ISSN No:-2456-2165

Hepatotoxicity of Farm Produce from Recently Remediated Crude Oil Polluted Site Amended Using Formulated Agrowastes

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Abstract:- Crude oil and petroleum products' environmental contamination has become a major public health concern, especially as no 100% clean up technology is known and locals keep cultivating food crops around recovered farm land barely immediately without due knowledge of toxic nature of harvested farm produce. This study examined acute hepatotoxicity effect of farm produce from a recently remediated crude oil polluted site amended using formulated agro-wastes. Thirty five (35) wistar albino rats were placed in seven groups with five rats each in this study. Group A received feed and water only, groups B and C received 1000mgkg^{-1} of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil respectively, groups E and F received 1000mgkg^{-1} of *T. occidentalis* and *T. triangulare* from polluted but amended soil respectively, while groups D and G received 500mgkg^{-1} each of *T. occidentalis* and *T. triangulare* from unpolluted and polluted but amended soil respectively for two weeks. Liver markers assay and liver histopathological evaluations were done using standard laboratory methods on sampled Wistar rats. Results revealed absence of hepatotoxicity from groups E to G, with AST (9.50±0.12 to 17.50±0.12 IU/l), ALT (10.00±1.44 to 14.5±0.23IU/l), ALP (136.50±0.12 to 118.50±0.12 IU/l), total protein (61.87±0.01 to 78.52±0.06 g/l) and Total Bilirubin (6.00±0.58 to 9.46±0.06 µmol/l) at levels not significantly different from the control @ $p \leq 0.05$ for groups E to G. This is suggestive that the sampled plants were devoid of probable acute hepatotoxicity effects on these experimental animals. This finding therefore indicates that the agro-waste formulation used for the bioremediation could be a green pathway in bioremediation technology.

Keywords:- *Hepatotoxicity, Talinum triangulare, Telfaria Occidentalis, Wistar Rats, Crude Oil Pollution.*

I. INTRODUCTION

The Liver remains a vital organ known for its detoxification abilities, clearing and filtering the blood of assorted poisonous and or toxic chemicals or metabolites emanating from the digestive tract. This makes it highly prone to damages and diseases. (Lu *et al.,* 2018). The role of the liver in general metabolic processes makes liver function test essential in several routine tests. This also calls for more care in handling the liver as virtually anything (especially toxicants) that enters the body through oral routes finds their way to the liver from which its either detoxified or it gets to the entire circulatory system where it causes more harm to cells, tissues and body organs. Liver damage is often linked to synthetic and metabolic dysfunctions (Srivastava, Shivanandappa, 2010). Petroleum product has been shown to be amongst watchlist of hepatic toxins. Crude oil has been reported to induce hepato-toxicity (Odinga and Ngelale, 2017). Odinga and Ngelale (2017) reported increased level of liver marker enzymes in wistar rats exposed to bonny light crude oil. Crude oil and other petroleum products have found their way into the body through several means such as dermal contact, inhalation, direct oral ingestion (by some locals who uses it for therapeutic purposes, repelling of witches etc (Orisakwe *et al.,*2004; Udoele, 1997; Dede *et al*., 2002; Arikpo *et al*., 2009 and Odinga and Ngelale, 2017) and indirect oral ingestion (from food crops harvested from crude oil contaminated site).

Environmental Contamination from oil spillages is no longer new globally especially at the Niger Delta region of Nigeria where indigents now witness such frequently either from equipment failure of industrial gadgets, oil leakages from tanks and pipelines and spillages from vandalized pipelines etc. this has also led to development of several clean up mechanisms (Dados *et al*. 2015 Nwaichi *et al.,* 2015 and Chioma *et al.,* 2017), one of which involves formulations from agrowastes. Concerns are now evident as no hundred percent clean up mechanism have been achieved and most contaminants seen in crude oil and other petroleum products are not readily metabolised in the body and as such tends to bioaccumulate and become toxic to vital body organs such as

ISSN No:-2456-2165

the liver. Reports have shown that some of these contaminants (such as heavy metals and Polycyclic Aromatic Hydrocarbons) could be more toxic through oral exposure routes. (Olua *et a*l., 2018). The predominant means of such exposure could be from agro products from contaminated soil. However, with the various remediation techniques developed and counting, the need to ascertain the safety of food products from remediated crude oil polluted farm lands becomes expedient. As this could help in reduction of probable health risks from such products. This study hence seek to determine the hepatotoxicity of extracts of *Tallinum triangulare* and *Telfaria occidentalis* obtained from crude oil polluted soil, bioremediated using selected agrowaste formulation on wistar albino rats. The sampled plants are known for their wide consumption by most households within the Niger Delta region.

II. MATERIALS AND METHODS

Thirty five wistar Albino rats of weight 120-170g purchased from the animal house of the Faculty of Basic medical sciences University of Port Harcourt, Port Harcourt Rivers State Nigeria, grouped into seven (7) with five (5) wistar albino rats each was used for this acute hepatotoxicity study. They were fed with growers mash (Top Feed Ltd.) and water *ad libitum through out the* 7 days acclimatization session.

The harvested *T. triangulare* and *T. occidentalis*) leaves were air dried and ground. The rotary extraction method was used to obtain the aqueous extract of *Talinum triangulare* and *Telfaria occidentalis.*

The extracts were administered to the wistar rats as shown in table 1. For a period of two (2) weeks.

Table 1 Experimental Animals Grouping

Groups	Treatment
A	Received distilled water and normal feed only
B	Received 1000mgkg^{-1} of *T. occidentalis*, obtained from unpolluted soil, water and feed only
C	Received 1000mgkg^{-1} of *T. triangulare*, obtained from unpolluted soil, water and feed only
D	Received 500 mgkg^{-1} each of *T. triangulare* and *T. occidentalis*, obtained from unpolluted soil, water and feed only
E	Received 1000mgkg^{-1} of *T. occidentalis*, obtained from agro waste best remediated soil, water and feed only
F	Received 1000mgkg^{-1} of *Talinum triangulare*, obtained from agro waste best remediated soil, water and feed only
G	Received 500mgkg^{-1} of *Talinum triangulare* and *T. occidentalis* each, obtained from agro waste best remediated soil, water and feed only

The wistar albino rats were sacrificed on day 14 of the experiment. Blood samples were obtained through jugular puncture and kept in lithium heparin bottle for laboratory liver marker enzymes evaluation. The weight of the experimental wistar albino rats were also measured before commencement of administration and prior to sacrifice of sampled rats. The liver marker enzymes activity was assayed using Randox methods. The liver was also harvested for histological study.

Data/Statistical Analysis

Statistical data analysis was carried out using SPSS Version 23.0 to evaluate the one way analysis of variance (Anova) @ $p \leq 0.05$.

III. RESULTS AND DISCUSSIONS

The Liver markers tells much on normal physiological condition of the liver they are significant in accessing general metabolic processes. The results of the effect of aqueous leaf extract of *T. triangulare* and *T. occidentalis* (planted and harvested from a bioremediated soil regime after 90 days treatment using formulated agro-wastes) on Hepatic markers of wistar rats is as shown in table 2. The result revealed AST (9.50±0.12 to 17.50±0.12), ALT (10.00±1.44 to 14.5±0.23), ALP (136.50±0.12 to 118.50±0.12) activities which were not statistically significantly different @ $p \leq 0.05$ except for values obtained in group C and D for AST activity, which was shown to be significantly different from the control group . Total Protein and Total Bilirubin levels were also shown not to be significantly different compared to control group depicting no inflammation of the hepatocytes on consumption of the harvested plant leaves grown on a crude oil polluted agricultural soil bioremediated using selected agrowaste formulation. This finding in suggestive that the soil status is devoid of contaminants at levels which might lead to acute toxicity effects. The results of the liver markers study was also in line with the liver photomicrographs which showed normal hepatocytes in wistar rats fed with plant extracts harvested from bioremediated soil regime as shown in plates 5 to 7.

ISSN No:-2456-2165

Table 2 Markers of Wistar Rats Fed with Plant Extracts

GROUP	AST(IU/l)	ALT(IU/l)	ALP(IU/l)	T.P(g/l)	TB (µmol/l)
A	9.50±0.12[a]	10.00±1.44[a]	118.50±0.12[a]	69.59±0.12[a]	6.00±0.58[a]
B	10.85±0.01[a]	11.30±0.12[a]	136.50±0.12[a]	78.52±0.06[a]	9.46±0.06[a]
C	17.50±0.12[b]	14.5±0.23[a]	131.75±0.12[a]	61.87±0.01[a]	7.15±0.01[a]
D	13.00±1.16[b]	12.5±0.17[a]	123.82±0.06[a]	66.34±0.17[a]	8.17±0.06[a]
E	10.34±0.12[a]	12.5±0.06[a]	120.45±0.23[a]	65.57±0.01[a]	8.23±0.12[a]
F	11.50±0.23[a]	11.65±0.01[a]	119.83±0.02[a]	63.56±0.02[a]	8.10±0.06[a]
G	10.28±0.12[a]	12.95±0.02[a]	122.52±0.01[a]	67.96±0.02[a]	7.11±0.01[a]

Values represents Mean±SEM, mean in the same column with same superscript alphabets are not significantly different. Where AST - (aspartate aminotransferase), ALT - Alanine Aminotransferase, ALP - Alkaline Phosphatase Activity, T.P – Total Protein, TB- Total Bilirubin.

This is an indication of no hepatocellular damage and no liver dysfunction as elevated levels of liver function markers are often found in blood circulation when the integrity of the liver is compromised (Green and Flamm, 2002). Bilirubin is the break down product of red blood cells and its serum concentration is used for the assessment of proper liver function. As shown in Table 2, the bilirubin level however did not show any significant difference across the wistar rats fed with the harvested plant extracts. This study has revealed that plants harvested from this remediated soil are devoid of toxicity effects on principal biochemical markers.

Table 3 Animal Grouping and Weight

Group	Average Weight (g)		
	Initial	Final	Net
A	122.33	130.67	8.33
B	112.67	127.33	14.67
C	116.67	128.67	12
D	124	137	13
E	142	167.67	25.67
F	127.33	149.8	22.47
G	133.33	171	37.67

However, Plate 1 (Control Group (A)), showed a histologically normal liver; with normal hepatocytes (Hep) and sinusoids containing normal capillaries and Kupffer cells while Plate 2 (Group B) to 4 (group D) showed histologically slightly distorted liver; revealing patent Central Vein and Hepatocytes with different stages of steatosis.

Photomicrographs of the Liver, Magnification X400 H&E

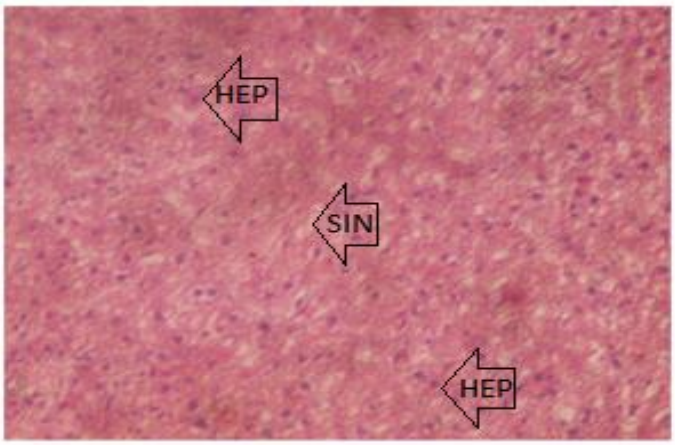

Plate 1: Control Group (A). Histologically normal liver; features includes; normal hepatocytes (Hep), sinusoids containing normal capillaries and Kupffer cells.

ISSN No:-2456-2165

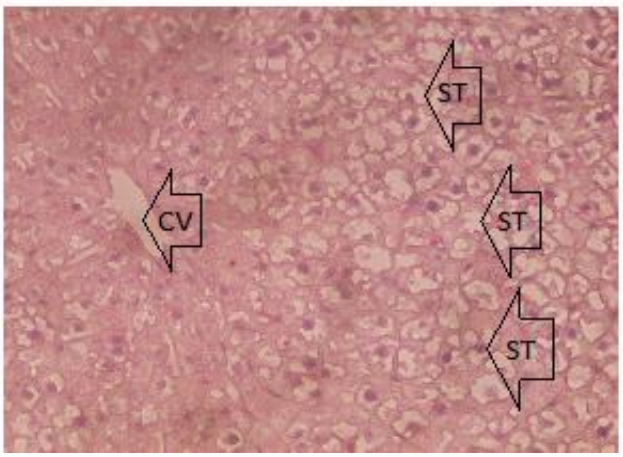

Plate 2: Group B. Histologically distorted liver; features includes; patent CV, Hepatocytes with different stages of steatosis (S.T)

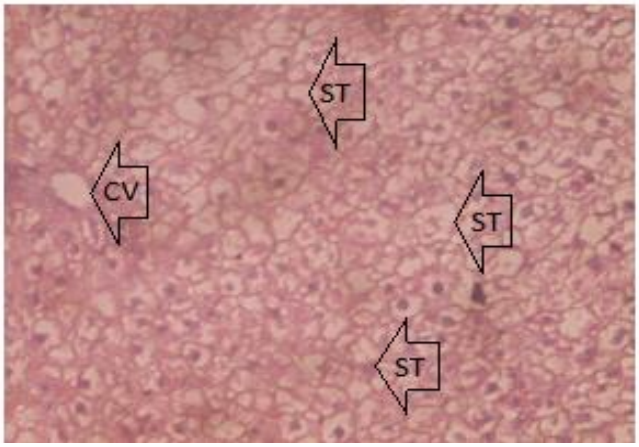

Plate 3: Group C. Histologically distorted liver; features includes; patent CV, Hepatocytes with different stages of steatosis (S.T).(Microvesicular, macrovesicular & council marls' body

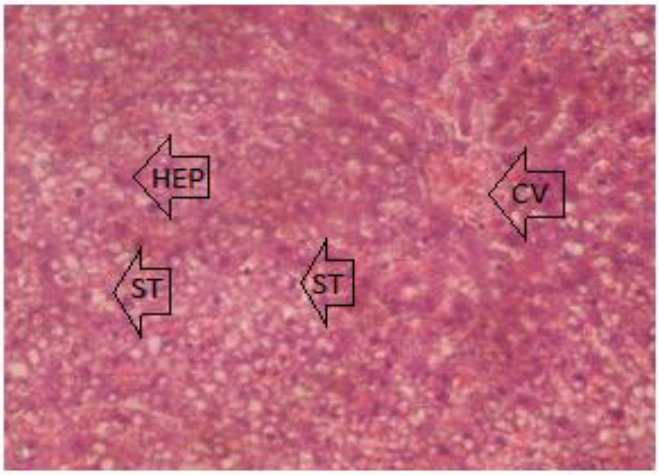

Plate 4: Group D. Histologically mildly distorted liver; features includes; partly normal hepatocytes (Hep), partly areas of hepatocytic steatosis (S.T). sinusoids containing Kupffer cells & normal capillaries, congested central vein.

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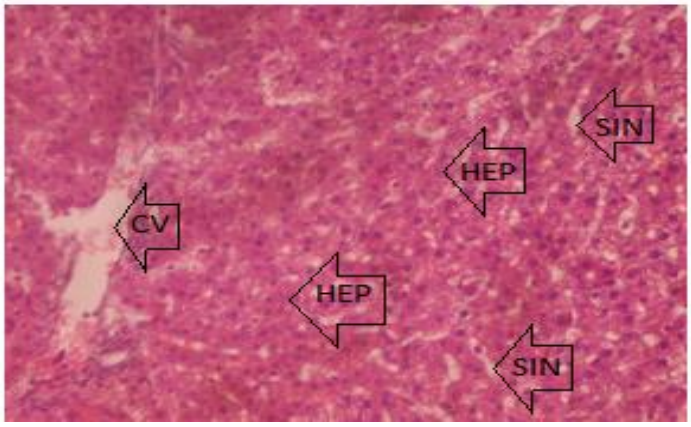

Plate 5: Group E. Histologically normal liver; features includes; normal hepatocytes (Hep), patent cv, sinusoids containing normal capillaries and Kupffer cells.

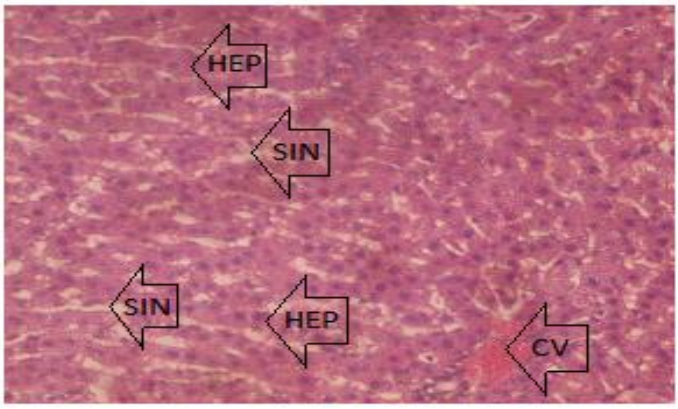

Plate 6: Group F. Histologically normal liver; features includes; normal hepatocytes (Hep), sinusoids containing normal capillaries & von Kupffer cells congested central vein.

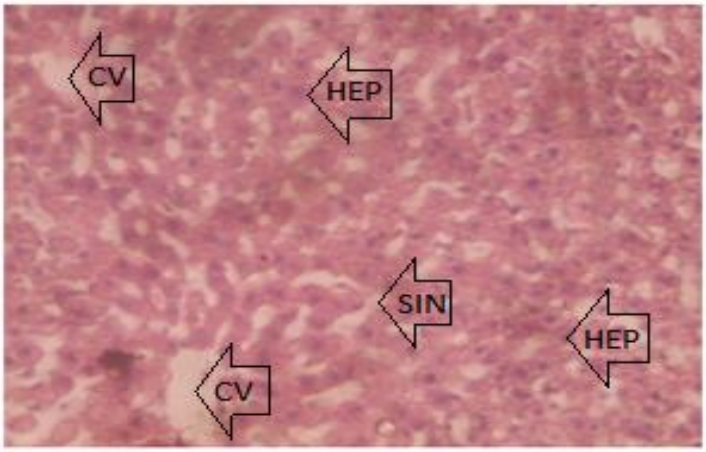

Plate 7 Group G Histologically normal liver; features include; normal hepatocytes (Hep), sinusoids containing normal capillaries patent central vein.

The results on body weight as shown in Table 3 revealed weight gain during the experimental period in all groups, with more notable weight gain observed in groups E, F and G fed with extracts from the plants harvested from the crude oil polluted farm land remediated using selected agro waste formulations. This however, suggests that the agro waste formulation could be rich in the essential nutrients, thereby facilitating enormous essential nutrient uptake by these plants which further could be evidenced in the observed notable weight gain in the wistar rats fed with extracts from these plants. The findings here contradict work by Ajibade *et al.,* (2013), who reported decrease in average body weights of wistar rats (relative to control) treated with aqueous leaf extract of *Talinum triangulare a*gainst Carbon tetrachloride induced hepatotoxicity. Generally leaf extracts of *T. triangulare* and *T. occidentalis* has been shown to have both preventive and ameliorative effects on hepatotoxicity (Adefolaju *et al.,* 2008; Ajibade *et al.,* 2013;

ISSN No:-2456-2165

Johnson *et al.*, 2017). The effective boost in weight gain could be from the agro waste formulation used for the bioremediation which is suggestive that bit could probably serve as good fertilizer or nutrient source for plants.

IV. CONCLUSION

This study revealed that the sampled aqueous leaf extracts of *T. triangulare* and *T. occidentalis* harvested from the bioremediated soil had no significant acute toxicity effect on hepatic tissues in wistar albino rats. This is hence suggestive that plant is devoid of any probable acute hepatotoxicity effects on these experimental animals. This finding therefore indicates that the agrowaste formulation used in this bioremediation could be a green pathway for enhancing bioremediation technology.

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