

Original Research Article

Toxicological evaluation of palm kernel oil (PKO) biodiesel-contaminated catfish on kidney of albino rats

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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⁺ and K⁺) 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 reduced relative kidney weight while serum levels of Na⁺, K⁺, urea, creatinine and kidney concentration of malodialdehyde (MDA) were elevated

28 significantly ($p < 0.05$) when compared with the controls. Activities of selected enzymes
29 of kidney studied were significantly lower in BD0.1 and BD0.25 rats relative to the
30 control. Particularly, the kidney activity of alkaline phosphatase (ALP) of control, and
31 rats fed with feed formulated with BD0.1 and BD0.25 are 188.24 ± 6.68 , 156.88 ± 9.84 ,
32 149.02 ± 6.77 U/mg protein, respectively. Histological examination revealed proliferation
33 of inflammatory cells in the kidney of experimental rat.

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

38 Keywords

39 Palm kernel oil; biodiesel; histopathology; kidney; haematology; oxidative stress

40 Introduction

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

47 Palm kernel oil (PKO) in Nigeria had hitherto been underutilized as edible oil. Available
48 records however ranked Nigeria as one of the world producers of palm kernel. Recently,
49 PKO has gained considerable recognition as raw material for production of biodiesel [2-
50 3]. Chemically, the PKO consist of triglyceride molecules of three long chain fatty
51 acids that are ester bonded to a single glycerol molecule. These fatty acids differ from
52 the length of carbon chains, the number, orientation and position of double bonds
53 in these chains. Alkaline catalysts such as NaOH and KOH are the most commonly
54 used in trans-esterification since their reaction is much faster than an acid-catalyzed
55 reaction [4]. Transportation of petroleum and petrochemicals is through pipelines that

Comment [u1]: Were there significant differences in the ALP levels among the groups?? What about the levels of other tissue enzymes??

Response
Yes. Included in the article

Comment [u2]: The inflammatory cells were not clearly demonstrated in the photomicrographs in the result section.

Response
The nephrocytes became inflammatory and these appear different from control. Expert histopathologist confirmed reading.

cut across land and water. Accidental 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 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.

Materials and Methods

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

Palm Kernel Oil (PKO)

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

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

87 *Preparation of Bio-diesel from PKO*

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

90 *Experimental Water and Fish Treatment*

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

100 At the end of the 30 h experimental period, the catfish were harvested, oven dried at
101 40°C and used as a source of protein (25%) to formulate diet for albino rats.
102 The diet for each group was formulated by mixing known quantities of sources
103 of each food class comprising corn starch (52%), oil (4%), maize cob (4%),
104 sucrose (10%) and vitamin/mineral mixture (5%). The food items were mixed
105 together and manually made into pellets to feed albino rats.

106 *Experimental Rats and Treatment*

107 Thirty albino rats of mean weight (51 ± 2.0 g) were procured from the animal house of
108 Department of Anatomy, University of Benin, Benin-City, Nigeria. All animals were
109 housed under standard laboratory conditions with free access to water *ad-libitum* and
110 balanced pellets food. The housing temperature was ($25 \pm 1^{\circ}\text{C}$) with 12 h dark

Comment [u3]: Male or female???

Response

Both sexes as the study is not gender sensitive

Comment [u4]: Mean weight???

Response

Mean weight. Thanks

111 /light cycle and 50% humidity. All ethical guidelines on the use of animals for
112 investigational purposes were followed and the experiment protocol was approved
113 by Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria ethics
114 committee.

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

122 At the end of the feeding exercise, the rats were anaesthetized by placing them in a jar
123 containing cotton wool soaked with chloroform before being sacrificed by jugular
124 puncture. The liver of was removed into a beaker containing ice cold 0.25 M sucrose
125 solution. The blood was obtained through their jugular veins. Each blood sample was
126 thereafter centrifuged at 3500 rpm for about 15 min using refrigerated centrifuge
127 RC650s and the serum obtained was preserved at -8⁰C until required for use. A portion
128 of the liver was homogenized for biochemical studies and enzyme assays. Haemoglobin
129 concentration of the blood of experimental animals was determined following the
130 method described by Mitruka and Rawnsley [8]. The RBC and WBC was done by the
131 method of manual counting, PCV by Microhaematocrit method, described by Muthaya
132 [9]. Other haematological parameters were determined as described by Tietz [10]. MDA
133 determination was based on method described by Bird et al. [11]. The method
134 described by Jollow *et al.* [12] was used to determine reduced glutathione (GSH)
135 concentration. Catalase (CAT) activity was determined according to the method of
136 Sinha [13]. The activity of superoxide dismutase (SOD) was determined by the method
137 of Misra and Fridovich [14]. The cytosolic glutathione s-transferase (GST) activity was
138 determined spectrophotometrically at 37⁰C (340nm) by the procedure described by
139 Habig *et al* [15]. Histopathological study, using hematoxylin and eosin (H&E) stain, of
140 the kidney obtained from experimental rats was carried out following the method
141 described by Drury and Wallington [16]. Protein concentration was determined by the

142 biuret reaction described [17]. The method used for assaying lactate dehydrogenase
143 (LDH) is based on that of Wroblewski and La Due [18] in which pyruvate is reversibly
144 reduced to lactate in the presence of nicotinamide adenine dinucleotide (reduced) as
145 co-enzyme. The method of Bessey et al. [19] as modified by Wright et al. [20] was
146 employed in the determination of alkaline phosphatase (ALP) and acid phosphatase
147 (ACP). Serum sodium and potassium were determined using flame photometry method.
148 The diacetyl monoxime method using thiosemicarbazide as described by Marsh et al.
149 [21] was used for serum urea determination. Serum creatinine was determined using
150 the method described by Brod and Sirota [22]. Histology and haematology studies were
151 done at the University of Benin Teaching Hospital (UBTH) Nigeria, while other studies
152 were carried out at the Environmental Science Laboratory of the Federal University of
153 Petroleum Resources, Effurun, Nigeria.

154 Statistical Analyses

155 All numerical results were obtained from the three (3) groups (control and treated). Data
156 obtained were presented as mean $\pm$ SEM and subjected to statistical analysis using a
157 one way analysis of variance (ANOVA) by employing the method of Steel and Torrie
158 [23]. Significant difference between the treatment means was determined at 95%
159 confidence level using Duncan's Multiple range test [24].

160 Results

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

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

Comment [u5]: Author should state the facility/laboratory where these analyses were carried out???

Response
Laboratories now indicated

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

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

173 Values are means ± SEM for 10 rats. ^{a,b,c} Row values with different superscripts are
 174 significantly different (p<0.05).

175 RBC=red blood count, HB=haemoglobin, MCV=mean corpuscular volume, MCH=mean
 176 corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration,
 177 PCV=packed cell volume, WBC=white blood count

178

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

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

182 Values are means ± SEM for 10 rats. ^{a,b,c} Column values with different superscripts are
 183 significantly different (p<0.05).

184 Effect of catfish exposed to PKO biodiesel on the activities of selected kidney enzymes
185 of rat is presented in Table 3. Activities of kidney ALP, ACP and LDH of control rat were
186 significantly higher ($p<0.05$) than those of test rats, while those of BD0.1 were not
187 significantly different from those of BD0.25 rats. Similarly, activities of antioxidant
188 enzymes, CAT, SOD and GST of control rat were significantly higher ($p<0.05$) than
189 those of test rats. Activities of the antioxidant enzymes of kidney of test rats were found
190 to decrease significantly ($p<0.05$) from BD0.1 to BD0.25 rats.

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

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

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

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

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

GROUP OF Rats	ALP	ACP	LDH	CAT	SOD	GST
Control	188.24±6.68 ^a	21.27±1.08 ^a	19.67±1.44 ^a	1.10±0.01 ^a	1.06±0.01 ^a	0.96±0.01 ^a
BD0.1	156.88±9.84 ^b	15.37±1.24 ^b	12.51±1.24 ^b	0.58±0.01 ^b	0.86±0.01 ^b	0.64±0.01 ^b
BD0.25	149.02±6.77 ^b	12.58±1.15 ^b	10.33±1.32 ^b	0.55±0.01 ^c	0.58±0.01 ^c	0.55±0.01 ^c

212 Values are means ± SEM for 10 rats. ^{a,b,c} Column values with different superscripts are
213 significantly different (p<0.05).

214 ALP: alkaline phosphatase, ACP: acid phosphates, LDH: lactate dehydrogenase, CAT:
215 catalase, SOD: superoxide dismutase, GST: glutathione-S-transferase

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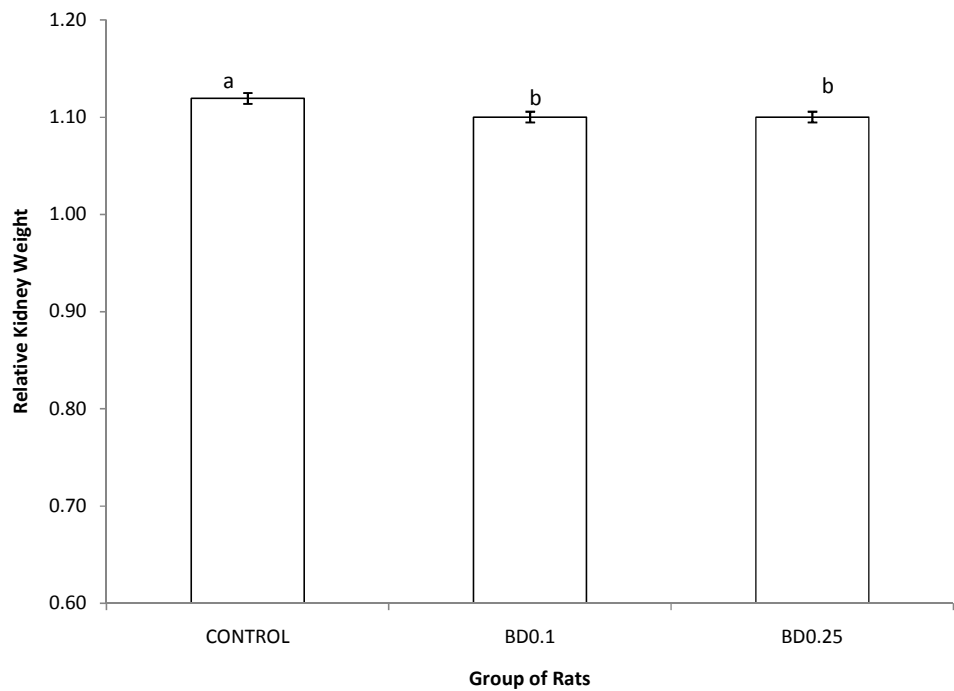
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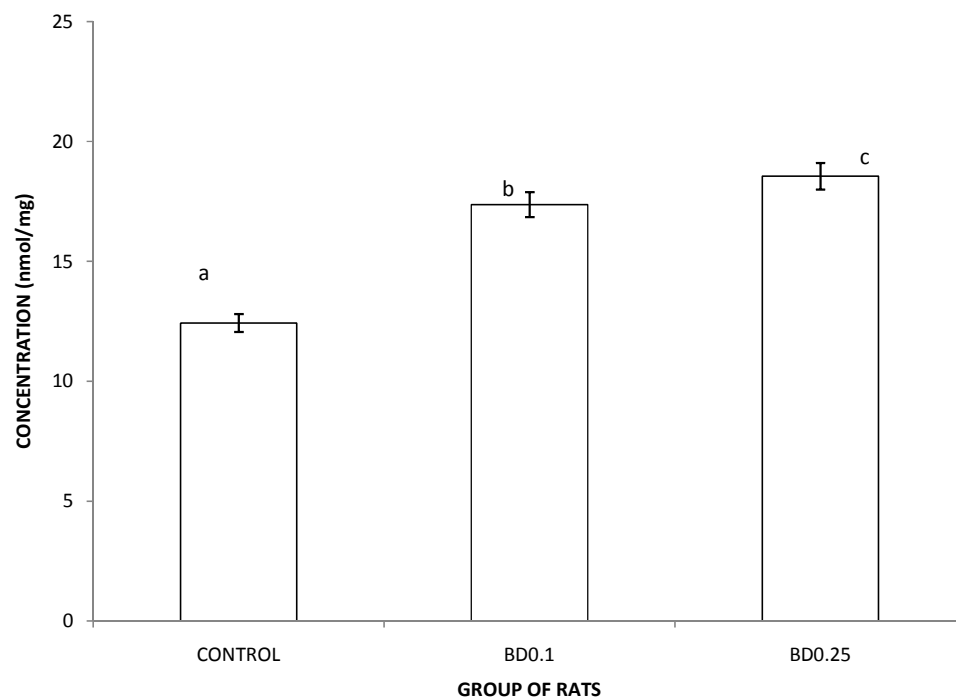
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233 Figure 1: Effects of catfish exposed to PKO biodiesel on relative kidney weight. Plotted
234 values are means $\pm$ SEM for 10 rats. ^{a,b,c} Column values with different superscripts are
235 significantly different (p<0.05).

236



237

238 Figure 2: Effects of catfish exposed to PKO biodiesel on kidney MDA. Plotted values are

239 means $\pm$ SEM for 10 rats. ^{a,b,c} Column values with different superscripts are significantly

240 different ($p < 0.05$).

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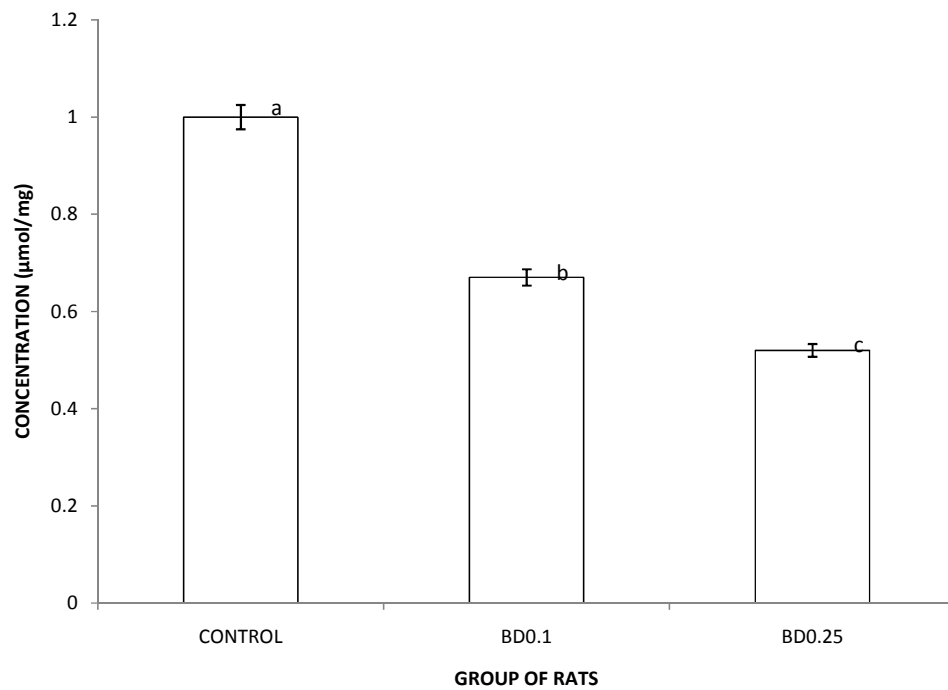
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 250 Figure 3: Effects of catfish exposed to PKO biodiesel on relative kidney GSH. Plotted
 251 values are means $\pm$ SEM for 10 rats. ^{a,b,c} Column values with different superscripts are
 252 significantly different ($p < 0.05$).

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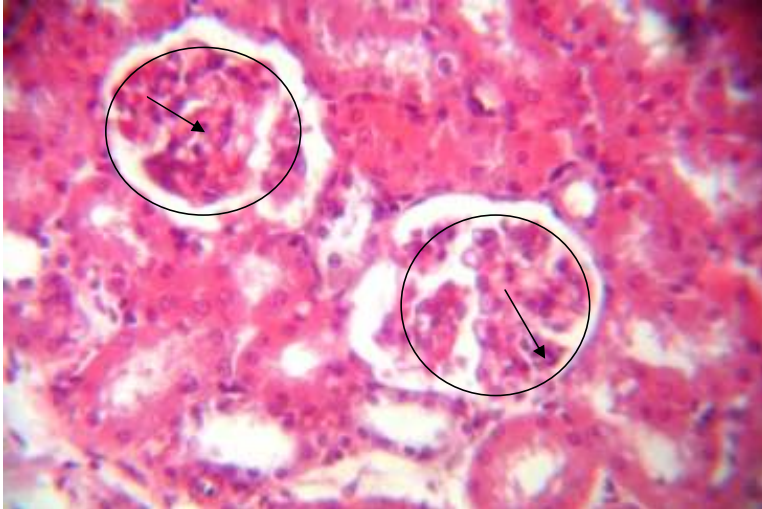
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Figure 4: Light **photo**micrograph (H&E, x40) of kidney of rats Fed with diet formulated with catfish. Transverse section reveals distinct renal tubules and corpuscles (straight arrows), prominent macula densa (circle) and distinct nuclei (bent arrows).

Comment [u6]: Which staining technique was used???

Response

Hematoxylin and eosin used as stain. Now indicated



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Figure 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.

Comment [u7]: This slide reading here is incorrect.
Response
Expert histopathologist confirmed correctness.

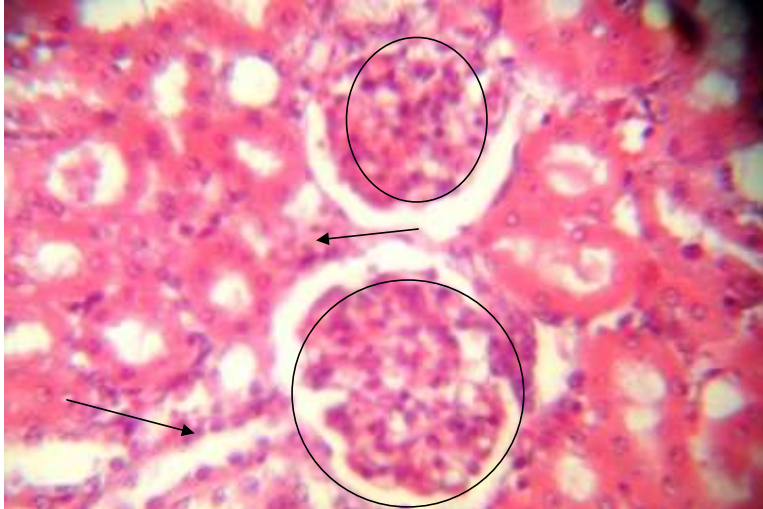


Figure 6: Light photomicrograph (H&E, x40) of kidney of rats Fed with diet formulated with catfish exposed to 0.25% v/v biodiesel contaminated water. Membranoproliferative glomerulonephritis (circles), showing mesangial cell proliferation, increased mesangial matrix view showing segmental sclerosis (arrows)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

Comment [u8]: There are no obvious hyaline insudation in this slide.
Response
 Insudation is demarcated and vacuolated.

289 Discussion

290 Aquaculture is the fastest growing food sector in the world, accounting for an estimated
291 43 % of all fish consumed by humans globally [25]. However, presence of biodiesel and
292 other synthetic chemicals, which are non-biodegradable, accumulate in the
293 environment to pose serious toxic threat to both aquatic animals and their
294 consumers [26]. The present study investigated the effect of catfish exposed to PKO
295 biodiesel on the kidney in albino rats.

296 Nutritional status of an individual is dependent on dietary intake and effectiveness of
297 metabolic processes. These can be determined by either or combinations of clinical,
298 anthropometric, biochemical or dietary methods. The value of blood components is an
299 indication of availability of nutrients for synthesis of blood cells. Data from this study
300 revealed that haematological properties of rat fed with catfish exposed to biodiesel
301 compete favourably with those of control (Table 1). Several studies have reported
302 changes in haematological parameters routinely used to determine stress associated
303 with environmental, nutritional, and/or pathological factors [7, 27].

304 In toxicological experiments, comparison of organ weights between treated and
305 untreated groups of animals have conventionally been used to evaluate the toxic effect
306 of the test article [28]. The reduced relative kidney weight (Figure 1) observed for test
307 rats in this study could portend toxic effect of catfish exposed to biodiesel.

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

315 The kidney has been reported to be very sensitive to food toxicity responding with
316 abnormal enzyme activities, anomalous concentrations of antioxidants and some serum

Comment [u9]: Statement appears to be ambiguous. Rephrase.

Comment [u10]: Did the author relate the decreased organ weight to the total body weight??? Author should present the organ and body weights differently for more clarity.
Response
The study is on the kidney, kidney body weight ratio is presented as relative weight. This is considered adequate for this study.

317 metabolites [27]. The decreased activity of ALP, ACP and LDH observed for the kidney
318 of test rats (Table 3) is likely to be as a result of loss of renal integrity and reduced
319 performance. In addition, a condition of oxidative stress may be linked with the reduced
320 activity of CAT, SOD and GST of kidney of test rat.

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

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

335 **Conclusion**

336 Conclusively, data from this study provided evidence that consumption of catfish
337 exposed to PKO biodiesel can, most likely, compromise renal integrity, reduce renal
338 performance, subject the kidney to oxidative stress and inflict varying degree of
339 inflammation on the kidney.

340 **Conflict of interest**

341 The author declares that there are no conflicts of interest.

342

343 **Acknowledgements**

344 The author acknowledges Prof O.B Oloyede and Prof (Mrs) A.T. Oladiji, Department of
345 Biochemistry, University of Ilorin, for their assistance.

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