



## Toxicological Evaluation of Palm Kernel Oil (PKO) Biodiesel-Contaminated Catfish on Kidney of Albino Rats

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### Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

### Article Information

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### ABSTRACT

**Aim:** To assess the toxicological and histopathological effects of feed formulated with catfish (*Clarias gariepinus*) exposed to palm kernel oil (PKO) biodiesel on kidney in albino rat.

**Study Design:** Randomized experimental controlled study

**Place and Duration of Study:** The study was carried out in Environmental Science laboratory of Federal University of Petroleum Resources Effurun between February and June, 2014.

**Methodology:** A total of 30 albino rats weighing 49 to 53 g and within the age range of 4-6 weeks were used for this study. Experimental rats were grouped into three, namely; Control, BD0.1 and BD0.25 placed on feed formulated with catfish exposed to borehole water mixed with different concentrations of PKO biodiesel (0 %v/v, 0.1 %v/v, 0.25 %v/v) for 30 days. Haematological properties, indices of oxidative stress, some enzymes of the kidney and selected serum indices of kidney functions (urea, creatinine, Na<sup>+</sup> and K<sup>+</sup>) were measured. Tissue activity of ALP, ACP, LDH, CAT, GST and SOD were also estimated using conventional methods. Analyses were carried out using the SPSS software package (version 11.5) and the results are expressed as mean ± SEM.

**Results:** The study showed that animals fed with fish exposed to PKO biodiesel significantly had

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reduced relative kidney weight while serum levels of Na<sup>+</sup>, K<sup>+</sup>, urea, creatinine and kidney concentration of malodialdehyde (MDA) were elevated significantly ( $p < 0.05$ ) when compared with the controls. Activities of selected enzymes of kidney studied were significantly lower in BD0.1 and BD0.25 rats relative to the control. Particularly, the kidney activity of alkaline phosphatase (ALP) of control, and rats fed with feed formulated with BD0.1 and BD0.25 are  $188.24 \pm 6.68$ ,  $156.88 \pm 9.84$ ,  $149.02 \pm 6.77$  U/mg protein, respectively. Histological examination revealed proliferation of inflammatory cells in the kidney of experimental rat.

**Conclusion:** Data from this study suggested that consumption of catfish exposed to PKO biodiesel can compromise renal integrity, reduce renal performance, subject the kidney to oxidative stress and inflict varying degree of inflammation on cellular architecture of the kidney.

**Keywords:** Palm kernel oil; biodiesel; histopathology; kidney; haematology; oxidative stress.

## 1. INTRODUCTION

Biodiesel has attracted considerable interest as an alternative fuel or extender for petrodiesel for combustion in compression-ignition (diesel) engines. Biodiesel is miscible with petrodiesel in any proportion and possesses several technical advantages over ultra-low sulfur diesel fuel (ULSD, <15 ppm S), such as inherent lubricity, low toxicity, derivation from a renewable and domestic feedstock, superior flash point and biodegradability, negligible sulfur content, and lower overall exhaust emissions [1].

Palm kernel oil (PKO) in Nigeria had hitherto been underutilized as edible oil. Available records however ranked Nigeria as one of the world producers of palm kernel. Recently, PKO has gained considerable recognition as raw material for production of biodiesel [2-3]. Chemically, the PKO consist of triglyceride molecules of three long chain fatty acids that are ester bonded to a single glycerol molecule. These fatty acids differ from the length of carbon chains, the number, orientation and position of double bonds in these chains. Alkaline catalysts such as NaOH and KOH are the most commonly used in trans-esterification since their reaction is much faster than an acid-catalyzed reaction [4]. Transportation of petroleum and petrochemicals is through pipelines that cut across land and water. Accidentals spills and pipeline damage result in pollution of land and water. Biodiesel, on the other hand, cannot be transported by pipelines due to its inferior storage and oxidative stability, lower volumetric energy content, inferior low-temperature operability versus petrodiesel. The mode of transportation is by truck, rail, boat or ships which pose greater risk of environmental

Palm kernel oil was purchased at the local market in Effurun, Nigeria. 100g PKO was used for the transesterification process. The ethanol

pollution. Spills of biodiesel on land can easily be washed by runoff to water and together with spills on water thereby contaminating water bodies and consequently endangering aquatic lives [5-6].

The African catfish, *Clarias gariepinus*, is a source of food to Nigerians; it is cultivated by many for commercial and subsistence purposes. Most of the ponds where *C. gariepinus* is cultivated serve as recipients to runoffs, while a few others are watered by boreholes. Components of runoffs are of serious health concern. Some species of fish may contain significant levels of these contaminants and other environmental contaminants. These substances are present at low levels in fresh waters and oceans, and they bioconcentrate in the aquatic food chain such that levels are generally highest in older, larger, predatory fish and marine mammals. Fish and seafood are a major source of human exposure to these contaminants [7].

Studies on the effect of biodiesel contaminated food are scanty. The paucity of information on toxicology of aquatic organisms exposed to biodiesel on consumers has necessitated the present study. This study, however, is aimed at investigating the effect of consumption of catfish exposed to PKO biodiesel on the kidney of albino rat.

## 2. MATERIALS AND METHODS

Reagents and solvents were of analytical grade and are products of British Drug House, Poole, England.

### 2.1 Perm Kernel Oil (PKO)

used (99% pure) is an analytical grade with boiling point of 78°C; while the NaOH used was also an analytical grade product of Aldrich Chemicals, England. The blender used was a

Dry and Wet mill Blender with a clear glass (1,250 cc capacity) containers and stainless steel cutting blades. Other major materials used include scales, translucent white plastic container with bung and screw-on cap, funnels, PET bottles and thermometer.

## 2.2 Preparation of Bio-diesel from PKO

Biodiesel was prepared from PKO in accordance with the method described by Aremu et al. [3]

## 2.3 Experimental Water and Fish Treatment

The Biodiesel from PKO was diluted with borehole water to obtain 0.25 and 0.1 %v/v. Twenty-four healthy juvenile catfish (*Clarias gariepinus*) of mean weight  $58.4 \pm 2.4$  g were obtained from a commercial fish pond at Ekpan in Delta State, Nigeria and acclimatized for ten days prior to the commencement of the experiment. The catfish were grouped into three (3) of eight catfish and were kept in 30L plastic aquaria. Control group served as control and the catfish here were cultured in borehole water while those in Groups BD0.1 and BD0.25 were exposed to the different mixtures (0.1% v/v and 0.25% v/v respectively) of Biodiesel from PKO. The catfish were fed *ad libitum* with commercial fish meal for 30hrs during which the experiment lasted.

At the end of the 30 h experimental period, the catfish were harvested, oven dried at 40°C and used as a source of protein (25%) to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class comprising corn starch (52%), oil (4%), maize cob (4%), sucrose (10%) and vitamin/mineral mixture (5%). The food items were mixed together and manually made into pellets to feed albino rats.

## 2.4 Experimental Rats and Treatment

Thirty albino rats of mean weight ( $51 \pm 2.0$  g) were procured from the animal house of Department of Anatomy, University of Benin, Benin-City, Nigeria. All animals were housed under standard laboratory conditions with free access to water *ad-libitum* and balanced pellets food. The housing temperature was ( $25 \pm 1^\circ\text{C}$ ) with 12 h dark /light cycle and 50% humidity. All ethical guidelines on the use of animals for investigational purposes were

followed and the experiment protocol was approved by Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria ethics committee.

The animals were grouped into three with each group containing ten rats. The rats in control group were fed on the control diet, which was formulated with catfish cultivated in borehole water. Animals in Groups BD0.1 and BD0.25 were fed on diet formulated with catfish exposed to the different mixtures of biodiesel (0.1 and 0.25 %v/v respectively). The feeding lasted for a period of thirty (30) days (after an acclimatization period of ten days) during which the weight and feed intake were monitored.

At the end of the feeding exercise, the rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. The liver of was removed into a beaker containing ice cold 0.25 M sucrose solution. The blood was obtained through their jugular veins. Each blood sample was thereafter centrifuged at 3500 rpm for about 15 min using refrigerated centrifuge RC650s and the serum obtained was preserved at  $-8^\circ\text{C}$  until required for use. A portion of the liver was homogenized for biochemical studies and enzyme assays. Haemoglobin concentration of the blood of experimental animals was determined following the method described by Mitruka and Rawnsley [8]. The RBC and WBC was done by the method of manual counting, PCV by Microhaematocrit method, described by Muthaya [9]. Other haematological parameters were determined as described by Tietz [10]. MDA determination was based on method described by Bird et al. [11]. The method described by Jollow et al. [12] was used to determine reduced glutathione (GSH) concentration. Catalase (CAT) activity was determined according to the method of Sinha [13]. The activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [14]. The cytosolic glutathione s-transferase (GST) activity was determined spectrophotometrically at  $37^\circ\text{C}$  (340nm) by the procedure described by Habig et al. [15]. Histopathological study, using hematoxylin and eosin (H&E) stain, of the kidney obtained from experimental rats was carried out following the method described by Drury and Wallington [16]. Protein concentration was determined by the biuret reaction described [17]. The method used for assaying lactate dehydrogenase (LDH) is

based on that of Wroblewski and La Due [18] in which pyruvate is reversibly reduced to lactate in the presence of nicotinamide adenine dinucleotide (reduced) as co-enzyme. The method of Bessey et al. [19] as modified by Wright et al. [20] was employed in the determination of alkaline phosphatase (ALP) and acid phosphatase (ACP). Serum sodium and potassium were determined using flame photometry method. The diacetyl monoxime method using thiosemicarbazide as described by Marsh et al. [21] was used for serum urea determination. Serum creatinine was determined using the method described by Brod and Sirota [22]. Histology and haematology studies were done at the University of Benin Teaching Hospital (UBTH) Nigeria, while other studies were carried out at the Environmental Science Laboratory of the Federal University of Petroleum Resources, Effurun, Nigeria.

### 3. Statistical Analyses

All numerical results were obtained from the three (3) groups (control and treated). Data obtained were presented as mean±SEM and subjected to statistical analysis using a one way analysis of variance (ANOVA) by employing the method of Steel and Torrie [23]. Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple range test [24].

### 4. RESULTS

Haematological properties of rats fed with catfish exposed to PKO biodiesel is presented in Table 1. No significance different ( $p>0.05$ ) was found among the test groups and relative to the control.

Table 2 presents serum concentration of some kidney function indicators of rats placed on diet formulated with catfish exposed to PKO biodiesel. Generally, concentrations of urea, creatinine, sodium and potassium ions of serum of test rats were significantly higher ( $p<0.05$ ) than those of control rats. The significant difference was found to widen as the concentration of biodiesel increased because serum concentrations of the kidney indicators of BD0.25 rats were significantly higher ( $p<0.05$ ) than those of BD0.1.

Effect of catfish exposed to PKO biodiesel on the activities of selected kidney enzymes of rat is presented in Table 3. Activities of kidney ALP, ACP and LDH of control rat were significantly higher ( $p<0.05$ ) than those of test rats, while

those of BD0.1 were not significantly different from those of BD0.25 rats. Similarly, activities of antioxidant enzymes, CAT, SOD and GST of control rat were significantly higher ( $p<0.05$ ) than those of test rats. Activities of the antioxidant enzymes of kidney of test rats were found to decrease significantly ( $p<0.05$ ) from BD0.1 to BD0.25 rats.

Fig. 1 presents the relative kidney weight of rats fed with catfish exposed to PKO biodiesel. The relative kidney weight of test was found to be significantly lower than that of control ( $p<0.05$ ).

The concentration of MDA of kidney of rats fed with catfish exposed to PKO biodiesel is shown in Fig. 2. It was generally observed that MDA concentration increased significantly ( $p<0.05$ ) as the level of biodiesel increased. Particularly, concentration of MDA of kidney of test rat was significantly higher than that of control ( $p<0.05$ ).

Fig. 3 presents the level of GSH of kidney of rats fed with catfish exposed to PKO biodiesel. GSH concentration of kidney of test rat was significantly lower ( $p<0.05$ ) than that of control, that of BD0.25 rat was also found to be significantly lower than that of BD0.1 rat ( $p<0.05$ ).

Figs. 4 – 6 presents light micrograph of kidney of rats fed with catfish exposed to PKO biodiesel. The kidney of control rats showed no visible lesion (Fig. 4), transverse section reveals distinct renal tubules and corpuscles, prominent macula densa and distinct nuclei. That of BD0.1 rat showed glomeruli which appear distinct with few proliferating inflammatory cells (Fig. 5) while that of BD0.25 (Fig. 6) revealed membranoproliferative glomerulonephritis, mesangial cell proliferation, and increased mesangial matrix slightly vacuolated in sclerotic layer.

### 5. DISCUSSION

Aquaculture is the fastest growing food sector in the world, accounting for an estimated 43 % of all fish consumed by humans globally [25]. However, presence of biodiesel and other synthetic chemicals, which are non-biodegradable, accumulate in the environment to pose serious toxic threat to both aquatic animals and their consumers [26]. The present study investigated the effect of catfish exposed to PKO biodiesel on the kidney in albino rats.

Nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes. These can be determined by either or combinations of clinical, anthropometric, biochemical or dietary methods. The value of blood components is an indication of availability of nutrients for synthesis of blood cells. Data from this study revealed that haematological

properties of rat fed with catfish exposed to biodiesel compete favourably with those of control (Table 1). Several studies have reported changes in haematological parameters routinely used to determine stress associated with environmental, nutritional, and/or pathological factors [7, 27].

**Table 1. Haematological properties of rats placed on diet formulated with catfish exposed to biodiesel polluted water over a period of 30 days**

Haematological properties	Group of rats		
	Control	BD0.1	BD0.25
RBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	9.27±0.14 <sup>a</sup>	9.05±0.22 <sup>a</sup>	9.04±0.25 <sup>a</sup>
Hb (gdL-1)	12.45±0.24 <sup>a</sup>	12.34±0.43 <sup>a</sup>	12.23±0.34 <sup>a</sup>
MCV (µ3)	49.36±3.11 <sup>a</sup>	47.53±2.78 <sup>a</sup>	46.43±3.52 <sup>a</sup>
MCH (µg)	13.77±0.85 <sup>a</sup>	14.30±0.69 <sup>a</sup>	14.72±0.76 <sup>a</sup>
MCHC (%)	31.10±1.84 <sup>a</sup>	30.87±1.98 <sup>a</sup>	31.60±2.31 <sup>a</sup>
PCV (%)	45.63±2.44 <sup>a</sup>	43.76±2.73 <sup>a</sup>	43.36±2.46 <sup>a</sup>
ESR (mm h <sup>-1</sup> )	0.92±0.01 <sup>a</sup>	0.91±0.01 <sup>a</sup>	0.91±0.01 <sup>a</sup>
Platelets (x 10 <sup>3</sup> mm <sup>-3</sup> )	206±4.49 <sup>a</sup>	204±5.99 <sup>a</sup>	201±5.77 <sup>a</sup>
WBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	11.28±0.56 <sup>a</sup>	12.14±0.78 <sup>a</sup>	12.28±0.89 <sup>a</sup>
Neutrophils (x 10 <sup>3</sup> mm <sup>-3</sup> )	2.62±0.23 <sup>a</sup>	2.61±0.22 <sup>a</sup>	2.61±0.21 <sup>a</sup>
Eosinophils (%)	0.03±0.001 <sup>a</sup>	0.03±0.001 <sup>a</sup>	0.03±0.001 <sup>a</sup>
Basophils (x 10 <sup>3</sup> mm <sup>-3</sup> )	0.02±0.00 <sup>a</sup>	0.02±0.001 <sup>a</sup>	0.02±0.001 <sup>a</sup>
Lymphocytes (x 10 <sup>3</sup> mm <sup>-3</sup> )	6.73±0.52 <sup>a</sup>	8.08±0.64 <sup>a</sup>	8.14±0.53 <sup>a</sup>
Monocytes (x 10 <sup>3</sup> mm <sup>-3</sup> )	0.03±0.00 <sup>a</sup>	0.03±0.001 <sup>a</sup>	0.03±0.001 <sup>a</sup>

Values are means ± SEM for 10 rats. <sup>a,b,c</sup> Row values with different superscripts are significantly different (p<0.05). RBC=red blood count, HB=haemoglobin, MCV=mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration, PCV=packed cell volume, WBC=white blood count

**Table 2. Serum concentration of selected kidney function indicators of rats placed on diet formulated with catfish exposed to biodiesel polluted water over a period of 30 days**

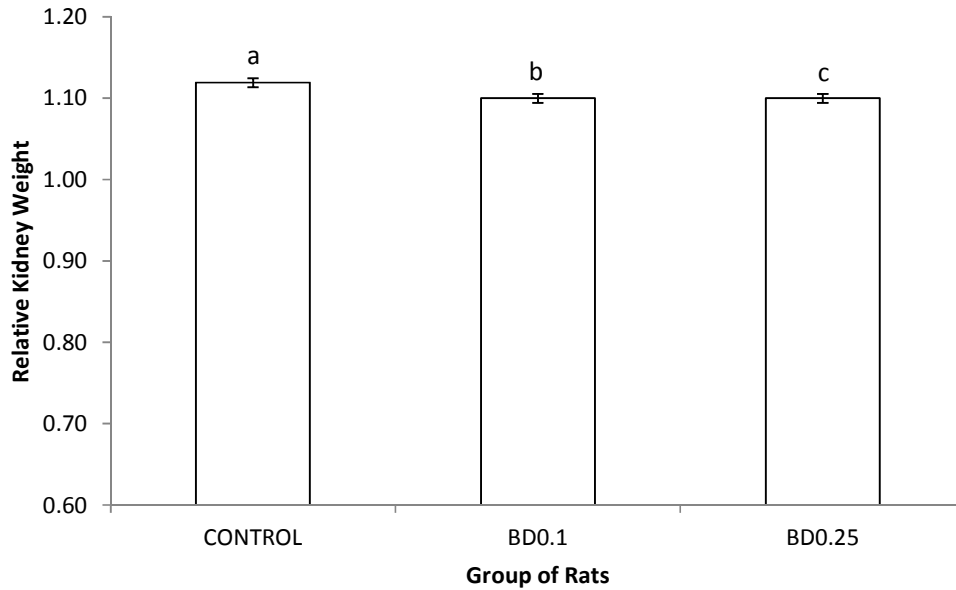
Group of rats	Urea (mmol/L)	Creatinine (mg/dL)	Na <sup>+</sup> (mmol/L)	K <sup>+</sup> (mmol/L)
Control	5.52±0.12 <sup>a</sup>	0.22±0.01 <sup>a</sup>	119±1.37 <sup>a</sup>	5.57±0.14 <sup>a</sup>
BD0.1	6.24±0.24 <sup>b</sup>	0.28±0.01 <sup>b</sup>	125±2.11 <sup>b</sup>	6.38±0.16 <sup>b</sup>
BD0.25	7.05±0.18 <sup>c</sup>	0.34±0.01 <sup>c</sup>	130±1.87 <sup>c</sup>	7.13±0.15 <sup>c</sup>

Values are means ± SEM for 10 rats. <sup>a,b,c</sup> Column values with different superscripts are significantly different (p<0.05)

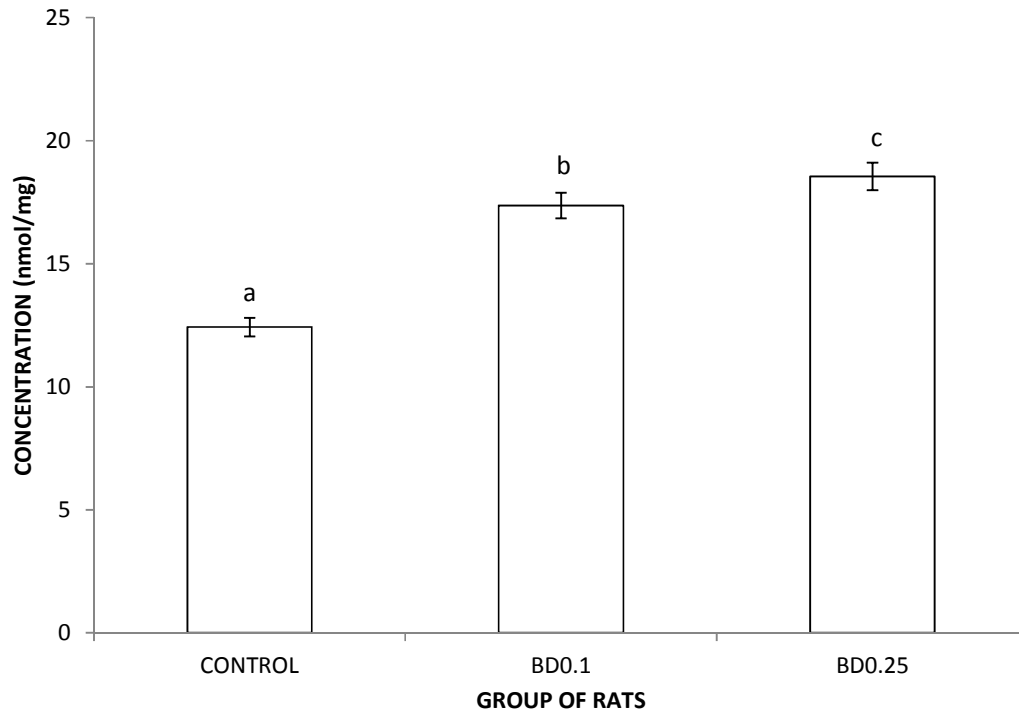
**Table 3. Specific activity (U/mg protein) of selected enzymes of the kidney of rats placed on diet formulated with catfish exposed to biodiesel polluted water over a period of 30 days**

Group of rats	ALP	ACP	LDH	CAT	SOD	GST
Control	188.24±6.68 <sup>a</sup>	21.27±1.08 <sup>a</sup>	19.67±1.44 <sup>a</sup>	1.10±0.01 <sup>a</sup>	1.06±0.01 <sup>a</sup>	0.96±0.01 <sup>a</sup>
BD0.1	156.88±9.84 <sup>b</sup>	15.37±1.24 <sup>b</sup>	12.51±1.24 <sup>b</sup>	0.58±0.01 <sup>b</sup>	0.86±0.01 <sup>b</sup>	0.64±0.01 <sup>b</sup>
BD0.25	149.02±6.77 <sup>b</sup>	12.58±1.15 <sup>b</sup>	10.33±1.32 <sup>b</sup>	0.55±0.01 <sup>c</sup>	0.58±0.01 <sup>c</sup>	0.55±0.01 <sup>c</sup>

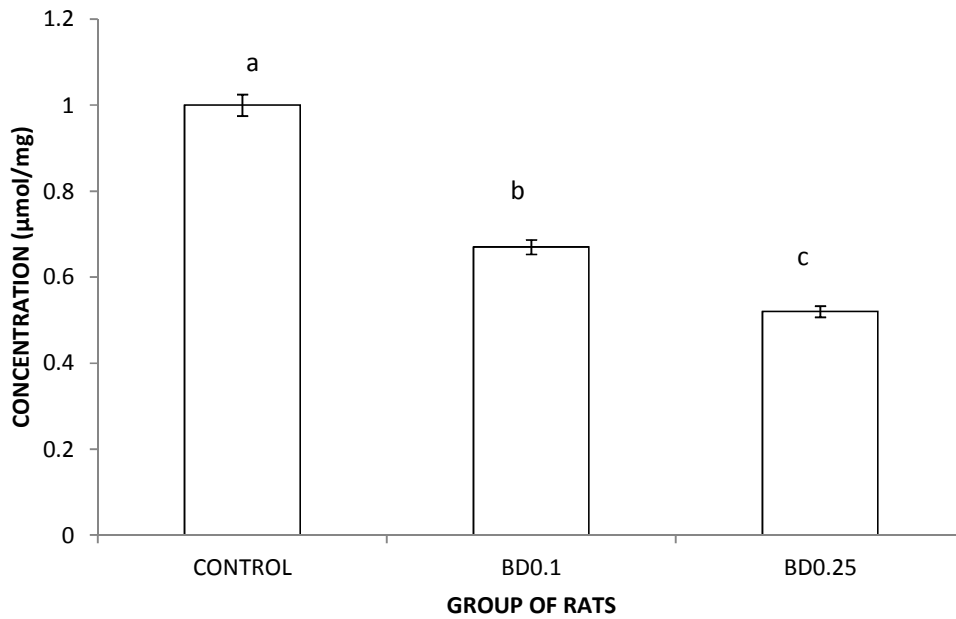
Values are means ± SEM for 10 rats. <sup>a,b,c</sup> Column values with different superscripts are significantly different (p<0.05). ALP: alkaline phosphatase, ACP: acid phosphates, LDH: lactate dehydrogenase, CAT: catalase, SOD: superoxide dismutase, GST: glutathione-S-transferase



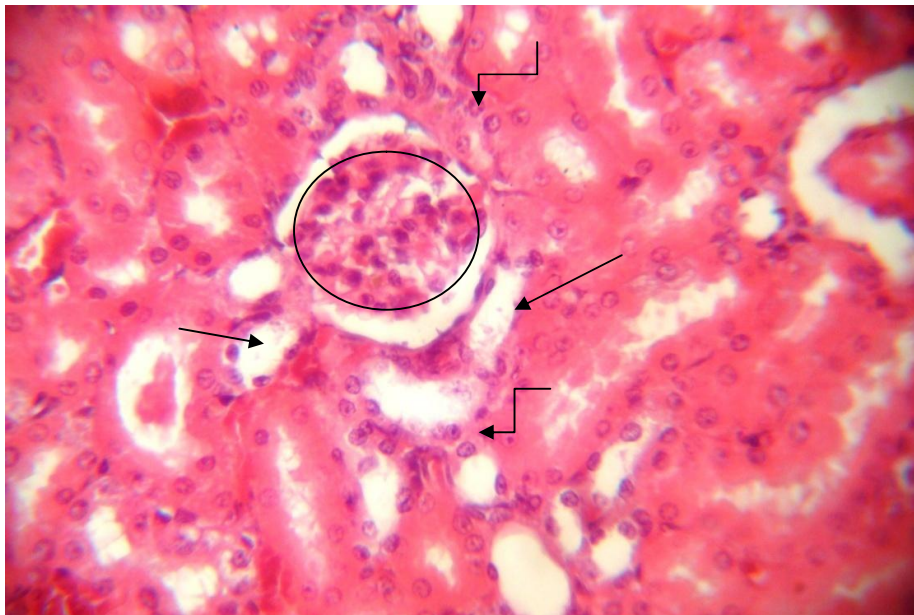
**Fig. 1. Effects of catfish exposed to PKO biodiesel on relative kidney weight. Plotted values are means ± SEM for 10 rats. <sup>a,b,c</sup> Column values with different superscripts are significantly different (p<0.05).**



**Fig. 2. Effects of catfish exposed to PKO biodiesel on kidney MDA. Plotted values are means ± SEM for 10 rats. <sup>a,b,c</sup> Column values with different superscripts are significantly different (p<0.05)**

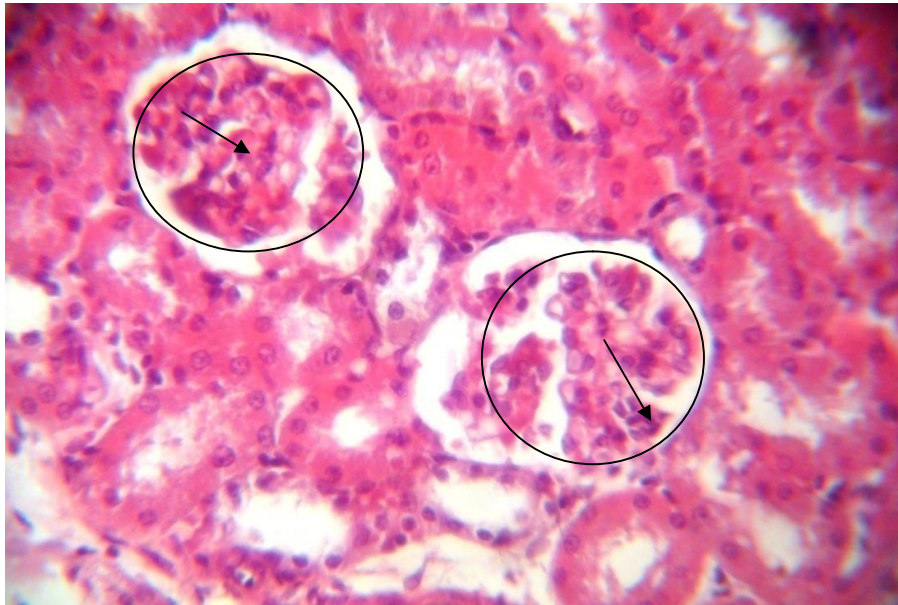


**Fig. 3. Effects of catfish exposed to PKO biodiesel on relative kidney GSH. Plotted values are means  $\pm$  SEM for 10 rats. <sup>a,b,c</sup> Column values with different superscripts are significantly different ( $p < 0.05$ )**

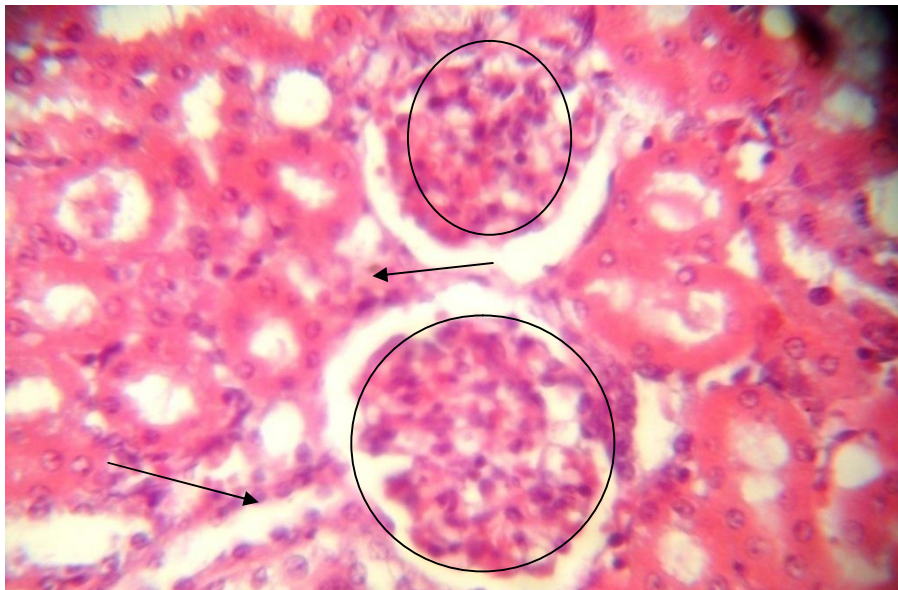


**Fig. 4. Light photomicrograph (H&E, x40) of kidney of rats Fed with diet formulated with catfish. Transverse section reveals distinct renal tubules and corpuscles (straight arrows), prominent macula densa (circle) and distinct nuclei (bent arrows)**





**Fig. 5.** Light photomicrograph (H&E, x40) of kidney of rats Fed with diet formulated with catfish exposed to 0.1% v/v biodiesel contaminated water. The glomeruli appear distinct with few proliferating inflammatory cells. The nucleus appears distinct



**Fig. 6.** Light photomicrograph (H&E, x40) of kidney of rats Fed with diet formulated with catfish exposed to 0.25% v/v biodiesel contaminated water. Membranoproliferative glomerulonephritis (circles), showing mesangial cell proliferation, increased mesangial matrix view showing segmental sclerosis (arrows) in two of seven glomeruli showing demarcated hyaline insudation slightly vacuolated in sclerotic layer

In toxicological experiments, comparison of organ weights between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of the test article

[28]. The reduced relative kidney weight (Fig 1) observed for test rats in this study could portend toxic effect of catfish exposed to biodiesel.



Elevated serum concentrations of Na<sup>+</sup> and K<sup>+</sup> in the serum of animals placed fed with catfish exposed to PKO biodiesel (Table 2) is suggestive of kidney dysfunction. Similarly, the elevated serum urea and creatinine of test rats lend credence to impaired renal function earlier suggested. It could be that the PKO biodiesel and/or its metabolic products in the tissues of the catfish are nephrotoxic. The measured substances, Na<sup>+</sup>, K<sup>+</sup>, urea and creatinine gives the best estimate of number of functioning nephrons and functional renal mass [29].

The kidney has been reported to be very sensitive to food toxicity responding with abnormal enzyme activities, anomalous concentrations of antioxidants and some serum metabolites [27]. The decreased activity of ALP, ACP and LDH observed for the kidney of test rats (Table 3) is likely to be as a result of loss of renal integrity and reduced performance. In addition, a condition of oxidative stress may be linked with the reduced activity of CAT, SOD and GST of kidney of test rat.

Malondialdehyde (MDA) is a lipid peroxidation product generated from reactive oxygen species (ROS), and as such is assayed in vivo as a biomarker of oxidative stress [30]. The significant increase in the levels of MDA of kidney of experimental rats (Fig. 2) lend credence to the view that biodiesel contaminated fish caused a reduction in the total antioxidant status of experimental rat by reactive oxygen species. In this study, catfish exposed to PKO biodiesel quickly depletes kidney glutathione levels (Fig. 3) it is, therefore, a potential agent which can lead to further lipid peroxidation.

In feeding experiments of this nature, it is equally important to conduct histopathological investigation to complement morphological, haematological, and biochemical examinations; hence the effect of catfish exposed to biodiesel on cells of vital organs such as the kidney was determined through histopathological examination (Figs. 4-6). The adverse effect of the cellular architecture of the kidney of test rats increased with increased level of biodiesel ranging from few proliferating inflammatory cells (Fig. 5) to membranoproliferative glomerulonephritis (Fig. 6).

## 6. CONCLUSION

Conclusively, data from this study provided evidence that consumption of catfish exposed to PKO biodiesel can, most likely, compromise

renal integrity, reduce renal performance, subject the kidney to oxidative stress and inflict varying degree of inflammation on the kidney.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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