NEPHROTOXICITY AND OXIDATIVE STRESS RESPONSE OF WISTAR RATS FED WITH PLANT EXTRACTS FROM BIOREMEDIATED CRUDE OIL IMPACTED SOIL.

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Abstract:
Nigerian Bonny-light crude oils known as light crude oils with relative water soluble hydrocarbons and a high percentage of lipophilic crude oil components are known to be potential lipophilic toxins or xenobiotics with potential electron uncoupling and energy inhibiting abilities. This endangers biological membranes, the kidney and other vital organs on exposure to crude oil. This hence necessitates prompt search for viable clean up methods for crude oil pollution, which remediation using selected agrowastes formulation was evaluated. This study investigated the nephrotoxicity and oxidative stress markers of wistar albino rats fed with *Telfaria ocidentalis* and *Talinum triangulare* leaf extracts harvested from crude oil polluted agricultural soil remediated using this selected agrowastes formulation. Results obtained revealed Catalase (CAT) activity with maximum value (19.961±0.19) in $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), and minimum (13.60±0.55) in group To_{UPS}. MDA has minimum value (1.57±0.12) in group $ToTt_{UPS}$, with maximum value (2.15±0.11) obtained from $ToTt_{BRS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare,* from best remediated soil). Values for SOD, GSH and GPX were not significantly different from the control. Renal markers' assay showed ranges for Urea (5.45±0.01-2.85±0.03U/l), Creatinine (70.50±0.29-60.0±0.29U/l) Potassium (4.60±0.00-3.50±0.23 g/l), Sodium (133.00±0.29-136.5±0.29g/l) chloride (99.5±0.2392.5±0.29g/l), and Bicarbonate (27.5±0.29-20.5±0.06g/l). Obtained values were shown not to be significantly different from the control group. This hence entails that farm produce from remediated soils using these selected agrowastes could have no probable acute toxicity effect on the kidney as no oxidative stress condition was also induced.

Keywords: Renal Toxicity, Bioremediation, Crude oil pollution, Oxidative stress markers, Agro-waste, Biostimulation. Toxicology.

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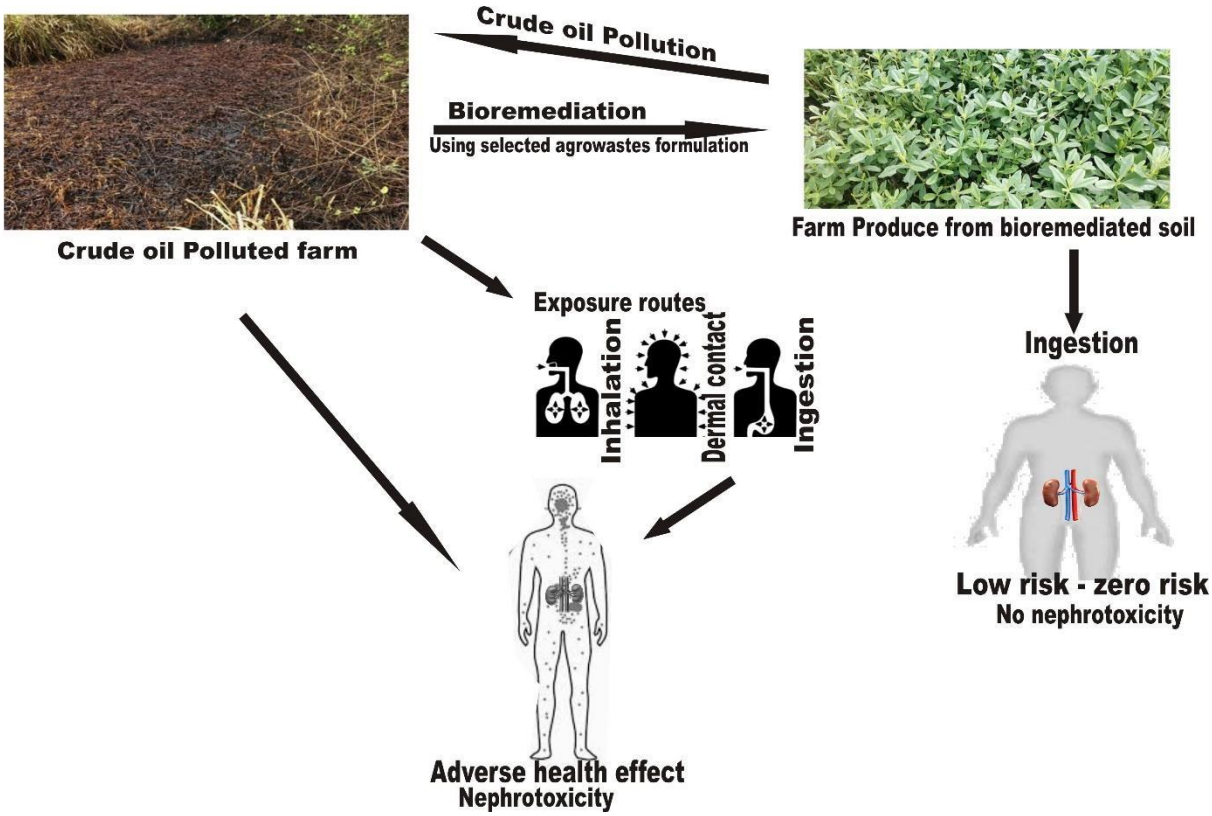

INTRODUCTION

Crude oil despite its high economic value, has become a night mare to exposed individuals due to reports of pronounced toxicity effects on exposed individuals [1]. Its exposure to humans has become of high public health concern as cases of pipeline vandalization, illegal refining, oil bunkering, fallen tankers, oil seepages and spillages are on increase especially in the Niger Delta region of Nigeria. Exposure to crude oil, crude oil fractions and other petroleum products has been shown to be deleterious, with documented reports of adverse health effects from previous studies revealing, nephrotoxicity [2,3], hepatotoxicity [4,5], cardiotoxicity [6,7] and hematoxicity [8] effects. Azeez *et al*. [9] also reported elevated oxidative stress markers in rats exposed to petroleum hydrocarbons. Reports have shown that crude oil and its products are exposed to human via dermal contacts, ingestion and inhalation thereby increasing probable exposure level and subsequent higher toxicity effects. Some of the major environmental contaminants and toxicant seen in crude oil include; heavy metals and polycyclic aromatic hydrocarbons, some of these compounds have been shown to settle within our environment (in air, land, water, dusts etc) each time there is crude oil spillage. Olua *et al*. [10] reported presence of some of these contaminants in dusts around public primary school in Rivers state which is situated within the Niger Delta region of Nigeria. This study also revealed that pupils and adults within these locations are prone to probable carcinogenic and non-carcinogenic health risks on exposure to the level of these pollutant seen in the sampled dusts [10] which occurred from industrial and domestic use of petroleum products, spillages or even during exploration and production processes of crude oil and its products.

These risks posed by exposure to spillage of petroleum products hence led to several studies on possible clean up technologies [11,12], to curtail the adverse health effect of crude oil on spillage in the environment. Several remediation techniques have been utilized in the clean-up [11,12,13] of crude oil pollution in our environment. These methods though successful does not give zero tolerance to the removal of this pollutant. This has also raised concerns as farmers step into such reclaimed polluted sites to continue with their agricultural practices without due knowledge of probable health risks consumption of farm produce from these reclaimed sites may pose. Investigations has been made on the hepatotoxicity of farm produce harvested from crude oil polluted sited remediated using selected agrowastes formulations [14]. This report revealed that the agrowastes formulations was able to facilitate bioremediation process to the degree that the observed pollutant levels in the soil was could not have any acute toxicity effect on the liver. Olua *et al*. [15] also reported that vegetables from such reclaimed site,

remediated using selected agrowastes formulations were devoid of acute toxicity effect on hematological parameters of wistar Albino rats [15]. However, no reports on the nephrotoxicity effects and oxidative stress markers of harvested products from such recently remediated agricultural soil, remediated using same selected agrowastes formulations. This study investigated the nephrotoxicity and oxidative stress markers of wistar albino rats fed with *Telfaria ocidentalis* and *Talinum triangulare* leaf extracts harvested from crude oil polluted agricultural soil remediated using selected agrowastes formulation.

2. MATERIALS AND METHODS

Sampling and Sample preparation

Telfaria occidentalis Seedlings and *Talinum triangulare* stems were obtained, planted and harvested using methods of Olua *et al*. [14]. The harvested leaves were air dried and ground into fine particles from which an aqueous extract was obtained using rotary extraction method.

Experimental Set up

Wistar albino rats (n=35) of 9-12 weeks old of comparable weight was used for this study. They were grouped into seven (n=5) and were acclimatized for seven days within which they were fed with growers mash (Top Feed Ltd.) and water *ad libitum* only. The experimental animals were subsequently fed and administered with *Telfaria occidentalis* and *Talinum triangulare* aqueous leaf extracts for two weeks acute toxicity study as follows; control (given feed and water only), To_{UPS},(given 1000mgkg^{-1} of *T. occidentalis*, from unpolluted soil), Tt_{UPS} (given 1000mgkg^{-1} of *Talinum triangulare*, from unpolluted soil), $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), To_{BRS} (given 1000mgkg^{-1} of *T. occidentalis*, from best remediated soil), Tt_{BRSs} (given 1000mgkg^{-1} of *Talinum triangulare*, from best remediated soil), $ToTt_{BRS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from best remediated soil). Blood samples from the experimental animals were collected via jugular puncture for biochemical analysis, while kidney samples were obtained for histological studies.

Biochemical assay

Lipid peroxidation was determined by assaying malondialdehyde (MDA) concentration using Ohkawa *et al*., [16] method. Superoxide dismutase and catalase activities were estimated by the methods of Misra and Fridovich, [17] and Clairborne, [18] respectively. Reduced glutathione (GSH) content was determined by Sedlak and Lindsay, [19] methods while glutathione peroxidase activity was measured using Olinescu and Nita, [20]. All the electrolytes (Na, K, Cl, HCO_3) analysed were estimated using automated machine (Mindray BS-800 Chemistry Analyzer).

Data/Statistical Analysis

Values obtained (n=3) were statistically analysed using SPSS Version 23.0 to evaluate the one way analysis of variance (Anova) @ $p \leq 0.05$. Results were presented as mean ± standard error of mean.

3 Results/Discussions

The Nigerian Bonny-light crude oils are known as light crude oils, as aromatic hydrocarbons accounts for about forty five percent (45%) of total hydrocarbons. These hydrocarbons are known to be relatively soluble in water [21], with potentially higher probable adverse toxicity effects. A high percentage of crude oil components is also known to be lipophilic in nature making biological membranes an easy target site for adverse health effects as lipophilic toxins or xenobiotics may could be potential electron uncouplers and energy inhibitors [22]. The kidney no doubt could be a potential target that could suffer considerable damage resulting to several abnormal clinical indication of renal diseases.

However, the results of the effect of the leave extract of sampled plants (harvested from crude oil polluted sites bioremediated using selected agrowastes formulations) on oxidative stress markers and Renal markers of Wistar albino rats (assayed to ascertain the acute renal toxicity effect of such plants from the remediated soil and dangers of early farming on crude oil polluted soil remediated using these selected agrowastes formulation) as shown in Tables 1 and 2 respectively, revealed Catalase (CAT) activity with maximum value (19.961±0.19) in $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), and minimum (13.60±0.55) in group To_{UPS}. MDA has minimum value (1.57±0.0.12) in group $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), with maximum value obtained from $ToTt_{BRS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare*, from best remediated soil) (2.15±0.11). Maximum value (38.44±0.19) was obtained for SOD in group Tt_{UPS}, with minimum value (25.37±0.83) seen in Control group. The result revealed maximum GSH values (786.52±0.29) in control Group while minimum

values (652.02±0.53) obtained in group $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), GPX was shown to be maximum (31.92±0.32) in group To_{UPS} and minimum (20.24±0.22) in control group. Obtained results however showed that the leaf extracts has no significant impact on oxidative stress markers when compared to the control, in Wistar albino rats fed with sampled plants' leaf extracts.

Table 1 Oxidative Stress Markers of Wistar Rats Fed with Plant Extracts

GROUP	**CAT(mg/ml)**	**MDA**(µmol/L)	**SOD(mg/ml)**	**GSH**(µmol/L)	**GPX**(µmol/L)
CONTROL	17.52±0.29^{a}	2.07±0.04^{a}	25.37±0.83^{a}	786.52±0.29^{a}	20.24±0.22^{a}
ToUPS	13.60±0.55^{a}	1.62±0.04^{a}	27.19±0.88^{a}	729.65±0.27^{a}	31.92±0.32^{a}
TtUPS	17.96±0.93^{a}	1.94±0.10^{a}	38.44±0.19^{a}	692.88±0.87^{b}	21.65±0.18^{a}
$ToTt_{UPS}$	19.61±0.19^{a}	1.57±0.0.12^{a}	26.49±0.23^{a}	652.02±0.53^{a}	24.34±0.90^{a}
ToBRS	16.24±0.74^{a}	1.90±0.12^{a}	34.48±0.30^{a}	775.38±0.49^{a}	26.98±0.58^{a}
TtBRS	16.35±0.33^{a}	2.09±0.78^{a}	31.47±0.51^{a}	781.60±0.68^{a}	21.77±0.79^{a}
$ToTt_{BRS}$	16.45±0.54^{a}	2.15±0.11^{a}	29.90±0.77^{a}	760.51±0.22^{a}	22.90±0.77^{a}

Values represents Mean±SEM, mean in the same column with same superscript alphabets are not significantly different. To_{BRS} = *T. occidentalis* planted on soil Polluted and treated with Rice Husk + Egg Shell + Plantain Peels formulation, Tt_{BRS} = *Talinum triangulare* planted on on soil Polluted and treated with Rice Husk + Egg Shell + Plantain Peels formulation, To_{UPS} = *T. occidentalis* planted on Unpolluted control soil, Tt_{UPS} = *Talinum triangulare* planted on Unpolluted control soil

Oxidative stress is a condition that arises when there is state of imbalance between production of free radicals and the antioxidant defense system that can trigger lipid perioxidation and damages to organs and other cellular components like the biomolecules [23,24]. The antioxidant enzymes (SOD, CAT, GPx and GSH) protect the cellular environment from the destructive effect of reactive oxygen species (ROS) which might be triggered by activities of toxic substances like Pb, Cd, PAHs etc. The results of the oxidative Stress Markers of Wistar Rats exposed to plants harvested from crude oil impacted soil remediated using agrowastes formulations revealed that the extracts did not induce oxidative damage in the treated wistar rats as no significant elevation of MDA (1.57±0.0.12 to 2.15±0.11 umol/ml) was observed at $p<0.05$. The SOD, CAT, GSH and GPx activities also recorded values which were not significantly different from the control groups. SOD has been reported as the first line of defence system against reactive oxygen species which scavenges superoxide radicals. SOD catalyses the dismutation of O_2^- to H_2O_2 and O_2.
Therefore, increased in SOD concentration is an indication of an induced oxidative stress which the body responded to by releasing the scavenging enzymes however no significant increase in SOD was observed in this study. Obtained results showed that the leaf extracts have no significant impact on oxidative stress when compared to the control, in the exposed Wistar albino rats.
The result has maximum Urea levels (5.45±0.01 U/l) obtained from Group Tt_{UPS} (given 1000mgkg^{-1} each of *Talinum triangulare* from unpolluted soil). while minimum values (2.85±0.03 U/l) was obtained in control group. Creatinine values was shown to be maximum (70.50±0.29 U/l) in group Tt_{UPS} (given 1000mgkg^{-1} each of *Talinum triangulare* from unpolluted soil). and minimum (60.0±0.29) in control group. Maximum value (4.60±0.00 g/l) was obtained for Potassium in group To_{UPS}, (given 1000mgkg^{-1} of *T. occidentalis*, from unpolluted soil), with minimum value (3.50±0.23 g/l) seen in group To_{BRS} (given 1000mgkg^{-1} of *T. occidentalis*, from best remediated soil). Sodium has minimum value (133.00±0.29 g/l) in group To_{UPS} (given 1000mgkg^{-1} of *Telfaria occidentalis* from unpolluted soil). with maximum value (136.5±0.29 g/l) obtained from Group $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil). The obtained results also revealed maximum chloride levels (99.5±0.23 g/l) in Group To_{UPS} ,(given 1000mgkg^{-1} of *T. occidentalis*, from unpolluted soil) while minimum values (92.5±0.29 g/l) was obtained in group $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil). Bicarbonate values was shown to be maximum (27.5±0.29g/l) in control group and minimum (20.5±0.06 g/l) in group Tt_{UPS} (given 1000mgkg^{-1} of *Talinum triangulare* from unpolluted soil). These values were shown not to be significantly different from the control group.

Table 2 Renal Markers of Wistar Rats Fed with Plant Extracts

GROUP	UREA (U/l)	CREA (U/l)	K (g/l)	Na (g/l)	Cl (g/l)	HCO_3 (g/l)
CONTROL	2.85±0.03[a]	60.0±0.29[a]	3.90±0.06 [a]	134.22±0.12 [a]	96.0±0.58[a]	25.0±0.29[a]
To_{UPS}	3.70±0.12[a]	65.50±0.29[a]	4.60±0.00 [a]	133.00±0.29 [a]	99.5±0.23 [a]	27.5±0.29[a]
TtUPS	5.45±0.01[a]	70.50±0.29[a]	3.75±0.03 [a]	134.5±0.29 [a]	95.0±0.58 [a]	20.5±0.06[a]
$ToTt_{UPS}$	5.10±0.06[a]	69.50±0.29[a]	3.50±0.23 [a]	136.5±0.29 [a]	92.5±0.29 [a]	20.5±0.29[a]
ToBRS	3.90±0.00[a]	61.0±0.58[a]	4.35±0.20 [a]	134.41±0.23 [a]	96.5±0.12 [a]	25.5±0.23[a]
TtBRS	2.95±0.03[a]	62.0±1.16[a]	4.15±0.03 [a]	135.05±0.03 [a]	96.5±0.17 [a]	24.0±0.23[a]
$ToTt_{BRS}$	3.90±0.12[a]	61.47±0.12[a]	3.95±0.02 [a]	134.78±0.01 [a]	98.0±0.58 [a]	23.0±0.03[a]

Values represents Mean±SEM, mean in the same column with same superscript alphabets are not significantly different. To_{BRS} = *T. occidentalis* planted on soil Polluted and treated with Rice Husk + Egg Shell + Plantain Peels formulation, Tt_{BRS} = *Talinum triangulare* planted on on soil Polluted and treated with Rice Husk + Egg Shell + Plantain Peels formulation, To_{UPS} = *T. occidentalis* planted on Unpolluted control soil, Tt_{UPS} = *Talinum triangulare* planted on Unpolluted control soil.

Photomicrographs of the Kidney, Magnification X400 H&E

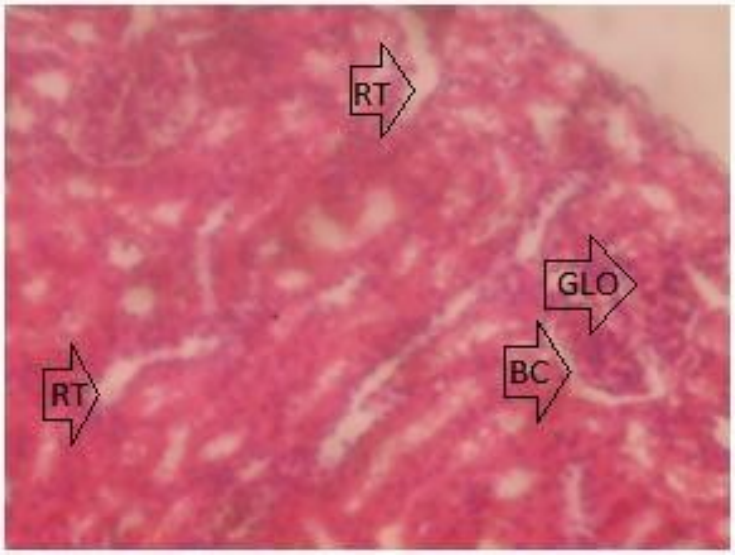

Plate 1: Control group. Histologically normal kidney; features includes; glomeruli containing tuft (glomerular messengial cells, capillaries and messengial matrix). Bowman capsular space (BC) surrounding the glomerulla tufts. Renal tubules (RT) lined by simple epithelial cells.

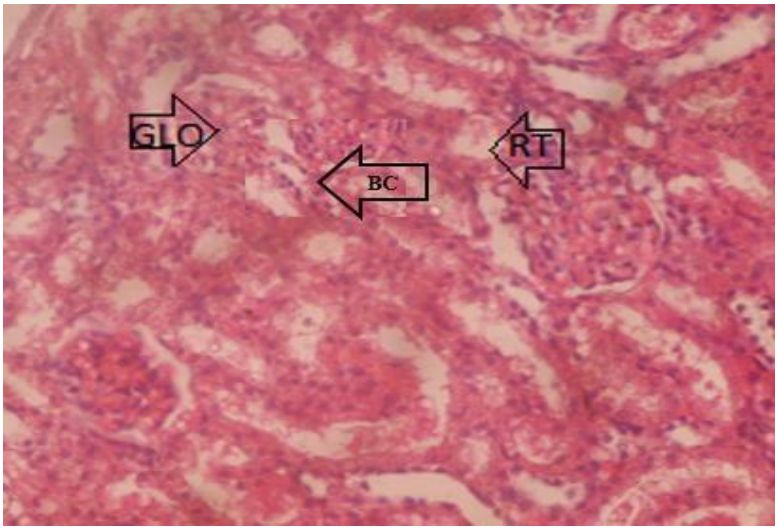

Plate 2: Group To_{UPS} Histologically normal kidney; features includes; glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells.

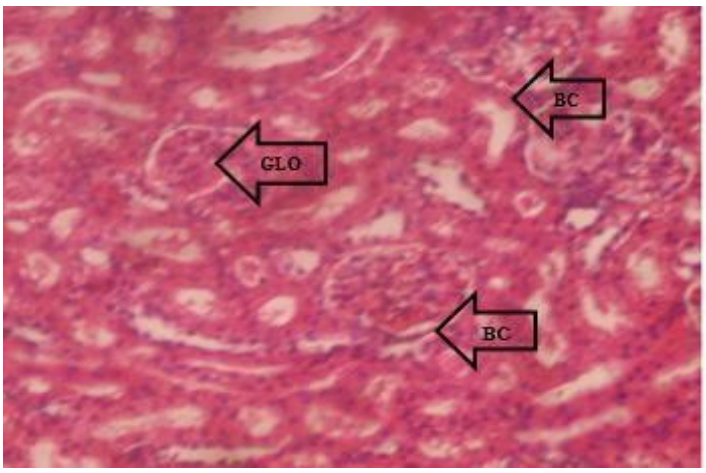

Plate 3: Group Tt_{UPS}. Histologically normal kidney; features includes; glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells.

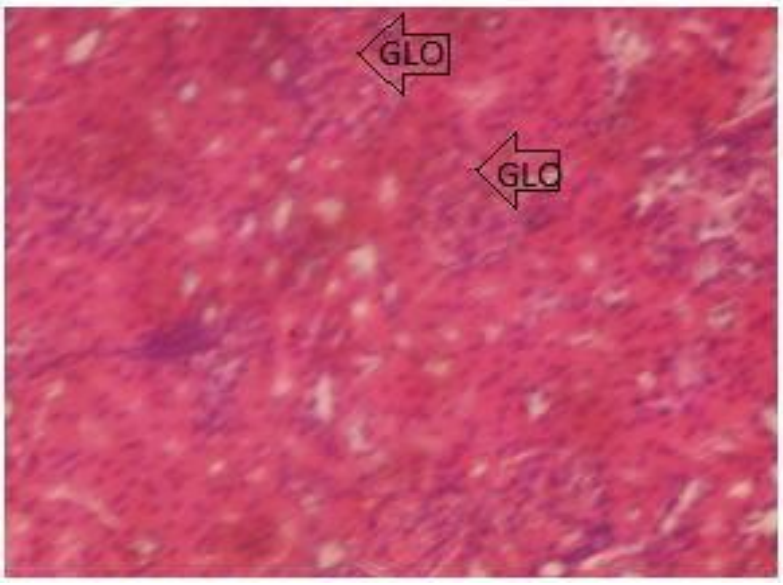

Plate 4: Group $ToTt_{UPS}$ (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), Histologically normal; features includes; Glomeruli (GLO) and Bowman capsular space (BC) arrowed

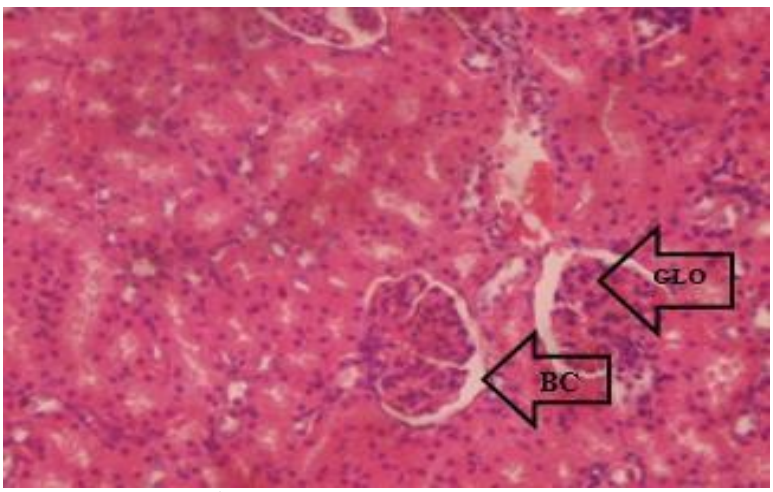

Plate 5: Group To_{BRS} (given 1000mgkg^{-1} of *T. occidentalis*, from best remediated soil). Histologically normal kidney; features includes; glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and

messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells.

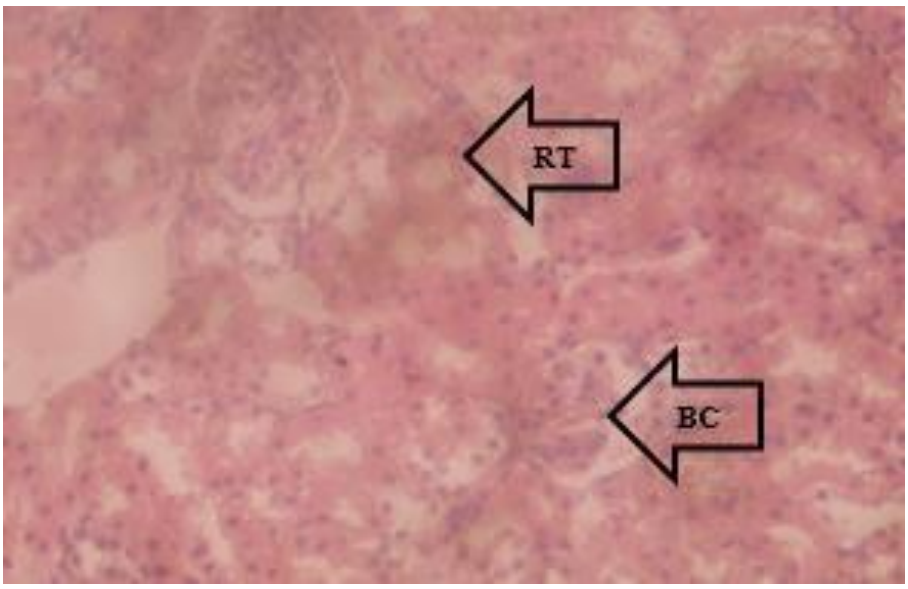

Plate 6: Group Tt_{BRS}. Histologically normal kidney; features includes; glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells.

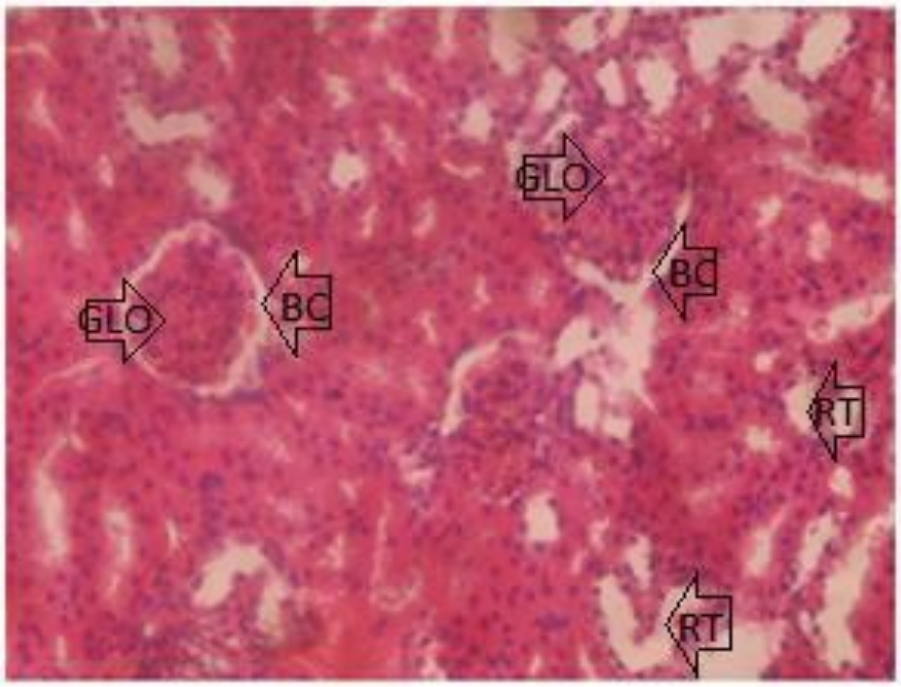

Plate 7: Group $ToTt_{BRS}$ Histologically normal kidney; features includes; glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells.

Obtained results however showed that *Talinum triangulare* (To_{BRS}) and *T. occidentalis* (Tt_{BRS}) aqueous leaf extracts has no significant impact on renal markers obtained from Wistar albino rats fed with these extracts as compared to the control at 95% degree of confidence.

Values obtained showed that the kidneys are devoid of any sort of inflammation. Electrolytes (Na^+,K^+, Cl^-, and HCO_3^-) are chemical substances or mineral element required for the functioning of organs in the body and mediate vital biochemical or metabolic processes in the body [25]. Renal failure is usually complicated by increased serum electrolytes (Na^+,K^+, Mg^{2+}) and decrease in some electrolytes [26].

The kidney's efficient integrity boils down to maintaining homeostasis via its role in eliminating metabolic wastes plus moderation of intracellular fluid volume, acid-base balance and electrolyte composition [3]. This consequently suggests that whatever injury resulting on body metabolism possibly will be indicative of poisonous insult to the kidney [27].

The results in this study revealed potent renal tissues as indicated in the kidney photomicrographs 1 to 7, with Plate 1: Control group. Showing histologically normal kidney; with its glomeruli containing tuft (glomerular messengial cells, capillaries and messengial matrix) and Bowman capsular space (BC) surrounding the glomerulla tufts. While Renal tubules (RT) were lined by simple epithelial cells. Plate 2, (Group To_{UPS}), 3 (Group Tt_{UPS}) and 4 (Group $ToTt_{UPS}$) (given 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil), showed histologically normal kidney;with glomeruli containing glomerular tuft (glomerular messengial cells, capillaries and messengial matrix). They also show patent Bowman capsular space (BC) surrounding the glomerullar tufts with patent Renal tubules (RT) lined by simple epithelial cells. Plate 5: Group To_{BRS} (given 1000mgkg^{-1} of *T. occidentalis,* from best remediated soil), 6: Group Tt_{BRS}) and 7: Group $ToTt_{BRS}$ revealed histologically normal kidney; with features as follows; glomeruli containing glomerular tuft
(glomerular messengial cells, capillaries and messengial matrix). Patent Bowman capsular space (BC) surrounding the glomerullar tufts. Patent Renal tubules (RT) lined by simple epithelial cells. The finding from this study is suggestive that *T. occidentalis* and *T. triangulare* harvested from these bioremediated site is devoid of any acute toxicity effect that could affect the morphology and function of the kidney. It could likewise be inferred that the effect of the extract on the kidney rather aided the potency of renal tissues as no significant difference in the electrolyte concentrations was observed rather slight increase and improved serum concentration of Na^+,K^+, Cl^-, and HCO_3^- was seen which suggests that the extracts could have enhanced and stimulated the release of serum electrolytes. Similar report by Kirdpon *et al.,* (2006) was observed on electrolytes change in children after *Aloe vera* consumption.

CONCLUSION

This study is suggestive that, the remediation technique applied using the selected agro-waste formulation as biostimulant was efficient, as farm produce from the remediated soil, did not show any acute toxicity effect on the kidney on ingestion of 1000mgkg^{-1} aqueous leaf extracts of *T. occidentalis* and *T. triangullare.* This could however indicate probable potentials for safe agricultural/ farming practices on such crude oil polluted soil remediated using this selected agrowastes formulations.

Conflicts of interest The authors declare no conflict of interest.

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