

Biochemical and histological studies of Albino rats fed vegetables from farmland nearby oil impacted sites in Rivers State, Nigeria

Igwe, K. O¹., Onyeike, E. N²., Uwakwe, A. A².

¹Department of Biochemistry, Federal University of Technology, Owerri, Nigeria

²Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria

Abstract: Biochemical and histological analysis were conducted to determine the effects of feeding albino rats with selected vegetables harvested from farmlands nearby oil impacted sites in Rivers state, Nigeria. One hundred and twenty albino rats were divided into six groups (Five rats each group) , each of the groups were fed each of the four selected vegetables. The vegetables were *Hibiscus esculentus*, *Telfairia occidentalis*, *Vernonia amygdalina* and *Talinum triangulare* while the farmlands were located at Bomu, Eleme, Umusoya, Rukpokwu and Omagwa. The result obtained was compared to values from rats fed similar edible vegetables harvested from non oil producing areas in Imo State. The biochemical indices of liver function determined include, total protein, albumin, globulin, total bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate amino transferase. Standard methods of analysis was used. The result of total protein concentration showed that serum of rats fed *Vernonia amygdalina* and *Talinum triangulare* from the study areas was significantly lower ($p < 0.05$) than values obtain from those from the control areas with values ranging from 53.47 ± 0.11 to 62.31 ± 0.10 g/dl. Serum of rats fed *Hibiscus esculentus* and *Telfairia occidentalis* harvested from the study areas had significantly higher albumin value while for *Talinum triangulare*, it showed lower value. For globulin, serum of rats fed *Telfairia occidentalis*, *Vernonia amygdalina* and *Talinum triangulare* from the study areas had significant lower values ($p < 0.05$). The result of total bilirubin concentration showed that serum of rats fed all vegetables had significantly higher values ($p < 0.05$) with values ranging from 18.26 ± 0.35 to 40.78 ± 0.33 mg/dl. For alkaline phosphatase concentrations, serum of rats fed all vegetables had significantly higher values ($p < 0.05$) with values ranging from 27.25 ± 0.05 to 53.87 ± 0.00 U/L. serum of rats fed *Vernonia amygdalina* and *Talinum triangulare* harvested from the study areas had significantly higher alanine aminotransferase concentration value while those fed *Hibiscus esculentus* and *Telfairia occidentalis* had lower values. finally, the result of aspartate amino transferase concentrations showed that serum of rats fed all vegetables had significantly higher values ($p < 0.05$) with values ranging from 23.22 ± 0.07 to 57.70 ± 0.06 U/L. Histopathological examination show mild inflammation of the liver of the rats fed vegetables from the study areas. The result indicate a compromise in liver of rats fed vegetables harvested from crude oil impacted farmlands.

Keywords: Oil spillage, edible vegetables, liver function.

1. INTRODUCTION

The ecosystem of the oil producing regions of Nigeria has been thoroughly disturbed by crude oil drilling, refining and other oil related industrial activities, these activities play a critical role in causing wide scale pollution of arable land, rivers, swamps and drinking water with hydrocarbon [1]. Rivers State is crisscrossed by several oil pipelines, alongside with wells and flow stations. These pipelines, wells and flow stations are mostly located nearby farmlands and gardens. Poor maintenance of oil infrastructure, sabotage of oil equipment, theft of oil and illegal refining are common conditions which has considerable impact that contributes to oil pollution in Rivers State [2].

Crude oil contains quite a number of toxic chemicals which is fast becoming a key instrument in causing a wide range of health effects in people and animals, depending on the level of exposure and susceptibility [3]. There is a growing body of literature that recognises the effect of some of these pollutants when absorbed by plants or organisms and are biotransformed into more toxic derivatives [4]. Other studies have shown that animal species that are not directly in contact with the oil spillage may be harmed via the food web.

There is evidence that prolonged ingestion of oil (either crude or refined) polluted plant material, seeds and water by animals do affect tissues and organs [5].

Evaporation, biodegradation and photo oxidation among other factors are important components which plays a key role in the transformation of the spilled oil physically and chemically. These processes helps in the removal of the hydrocarbon molecules from the geosphere [6], [7]. In the process of transformation of the hydrocarbons, less complex hydrocarbons are formed. If these process is complete, the remaining waste or by- product are mainly carbon dioxide, water, fatty acid and paraffin. However, these processes are usually too slow to bring about non- toxic and immobile hydrocarbon [8]. This process may be so slow that hydrocarbons may remain in soils and water for decades, enough time for it to runoff into nearby farmland and are bioaccumulated by vegetables. The bioaccumulation factors of pollutants depends on the nature of the pollutants, vegetable species, soil pollution level and soil characteristics [9].

Inorganic anionic pollutants such as chlorides, phosphates, nitrates and sulphates are dominant features in soil disturbed by oil drillings and crude oil associated pollution [10]. The polycyclic aromatic hydrocarbon which is a fundamental property of crude consists of fused aromatic rings. A primary concern of polycyclic aromatic hydrocarbon is their ability to become carcinogens, mutagens, and teratogens, for example benzene [11].

Heavy metals is a major area of interest within the field that is polluted, they are non-biodegradable and persistent environmental contaminants which may be carried along with the runoff and deposited in nearby gardens of farmlands. There is evidence that vegetables take up heavy metals by absorbing them from deposits on contaminated soil [12].

Recently, there has been renewed interest in vegetables as staple part of human meal, taken as food in raw and cooked forms. Prolonged consumption of unsafe concentrations of these pollutants through these vegetables may lead to their chronic accumulation in liver of animals and causing disruption of numerous biochemical processes, leading to liver damages [13].

In light of recent events in consumption of contaminated vegetables, it is becoming extremely difficult to ignore the existence of pollutants in vegetables harvested nearby oil spilled areas. Researchers have not treated pollutants arising from leached oil spillage in much detail. Previous published studies are limited to different humans' health risks occurred by consumption of contaminated home grown vegetables harvested at different times [9].

The purpose of this investigation is to assess the liver function and protein indices of rats fed selected edible vegetables harvested from farmland nearby oil impacted sites in Rivers State.

2. MATERIALS AND METHODS

A. Vegetable Samples Collection

Four vegetables *Abelmoschus esculentus*, (Okra) *Telfairia occidentalis* (pumpkin) *vernonia amygdalina* (Bitter Leaf) and *Talinum triangulare* (Water Leaf) were harvested from farmland around oil spilled sites in five communities of Rivers State viz Umusoya, Eleme, Omagwa, Rukpowu, and Bomu while same vegetables from non-oil producing communities in Imo State were used as control.

B. Preparation of Vegetable Samples.

The harvested vegetables were sorted to remove extraneous materials spoilt and unhealthy ones. The sorted vegetables were thoroughly washed, slightly boiled for about 2 minutes, dried and then ground then used for formulation of diet.

C. Experimental rats and treatments

Estimation of Liver function markers

Serum albumin (ALB) was determined by the method of of Doumas *et al* [14], serum total protein (TP) by the method of Tietz, [15], serum bilirubin by colourimetric method based on the method described by Jendrassik and Grof, [16], for the *invitro* determinations in serum using Randox laboratory test kit (Antrim, UK). Globulin was calculated thus; serum

globulin = total protein – serum albumin (TP-ALB). The estimation of alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) activities was done using Reitman and Frankel, [17] method for the quantitative *in-vitro* determinations in serum using Randox laboratory test Kit (Antrim, UK).

Histopathological analysis of rat liver tissues were carried out using the method of Conn [18] as modified by Kumar and Gill [19].

3. RESULT

Table 1: Total protein concentrations (g/dl) in serum of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	62.31 ± 0.10 ^a	54.40 ± 0.16 ^c	70.23 ± 0.06 ^d	60.26 ± 0.05 ^c
RUKPOKWU	61.39 ± 0.08 ^b	53.47 ± 0.11 ^d	73.29 ± 0.15 ^a	61.16 ± 0.11 ^a
OMAGWA	62.27 ± 0.11 ^{ac}	55.54 ± 0.12 ^a	75.11 ± 0.03 ^b	70.11 ± 0.04 ^d
BOMU	61.62 ± 0.64 ^{abcd}	55.47 ± 0.11 ^{ab}	72.22 ± 0.08 ^c	62.13 ± 0.07 ^e
ELEME	62.25 ± 0.10 ^{acd}	55.53 ± 0.09 ^{ab}	74.41 ± 0.51 ^{ab}	61.33 ± 0.19 ^{ab}
CONTROL AREA	71.44 ± 0.06 ^e	76.21 ± 0.07 ^e	72.39 ± 0.10 ^c	60.96 ± 0.04 ^{ab}

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 2: Albumin concentrations (g/dl) in serum of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	30.79 ± 0.06 ^a	33.55 ± 0.07 ^a	35.15 ± 0.03 ^a	37.61 ± 0.15 ^a
RUKPOKWU	31.01 ± 0.12 ^{ab}	32.84 ± 0.11 ^b	34.82 ± 0.06 ^b	36.86 ± 0.06 ^b
OMAGWA	30.80 ± 0.11 ^{abc}	33.57 ± 0.13 ^{ac}	35.42 ± 0.19 ^{ac}	37.72 ± 0.06 ^{ac}
BOMU	31.24 ± 0.32 ^{abcd}	32.84 ± 0.12 ^{bd}	34.36 ± 0.19 ^b	36.55 ± 0.14 ^b
ELEME	30.69 ± 0.10 ^{acd}	33.47 ± 0.06 ^{ac}	35.13 ± 0.04 ^{ac}	37.52 ± 0.18 ^{ac}
CONTROL AREA	34.62 ± 0.02 ^e	32.68 ± 0.03 ^{bd}	33.17 ± 0.01 ^d	30.16 ± 0.03 ^d

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 3: Globulin concentrations (mg/dl) in serum of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	31.17 ± 0.06 ^a	22.18 ± 0.07 ^a	31.20 ± 0.06 ^a	24.18 ± 5.49 ^a
RUKPOKWU	30.23 ± 0.04 ^b	22.22 ± 0.07 ^{ab}	30.85 ± 0.10 ^b	29.68 ± 0.28 ^{ab}
OMAGWA	31.15 ± 0.05 ^{ac}	22.16 ± 0.02 ^{abc}	34.14 ± 0.04 ^d	32.28 ± 0.12 ^a
BOMU	31.28 ± 0.26 ^{acd}	22.36 ± 0.25 ^{abcd}	31.15 ± 0.13 ^{abc}	30.21 ± 0.07 ^{abc}
ELEME	30.63 ± 0.60 ^{abcd}	22.22 ± 0.06 ^{abcd}	31.55 ± 0.50 ^{abc}	30.18 ± 0.16 ^{abc}
CONTROL AREA	36.81 ± 0.09 ^e	43.50 ± 0.09 ^e	36.48 ± 0.09 ^e	30.78 ± 0.07 ^a

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 4: Total bilirubin concentrations (mg/dl) in serum of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	36.40 ± 0.04 ^a	40.22 ± 0.03 ^a	18.79 ± 0.07 ^a	24.66 ± 0.22 ^a
RUKPOKWU	36.33 ± 0.07 ^{ab}	40.16 ± 0.10 ^{ab}	18.26 ± 0.35 ^{ab}	24.55 ± 0.20 ^{ab}
OMAGWA	36.70 ± 0.27 ^{abc}	40.78 ± 0.33 ^{abc}	18.93 ± 0.10 ^{abc}	24.79 ± 0.20 ^{abc}
BOMU	36.39 ± 0.04 ^{abcd}	40.22 ± 0.01 ^{abcd}	18.79 ± 0.06 ^{abcd}	24.85 ± 0.03 ^{abcd}
ELEME	36.99 ± 1.33 ^{abcd}	40.21 ± 0.08 ^{abcd}	18.73 ± 0.17 ^{abcd}	24.79 ± 0.13 ^{abcd}
CONTROL AREA	15.10 ± 0.01 ^e	9.91 ± 0.08 ^e	13.13 ± 0.05 ^e	7.90 ± 0.05 ^e

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 5: Alkaline phosphatase concentrations (U/l) in plasma of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	28.53 ± 0.09 ^a	53.57 ± 0.19 ^a	44.28 ± 0.07 ^a	27.31 ± 0.08 ^a
RUKPOKWU	28.60 ± 0.19 ^{ab}	53.87 ± 0.00 ^{ab}	44.28 ± 0.10 ^{ab}	27.30 ± 0.00 ^{ab}
OMAGWA	28.60 ± 0.13 ^{abc}	53.71 ± 0.13 ^{abc}	44.36 ± 0.11 ^{abc}	27.31 ± 0.06 ^{abc}
BOMU	28.46 ± 0.14 ^{abcd}	53.61 ± 0.19 ^{abcd}	44.29 ± 0.11 ^{abcd}	27.30 ± 0.15 ^{abcd}
ELEME	28.45 ± 0.19 ^{abcd}	53.37 ± 0.20 ^{abcd}	44.25 ± 0.12 ^{abcd}	27.25 ± 0.05 ^{abcd}
CONTROL AREA	18.25 ± 0.06 ^e	12.29 ± 0.13 ^e	33.33 ± 0.89 ^e	7.32 ± 0.10 ^e

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 6: Alanine aminotransferase concentrations (U/l) in plasma of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	24.68 ± 0.06 ^a	28.58 ± 0.05 ^a	27.19 ± 0.11 ^a	26.26 ± 0.14 ^a
RUKPOKWU	24.59 ± 0.06 ^{ab}	28.54 ± 0.14 ^{ab}	27.04 ± 0.04 ^{ab}	26.17 ± 0.09 ^{ab}
OMAGWA	24.73 ± 0.06 ^{abc}	28.70 ± 0.11 ^{abc}	27.27 ± 0.16 ^{abc}	26.13 ± 0.08 ^{abc}
BOMU	24.48 ± 0.12 ^{abcd}	28.50 ± 0.14 ^{abcd}	27.10 ± 0.06 ^{abcd}	26.10 ± 0.04 ^{abcd}
ELEME	24.56 ± 0.09 ^{abcd}	28.59 ± 0.17 ^{abcd}	27.09 ± 0.09 ^{abcd}	26.10 ± 0.07 ^{abcd}
CONTROL AREA	17.37 ± 0.07 ^e	17.61 ± 0.06 ^e	26.12 ± 0.02 ^e	13.06 ± 0.04 ^e

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

Table 7: Aspartate amino transferase concentrations (U/l) in plasma of albino rats fed vegetables from the areas studied.

Location	<i>Talinum triangulare</i>	<i>Vernonia amygdalina</i>	<i>Telfairia occidentalis</i>	<i>Hibiscus esculentus</i>
UMUSOYA	31.59 ± 0.20 ^a	57.38 ± 0.17 ^a	23.28 ± 0.20 ^a	36.45 ± 0.18 ^a
RUKPOKWU	31.67 ± 0.09 ^{ab}	57.43 ± 0.17 ^{ab}	23.28 ± 0.16 ^{ab}	36.48 ± 0.16 ^{ab}
OMAGWA	31.81 ± 0.12 ^{abc}	57.56 ± 0.12 ^{abc}	23.50 ± 0.29 ^{abc}	36.50 ± 0.16 ^{abc}
BOMU	31.56 ± 0.03 ^{abcd}	57.50 ± 0.12 ^{abcd}	23.22 ± 0.07 ^{abcd}	36.42 ± 0.16 ^{abcd}
ELEME	31.58 ± 0.07 ^{abcd}	57.70 ± 0.06 ^{abcd}	23.26 ± 0.15 ^{abcd}	36.53 ± 0.10 ^{abcd}
CONTROL AREA	28.17 ± 0.03 ^e	30.37 ± 0.06 ^e	20.54 ± 0.09 ^e	17.52 ± 0.09 ^e

Values are Means ± standard deviations of triplicate determinations.

Values in each column with different superscript letters differ significantly at 5% level ($p < 0.05$).

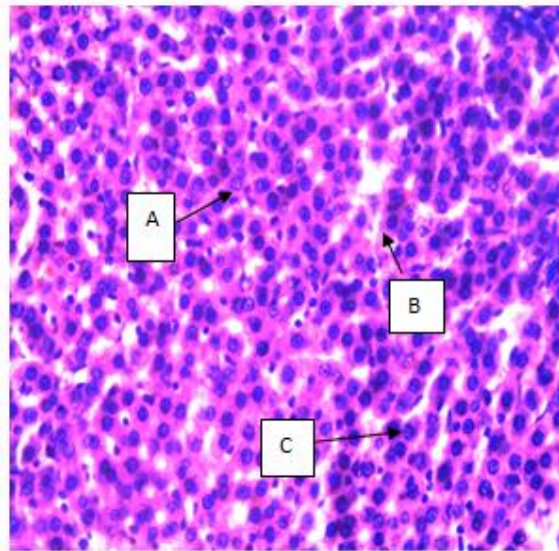


Plate 1: Microscopic representation of the liver of rats fed vegetables from the control areas. Normal liver with hepatocytes (A), central vein B, and portal triad (C) {X40 H & E}

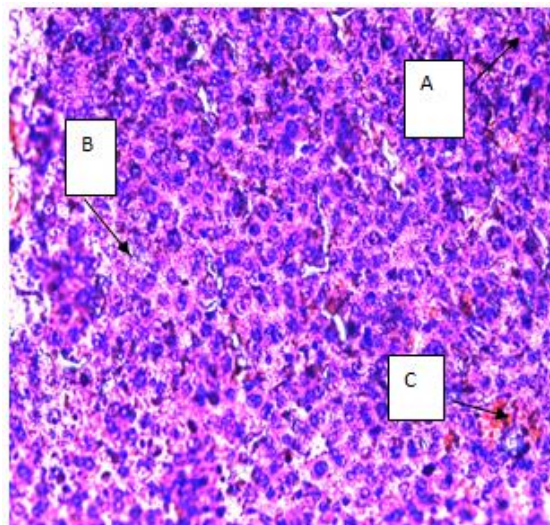


Plate 2: Microscopic representation of the liver of rats fed vegetables from Umusoya. hepatocytic degeneration (A), cyto-architectural distortions (B), and mild necrosis (C) {X40 H & E}

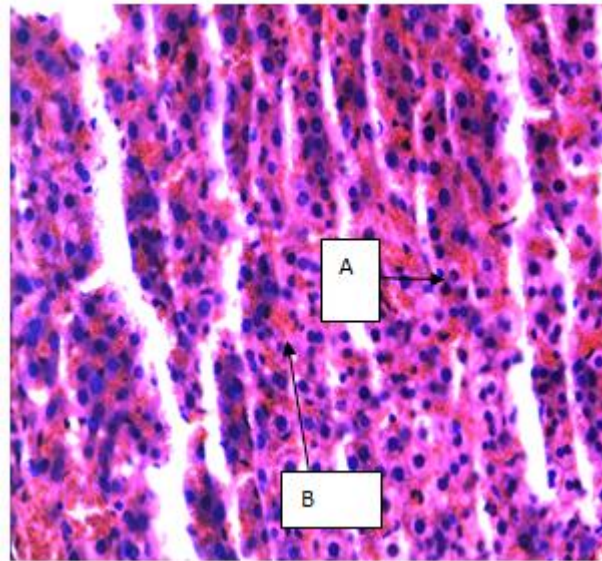


Plate 3: Microscopic representation of the liver of rats fed vegetables from Eleme. multinucleation (A), and hepatocytic development amidst an haemorrhagic background (B), {X40 H & E}

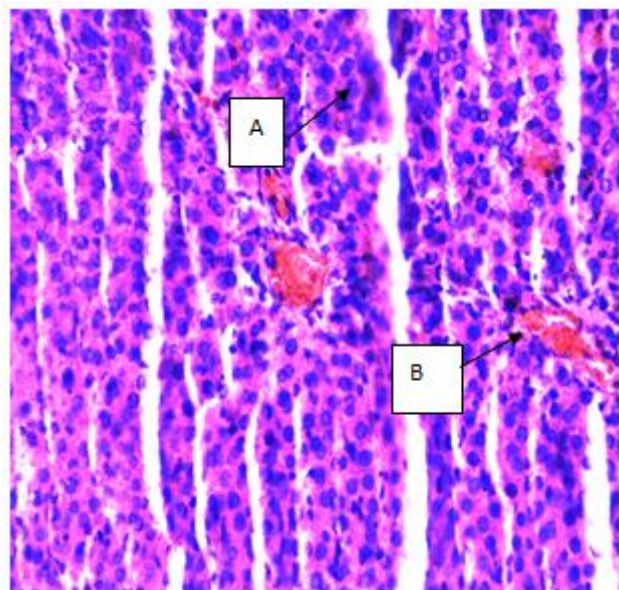


Plate 4: Microscopic representation of the liver of rats fed vegetables from Omagwa. Multinucleation (A), and hepatocytic development amidst an haemorrhagic background (B), {X40 H & E}

The results of total protein, albumin, globulin and total bilirubin in serum of albino rats fed selected edible vegetables harvested from farmland within the areas studied are shown in Tables 1 – 4.

The total protein concentration in the serum of rats fed the edible vegetables is given in Table 1. The total protein concentration in serum of rats fed *Talinum triangulare* and *Vernonia amygdalina* from the control area had significantly higher ($p < 0.05$) values of 71.44 ± 0.06 g/dL and 76.21 ± 0.07 g/dL than values in serum of rats fed *Talinum triangulare* and *Vernonia amygdalina* from the areas studied. Also total protein concentration in serum of rats fed *Telfairia occidentalis* and *Hibiscus esculentus* from Omagwa were higher than values in serum of rats fed *Telfairia occidentalis* and *Hibiscus esculentus* from the other areas studied although the differences were not significant.

The albumin concentration in the serum of rats fed the edible vegetables is given in Table 2. The albumin concentration in serum of rats fed *Talinum triangulare* from the control area had significantly higher ($p < 0.05$) values of 34.62 ± 0.02 mg/dL than values in serum of rats fed *Talinum triangulare* from the areas studied. Also albumin concentration in serum of rats fed *Telfairia occidentalis* and *Hibiscus esculentus* from the control area had significantly lower ($p < 0.05$) values of

33.17 ± 0.01 mg/dL and 30.16 ± 0.03 mg/dL than the values in serum of rats fed same vegetables from the areas studied. Finally albumin concentration in serum of rats fed *Vernonia amygdalina* from the control area was lower than values in serum of rats fed *Vernonia amygdalina* from the areas studied although the difference was not significant.

The globulin concentration in the serum of rats fed the edible vegetables is shown in Table 3. The globulin concentration in serum of rats fed *Talinum triangulare*, *Vernonia amygdalina* and *Telfairia occidentalis* from the control area had significantly higher ($p < 0.05$) values of 36.81 ± 0.09 mg/dL, 43.50 ± 0.09 mg/dL and 36.48 ± 0.09 mg/dL than values in serum of rats fed same vegetables from the areas studied. Also globulin concentration in serum of rats fed *Hibiscus esculentus* from the control area was lower than values in serum of rats fed *Hibiscus esculentus* from the areas studied although the difference was not significant.

The total bilirubin concentration in the serum of rats fed the edible vegetables is presented in Table 4. The total bilirubin concentration in serum of rats fed all the vegetables from the control area had significantly lower ($p < 0.05$) values (with values ranging from 7.90 ± 0.05 to 15.10 ± 0.01 mg/dL) than values in serum of rats fed all the vegetables from the areas studied.

The result of alkaline phosphatase, alanine aminotransferase and aspartate amino transferase of albino rats fed selected edible vegetables harvested from farmland within the areas studied is shown in Table 5 – 7. The alkaline phosphatase concentration in plasma of rats fed the vegetables is presented in Table 5. The alkaline phosphatase concentration in plasma of rats fed all the vegetables from the control area had significantly lower ($p < 0.05$) values (with values ranging from 7.32 ± 0.10 to 33.33 ± 0.13 U/I) than values in plasma of rats fed all the vegetables from the areas studied.

The alanine aminotransferase concentration in plasma of rats fed the vegetables is given in Table 6. The alanine aminotransferase concentration in plasma of rats fed all the vegetables from the control area had significantly lower ($p < 0.05$) values (with values ranging from 13.06 ± 0.04 to 26.12 ± 0.02 U/I) than values in plasma of rats fed all the vegetables from the areas studied.

The aspartate amino transferase concentration in plasma of rats fed the vegetables is shown in Table 7. The aspartate amino transferase concentration in rats fed all the vegetables from the control area had significantly lower ($p < 0.05$) values (with values ranging from 17.52 ± 0.09 to 30.37 ± 0.06 U/I) than values in plasma of rats fed all the vegetables from the areas studied.

Plates 1-4 show the results of the histological examination of the livers of control and test rats. The liver of rats fed vegetables from the control areas (Plate. 1) showed normal hepatocytes with prominent central vein and portal triad. Histological evaluation of the liver of rats fed vegetables from the study areas showed congestion (increase in blood flow) in the portal region as well as the sinusoids. Dilation (widening) of the blood vessels of the portal zone of the liver was observed. There was also multinucleation, and hepatocytic development amidst haemorrhagic background indicating features of a compromised liver.

4. DISCUSSION

Several reports have shown that the liver makes most of the proteins found in blood including albumin and other proteins. The liver uses enzymes to synthesize proteins, an excess of two of these enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are elevated in serum when liver cells have been damaged [20].

Plasma proteins like albumin are exclusively synthesized by the liver and has a circulating half-life of approximately three weeks. Reduction in albumin (normal > to 3.5 gm/dl) usually indicates liver disease of more than three weeks duration [21].

The Liver has to perform different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver but the tests of the liver's capacity to transport organic anions and to metabolize drugs can be assessed by determining Serum bilirubin [22].

This study set out to determine whether selected vegetables harvested from farmland nearby oil impacted sites in Rivers state, Nigeria will affect the liver of rats fed with these vegetables.

Total protein was significantly higher in the blood of rats fed *Talinum triangulare* and *Vernonia amygdalina* from the control area. Exposure to pollutants in the edible vegetables might have resulted in reduction of total protein values. It was

reported that in stressful environment, protein catabolism takes place resulting in reduced protein levels, which signifies poor liver functions and some kidney diseases [23]. Serum total protein levels is a rough measure of protein status but reflects major functional changes in kidney and liver functions [24].

Albumin was significantly higher in the blood of rats fed *Talinum triangulare* from the control area. The liver synthesizes proteins, among which is albumin. The decrease in albumin levels in liver of rats fed polluted vegetables could be attributed to changes in protein and free amino acids metabolism and their synthesis in the liver [25]. This adverse effect might be caused by the interference of heavy metals with protein synthesis or by the binding of heavy metals to some metal-binding proteins and their removal through detoxification processes [26]. In an attempt to clarify the mechanism involved, Hoffman *et al.* [27] reported that lead caused a disruption in protein and RNA synthesis.

Globulin was significantly higher in the blood of rats fed *Talinum triangulare*, *Vernonia amygdalina* and *Telfairia occidentalis* from the control areas. The decreased of globulin concentration by rats fed these polluted diet may indicate an immunodepressive response [28]. Liver is the site of albumin synthesis but globulin is formed by the lymphatic system to some extent [29]. Decreased serum concentrations of albumin and globulin as observed in this study in the rats fed polluted vegetables lend credence to the submission that the liver function may be impaired. Both globulin and albumin are also produced by the liver. If the liver is damaged, it can no longer produce these proteins. The results presented on serum proteins are consistent and all pointing to the fact that the liver may have been damaged by consumption of the contaminated vegetables.

The concentrations of the total proteins, bilirubin and albumin in the serum may

Indicate the state of the liver and the type of damage [30].

This study also demonstrated that toxic metabolites in the edible vegetable (Total bilirubin) might be responsible for the significantly higher value in rats fed all the polluted vegetables. The observed increase in bilirubin concentration confirms the fact that there may be evidence of liver dysfunction, which may be as a result of the effect of the toxic components of crude oil in the formulated diets. This assertion is supported by the work of Ovuru and Ekweozor, [31] who reported an increase in total serum bilirubin concentration in semi adult rabbits exposed to crude oil contaminated diet and attributed this to a metabolic disturbance in the liver arising from defective conjugation and/ or excretion of bilirubin. Serum bilirubin, albumin and globulin concentrations are some biochemical indices for monitoring liver function in the blood. Abnormal levels of these proteins have been reported to be associated with haemolysis or increased breakdown of RBC and/ or liver damage [32]. Bilirubin is a breakdown product of heme (a part of haemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. It does this by the following mechanism: bilirubin is taken up into hepatocytes, conjugated (modified to make it watersoluble) and secreted into the bile, which is excreted into the intestine. Increased total bilirubin causes jaundice and can signal a number of problems. Studies had shown that if direct (that is, conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin and the location of the problem is upstream of bilirubin excretion. Anemia, viral hepatitis, or cirrhosis can be suspected. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones or cancer should be suspected [33]. In this study, rats fed vegetables from the study areas presented elevated serum concentrations of total bilirubin suggesting that the liver is not able to excrete bilirubin which is an evidence of liver dysfunction.

There was a significant higher value ($p < 0.05$) in ALP concentration of rats fed all the vegetables from study areas when compared to the ones fed the vegetables from the control area. ALP is a hydrolytic enzyme responsible for removing of phosphate group from many types of molecule including nucleotides, protein and alkaloids. An increase in ALP level is an indication that there has been an obstruction of the bile duct. ALP may increase as a result of Pagets disease of the bone. An increase in ALP level may also be as a result of celiac diseases [34]. ALP is present in all the tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone and placenta. Normal ALP levels are approximately 20-140u/l and it increases in active bone formation as ALP is a byproduct of osteoblast activity [35]. ALT concentration also shows a significant increase ($p < 0.05$) in rats fed the different vegetables from study areas compared to rats fed vegetables from control area. Activities of ALT rises in disease as associated with death of the hepatocytes like viral hepatitis [36]. ALT level usually increases where liver has been diseased or damaged, additional ALT is released into the blood stream. ALT is commonly used as a way for screening liver problems. Reference level of ALT ranges from 10-40u/l [37]. There was a significant increase ($p < 0.05$) in AST concentration of rats fed vegetables from study areas

compared to those fed vegetables from control area. The increase in AST level was higher than normal AST level. AST is an enzyme found in the liver, heart, skeletal muscle, kidney, brain and red blood cell. It is commonly measured clinically as a marker for liver health. AST reference ranges in man are about 8-40u/l and 6-34u/l in female [38]. AST level increases when there is disease affecting organs of the body such as myocardial infarction, acute pancreatitis, acute hemolytic anemia and acute renal diseases (Berg et al 2007). This increase may be because there has been accumulation of toxic substances in the body.

Histological evaluation of the liver of rats fed vegetables harvested from farmland near oil impacted sites revealed increased activities in the blood vessels of the liver as well as inflammation especially around the portal zone the was also cyto-architectural distortions, multinucleation and mild necrosis. The rats fed vegetables from the control areas however had normal liver with no inflammation.

5. CONCLUSION

The study has therefore shown that pollutants from the farm land might have been bioaccumulated by the edible vegetables in the study areas and that these vegetables when consumed by albino rats had adverse effect on biochemical and histological parameters.

REFERENCES

- [1] Odokuma, L.O. and Okpokwasili, G.C. (1992): Role of composition in the biodegradation of dispersants. *Waste Mang.* 12, 12-43
- [2] Annon (2006). Niger Delta Natural Resource Damage Assessment and Restoration Project. Phase 1- Scoping Report. Federal ministry of Environment, Abuja Nigeria Conservation Foundation, Lagos, WWF UK, CEESP-IUCN Commission on Environment, Economic and Social Policy
- [3] C.O. Ujowundu, F.N. Kalu, L.A. Nwaogu, E.U. Ezeji and K.O. Igwe (2012). Management of Changes in Liver Chemistry in Male Rats Acutely Exposed to Crude Petroleum Oil. *Journal of Applied Pharmaceutical Science* 02 (06): 116-120
- [4] Duffus, J. H. (1980). *Environmental toxicology*. Edward Arnold Pub. Ltd., London.
- [5] George, O.S. and Sese, B.T. (2012): Effects of crude oil contaminated feed on performance and organ weights of growing rabbits in the Niger Delta Area of Nigeria. *Adv. in food and energy security* 2: 43-49.
- [6] Douglas, G.S., Bence, A.E., Prince, R.c., Mcmillen, S.J., Butler, E.L. (1996). Environmental Stability of Selected Petroleum Hydrocarbon Source and Weathering ratios. *Environ.Sci. Technol.*, 30, 2332
- [7] Garrett, R. M., Pickering, I. J., Haith, C. E., Prince, R. C. (1998). Photooxidation of crude oils. *Environ. Sci. Technol.*, 32, 3719.
- [8] Apajalahti, J.A. and Salkinoja-Salonen, M.S. (1986). Degradation of polychlorinated phenols by *rhodococcus chlorophenicus*. *Applied microbiology and Biotechnology* 25, 62-67
- [9] Emese Sipter, Rita Auerbach, Katalin Gruiz and Gabriella Mathe-Gasper (2009). Change of Bioaccumulation of toxic metals in vegetables. *Communications in soil science and plant Analysis*. 40(6): 285-293.
- [10] Powell, M.J and Florence, L.A (1993) Characteristics Associated with differences between undisturbed soils. *Soil Biol Biochem*. pp 1499-1511 National Research Council.
- [11] Fetzer J.C. (2000). *The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons*. New York: Wiley.
- [12] Kachenko, A. G. and Singh, B., (2006). Heavy metals contamination in vegetables grown in Urban and metal smelter contaminated sites in Australia, *water, Air and Soil pollution*. 169: 101-123
- [13] Sharma, R. J., Agrawal, M. and Marshall, F. M. (2009). Heavy Metals in Vegetables collected from production and market sites of a tropical urban area of India. *Food and Chemical Technology*, 47:583-591
- [14] Dumas, B. T; Watson, W, A. and Briggs, H. G. (1971). Albumin standard and measurement of serum albumin with bromocresol green (BCG). *Clinical chemical Acta*, 31:87-90

- [15] Tietz, N. W. (1995). Clinical guide to laboratory test. 3rd Edn. W. B. Saunders, Philadelphia, USA
- [16] Jendrassik, L and Grof, P (1938). Colorimetric Method of Determination of bilirubin. *Biochem Z.* 297:81-82
- [17] Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. *American journal of Clinical Pathology*, **28**:56 -63.
- [18] Conn, H. J. (1969). Biological Stains. In Lillie, R.D. (Ed.) 8th edition. Williams and Wilkins, Baltimore. Pp. 269 – 281.
- [19] Kumar, G. L. and Gill, G. W. (2010). Manual versus automated special staining protocols. In Kumar, G. L. (ed.), Special stains and H&E 2nd ed, Dako, California pp 1 – 44.
- [20] Owu, D.U., Osim, E.E. and Ebong, P.E. (1998). Serum liver enzymes profile of wistar rats following chronic consumption of fresh or thermo-oxidized palm oil diets. *Acta. Tropical*, 69: 65-73.
- [21] Peters, T. (1996). All about albumin, biochemistry, genetics and medical application. San Diego:Academic press
- [22] Friedman S. F., Martin, P. and Munoz J. S. (2003). Laboratory evaluation of the patient with liver disease, Hepatology, a textbook of liver disease. Philadelphia: Saunders publication
- [23] Sarada, S.K.S., Dipti, P., Anju, B., Pauline, T., Kain, A.K., Sairam, M., Sharma, S.K., Ilavazhagan, G., Kumar, D. and Selvamurthy, W. (2002), "Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats", *J. Ethnopharmacol.*, 79(2), 149-153.
- [24] Agrawal, R, and Johri, G. N. (1990). Serum protein changes in lead exposed mice infected with *Hymenolepis nana*. *J Hyg. Epidemiol. Microbiol. Immunol.* 34, 387-390.
- [25] Rivarola V.A., Balegno H.F(1991). Effect of 2,4-dichlorophenxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicol. Lett.*; 56: 151-7.
- [26] Cawson R.A., McCarcken A.W., Marcus P.B. (1982). Pathological mechanisms and human disease. The C.V. Mosby Company. St. Louis.
- [27] Hoffman E.O., Trejo R.A., Luzio N., Lamberty J. (1972) Ultrastructural alterations of liver and spleen following of acute lead administration in rats. *Exp. Mol. Pathol.*; 17: 159-70
- [28] Shehata S.A., Askar A.A., Mohamed M.S. (2003). Reducing of the dietary toxicity of T-2 toxin and diacetoxyscripenol(DAS) by garlic in fish. *J. Agric. Sci. Mansoura Univ.*, 28(10): 7169-7182.
- [29] Jones, E. and Bark, P., (1979). Chemical Diagnosis of Diseases, Biochemical Press, Amsterdam, N.Y., Oxford, 325
- [30] Yakubu, M. T. , Akarji, M. A. and Oladiji, A. T. (2005) Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ext. Heim) stem in male albino rats. *Asian J. Androl.* 7: 399-404
- [31] Ovuru S.S and Ekweozor I.K.E (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. *African Journal of Biotechnology* 3 (6): 346-348.
- [32] Islam M.S, Lucky NS, Islam M.R, Ahad A, Das B.R, Raham M.M, Siddiui M.S.I (2004). Haematological parameters of Fayoumi, Assil and local chickens reared in sylhet region in Bangladesh. *Int. J. Poult. Sci.* 3(2):144-147.
- [33] Sunmonu, T. O. and Oloyede (2007). Biochemical assessment of the effects of crude oil contaminated catfish (*clarias gariepinus*) on the hepatocytes and performance of rat. *African journal of Biochemistry Research*, vol 1 (5): 083-089
- [34] Azad M (2005). Toxicity of water soluble fractions of crude oil on *Metamysidopsis insularis*, an indigenous tropical mysid species. *Environmental Monit. and Assess.* 104:37-40.
- [35] Nivedita G, Vatsal M, Madhuri k, Pushph S, Simta K, Vaman CR (2002). Toxicity study of diethyl phthalate on freshwater. *Fish Girrihinamri gala. J. Ecotox. Environ. Safety*, 53:255-258.)

- [36] Gabriel U.U and George A. (2005). Plasma enzymes in *Clarias gariepinus* exposed to chronic level of round up (glyphosate). J. Environ. Ecol. 23(2):271-276.
- [37] Ghouri N, Preiss, D and Sattar, N. (2010). Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology* 52(3):1156-61
- [38] Muriana, F. J., Alvarez-Ossario, M. C. and Relimpio, A.M.(1991). Purification and characterization of aspartate aminotransferase from the halophile archaeobacterium *Haloferax mediterranei*. *Biochem J* 278, 149- 154
- [39] Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007): *Biochemistry*. W.H.Freeman and Company, New York. Pp742-747.