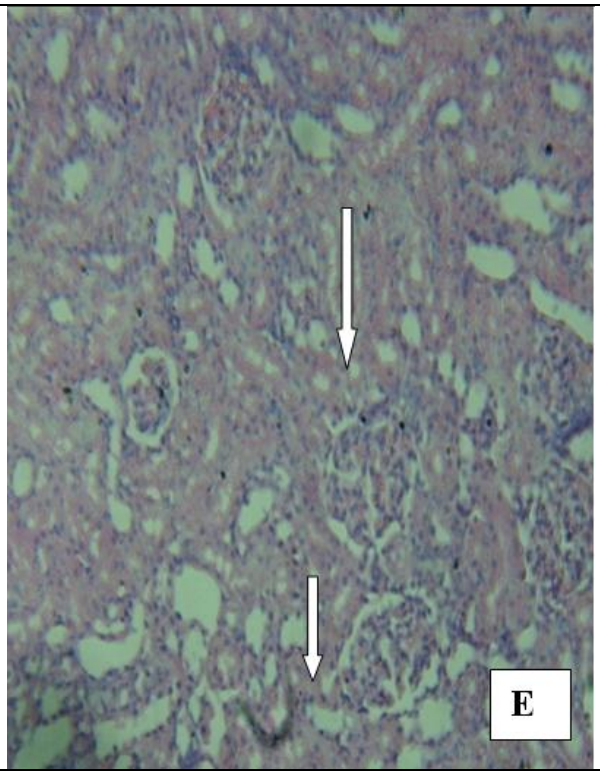


Plate E: (Group 5-gentamicin) section of kidney tissue shows normal Bowman's capsules (Short arrow) and tubules architecture but with unremarkable presence of lymphocytes (Long arrow) (x100, H&E)

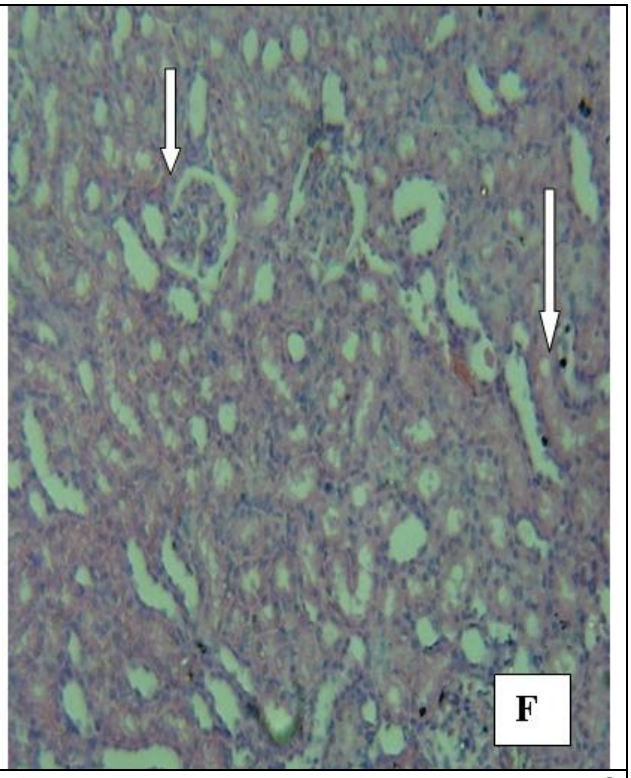


Plate F: (Group 1-Paraquat) section of kidney tissue shows normal kidney histology. Bowman's capsules (Short arrow) and tubules (Long arrow) are normal (x100, H&E),

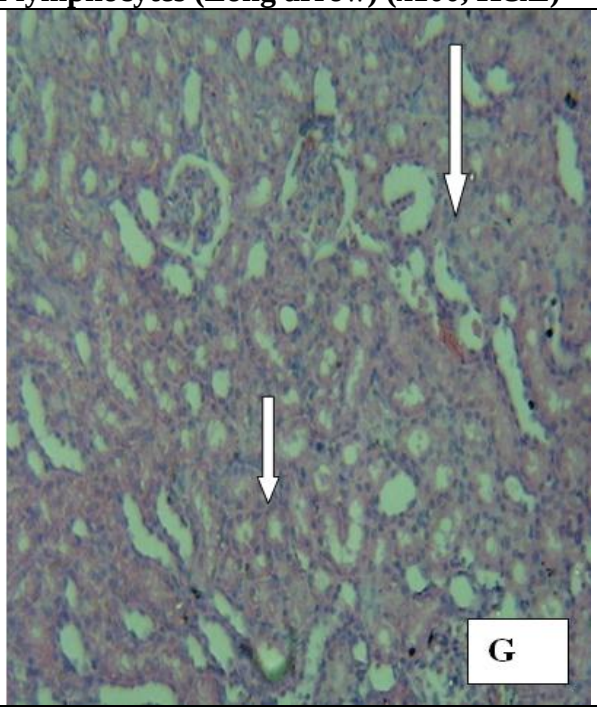


Plate G: (GROUP 2-Paraquat) section of kidney tissue shows mildly distorted Bowman's capsules (Short arrow) and tubules architecture with early sign of fibrosis(Long arrow) (x100, H&E)

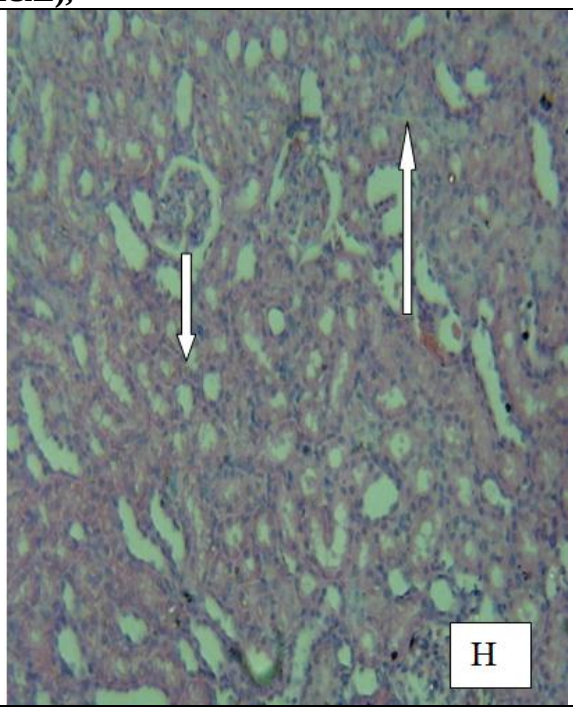


Plate H: (GROUP 3-paraquat) section of kidney tissue shows normal tubules architecture with early sign of fibrosis (Long arrow) (x100, H&E)

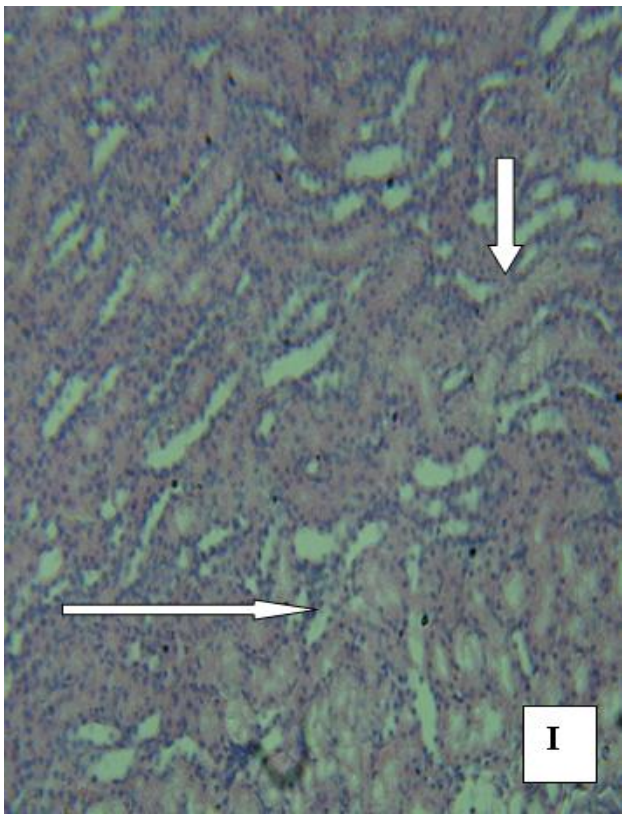


Plate I: (GROUP 4-paraquat) section of kidney tissue shows normal Bowman's capsules (Short arrow) and tubules architecture but with unremarkable cellular hyperplasia (Long arrow) (x100, H&E).

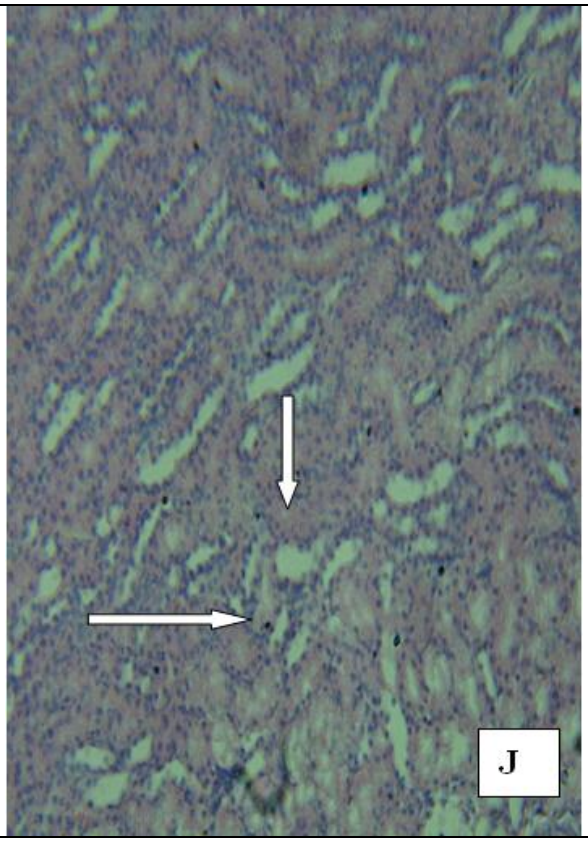


Plate J: (GROUP 5- paraquat) section of kidney tissue shows normal kidney histology. Bowman's' capsules (Short arrow) and tubules (Long arrow) are normal (x100, H&E).

Paraquat was first used in agriculture for the first time in 1962 (Han et al., 2014). Paraquat has been recorded to cause nephrotoxicity through oxidative stress, inflammation, apoptosis, and direct damage to renal tubules (Wei et al., 2014). Paraquat exerts its herbicide action by preventing reduction of $NADP^+$ to NADPH during photosynthesis (Atashpour et al., 2017). Renal toxicity attributive to aminoglycosides is of major clinical concern because of their widespread use (Shalini et al., 2016). Reactive oxygen species (ROS) have been implicated in the pathogenesis of gentimicin-induced kidney injury. This results in severe tissue damage and degeneration. Cr and urea are considered the most important indicators of kidney function. (Shalini et al., 2016). The results of the present study revealed that the beta glucan enriched fractions of PTR schlerotia possesses significant nephroprotective effect against gentamicin and paraquat -induced nephrotoxicity. Preliminary phytochemical studies showed high presence of carbohydrates and saponins and little presence of protein and tannins. Most of the bioactive polysaccharides previously isolated from mushroom are water-soluble (1→3)- β-D-glucans with (1→ 6)-β-linked side branches (Schmid *et al*., 2001). Thus, for the

present study, the β-D glucan- rich polysaccharide fraction of *P. tuberregium* sclerotia was extracted and subjected to evaluation for nephroprotective activities. The significantly high blood urea in the gentamicin alone group suggests kidney injury. The administration of BGPTS prevented gentamicin-induced nephrotoxicity, significantly ($P < 0.05$) reducing urea accumulation in the BGPTS 100mg/kg (mid dose) and BGPTS 250mg/kg (high dose) groups. The effect of ROS in the body is usually suppressed by antioxidant enzyme systems. The suppression of gentimicin-induced nephrotoxicity by the extract may have resulted from the anti-oxidant properties (Okokon *et al.*, 2011, Nworu et al., 2014). They attributed the activities to antioxidant potentials via mopping up trichloromethyl free radicals as they are formed**,** thereby limiting their damaging effects on hepatocytes.there was equaly a significant reduction in the chloride and soduim ions measure between the treatment group and the negative control group. No significant reduction was observed while measuring for potassium ion.

In this study, we observed a significant increase in serum urea and Creatinine in the group that received only paraquat compared to the control/treatment groups which is consistent with previous studies.

Histopathological examination of rat kidney sections of gentamicin alone(Plate A) group showed impaired renal morphology throughout, with severe tubular degeneration, presence of inflammatory exudates, fibrosis and areas of necrosis. Kidneys from animals concurrently treated with 50 mg/kg BGPTS showed moderate degenerative changes in the glomeruli and tubules, while animals treated with 100 mg/kg BGPTS showed significant nephroprotection with minimal degenerative changes in the glomeruli and tubules. The high dose 250 mg/kg BGPTS provided the highest protection, with near normal appearance of glomeruli, interstitium, and tubules in the kidney, indicating significant nephroprotection (Plate B,C,D,E,G,H,I,J).

CONCLUSION AND RECOMMENDATIONS

The findings reported in the study indicate that the oral administration of enriched fraction of beta glucan from the sclerotia of *Pleurotus tuberreguim* exhibited significant nephroprotective effects against organ damages induced by various toxicants. We equally recommend that more concerted efforts are still needed for the isolation, characterization and biological evaluation for the active principles of the extract.

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