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Toxicological evaluation of the effect of African cat fish (*Clarius gariepinus*) from water contaminated with biodiesel on the liver of Albino rats

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Abstract

This study investigated the effect of African cat fish (Clarias gariepinus) from water contaminated with biodiesel on the liver of albino r*ats.* The Biodiesel fr*om PKO was diluted with distilled*water to obtain 0.25 and 0.1 %v/v. Twenty-four healthy juvenile catfish (Clarias gariepinus) were obtained from a commercial fish pond 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. Group A served as control and the catfish here were cultured *in distilled* water while those in Groups B and C 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 ofprotein (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%). Generally, it was observed that concentrations of direct and total bilirubin of rats increased significantly ($p<0.05$) as the level of biodiesel increased while the serum concentrations of albumin and globulin decreased significantly ($p<0.05$) with increasing level of biodiesel. There was no significant difference ($p>0.05$) between the concentration of serum total bilirubin of BD0.1 rats and the control, while that of BD0.25 rats was significantly higher than that of control ($p<0.05$). Specific activities of alanine transaminase (ALT), asparatate transaminase (AST) and Gama glutamyl transpeptidase (GGT) of serum of BD0.1 and BD0.25 rats were not significantly different($p>0.05$) but significantly lower than those of control rats. Elevated serum bilirubin of rats observed in this study suggested liver dysfunction which may not be unconnected with catfish exposed to biodiesel from PK*O*. Abnormal values of serum GGT, ALT and AST also indicated that the catfish exposed to PKO biodiesel might have altered protein metabolism, among others, at the subcellular level and this may be indicative of impairment of the function of the tissues. The observed decreased activity of SOD, CAT and GST in the liver of rats fed with catfish exposed to PKO biodiesel was indicative of oxidative stress.

Keywords: Biodiesel; PKO; Clarius gariepinus; Albino rats; liver; ALT; AST; GGT; Toxicity.

1. Introduction

In a quest to satisfy the increasing demand of conventional fuel and monitor the Environmental impact associated with it; the production of synthesized (biofuel) fuel has also been discovered and used which in most time considered being more environmental friendly compared to the already existing fossil fuel. The word fuel is for any substance or material that is combustible and produces heat or energy (Ratcliff, 2000). Example of such material is coal, petrol, gasoline,

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plastic, nylon, woods, charcoal, just to mention but a few. Thus, the word biofuel is used majorly for biological produced fuel.

Biodiesel is an alternative fuel similar to conventional or 'fossil' diesel. Biodiesel can be produced from straight vegetable oil, animal oil/fats, tallow and waste cooking oil (Alternative Fuel Data Center, 2012). The process used to convert these oils to Biodiesel is called transesterification. The largest possible source of suitable oil comes from oil crops such as rapeseed, palm or soybean. In the UK rapeseed represents the greatest potential for biodiesel production. Most biodiesel produced at present is produced from waste vegetable oil sourced from restaurants, chip shops, industrial food producers such as Birdseye etc. Though oil straight from the agricultural industry represents the greatest potential source it is notbeing produced commercially simply because the raw oil is too expensive. After the cost of converting it to biodiesel has been added on it is simply too expensive to compete with fossil diesel. Waste vegetable oil can often be sourced for free or sourced already treated for a small price. (The waste oil must be treated before conversion to biodiesel to remove impurities).

The scope of this study relies on the importance of determining the physicochemical effects and characteristics of Palm kernel oil (PKO) biodiesel-contaminated catfish on the liver of human using albino rat (Rattus novergicus) as model. This study will as well be used as a reference material for further investigation on the toxic effect of PKO biodiesel on all living things as well other area of toxicological evaluation.

Rodents are used as models in medical testing in that their genetic, biological and behavior characteristics closely resemble those of humans, and many symptoms of human conditions can be replicated in mice and rats (Maxwell, 2016). "Rats and mice are mammals that share many processes with humans and are appropriate for use to answer many research questions," said Jenny Haliski, a representative for the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (Medina, 2010).

Over the last two decades, those similarities have become even stronger. Scientists can now breed genetically-altered mice called "transgenic mice" that carry genes that are similar to those that cause human diseases. Likewise, select genes can be turned off or made inactive, creating "knockout mice," which can be used to evaluate the effects of cancer-causing chemicals (carcinogens) and assess drug safety, according to the FBR. Rodents also make efficient research animals because their anatomy, physiology and genetics are well-understood.

This investigation is carried out to assess the effect of cat fish from water contaminated with biodiesel on the liver of albino rats based on; Serum levels of some indicators of liver function such as bilirubin, globulin, albumin e.t.c., levels of malodialdehyde, a product of lipid peroxidation, and reduced glutathione, a non-enzymic antioxidant and Specific activities of selected enzymes of the liver such as ALT, AST, GGT, GST, CAT, SOD, GSH e.t.c.

The study is carried out to examine the danger posed by consuming fishes from contaminated water especially those in water body exposed to oil spillage; rivers used as dump sites by industries; dirty pond where water runoff from waste site is used in their cultivation etc.

2. Methods

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

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 78oC; 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.

20.0g of ethanol was measured and poured into a plastic container after which 1.0g of NaOH was carefully added. The container was swirled round thoroughly for about 2 min repeatedly about six times for complete dissolution of NaOH in the ethanol to form sodium ethoxide.100.0 g of PKO was measured out, pre-heated to 60oC in a beaker and poured into the blender. Sodium ethoxide from the plastic container was carefully poured into the PKO, the blender lid was secured tightly and the blender switched on while agitation in the blender was maintained for 90 min.

The mixture was poured from the blender into a PET bottle for settling and the lid was screwed on tightly. The reaction mixture was allowed to stand overnight while phase separation occurred by gravity settling. The PKO biodiesel was carefully decanted into a PET bottle leaving the glycerol at the base. The biodiesel was washed with water. The procedure was replicated three times and average biodiesel yield as well as glycerol yield was measured on weight basis

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) 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. Group A served as control and the catfish here were cultured in distilled water while those in Groups B and C 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 40oC 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.

Thirty albino rats of (51±2.0g) 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 ± 1°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 Group A served as the control and they were fed on the control diet, which was formulated with catfish cultured in borehole water. Animals in Groups B and C 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.

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 (1960). Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple range test (1955)

3. Results

The serum levels of some indicators of liver function were monitored. Table 3.1 presents the results of serum concentrations of bilirubin, albumin and globulin of rats placed on feed formulated with catfish exposed to biodiesel over a period of 30 days. Generally, it was observed that concentrations of direct and total bilirubin of rats increased significantly ($p<0.05$) as the level of biodiesel increased while the serum concentrations of albumin and globulin decreased significantly ($p<0.05$) with increasing level of biodiesel. The concentration of direct bilirubin in BD0.25 rats is about 2 folds that of the control. There was no significant difference ($p>0.05$) between the concentration of serum total bilirubin of BD0.1 rats and the control, while that of BD0.25 rats was significantly higher than that of control ($p<0.05$). The serum globulin concentration of control rats is about 1 ½ fold that of the BD0.25 and BD0.1 rats.

Figure 3.1 presents the level of serum malondialdehyde (MDA) of rats placed feed formulated with catfish exposed to biodiesel. The general trend observed was that serum level of MDA increases significantly ($p<0.05$) with increasing level of biodiesel. The serum MDA levels of test rats were about 1 ½ fold that of the control.

Results of concentration of serum reduced glutathione (GSH) of rats placed on diet formulated with catfish exposed to biodiesel over a period of 30 days is presented in Figure 2. Here, concentration of serum GSH of test rats was significantly lower than that of the control ($p<0.05$). GSH concentration of serum of control rats is twice that of the rats placed on diet formulated with catfish exposed to biodiesel.

Specific activities of selected liver enzymes of rats exposed to biodiesel were assayed and the result presented in Table

Specific activities of alanine transaminase (ALT), asparatate transaminase (AST) and Gama glutamyl transpeptidase (GGT) of serum of BD0.1 and BD0.25 rats were not significantly different ($p>0.05$) but significantly lower than those of control rats. Conversely, specific activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase (GST) of serum of BD0.1 is significantly higher than that of BD0.25 (p<0.05) while both are significantly lower than that of the control rats (p<0.05). Generally, specific enzyme activities of the selected enzymes decreased as the level of biodiesel increased.

Table 1 Serum concentration of selected liver function indicators of rats placed on diet formulated with catfish exposed to biodiesel polluted water over a period of 30 days.

	Direct		**Albumin**	**Globulin**
Group of Rats	(mM/L)	Total (mM/L)	(g/dL)	(g/dL)
CONTROL	1.04±0.02 a	5.45±0.21 a	16.89±1.35 a	10.54±1.09 a
BD0.1	1.26±0.02b	6.02±0.52 ab	14.66±1.64b	7.72±1.30 b
BD0.25	1.94±0.02c	6.56±0.64b	12.31±1.55 b	6.48±0.97 b

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

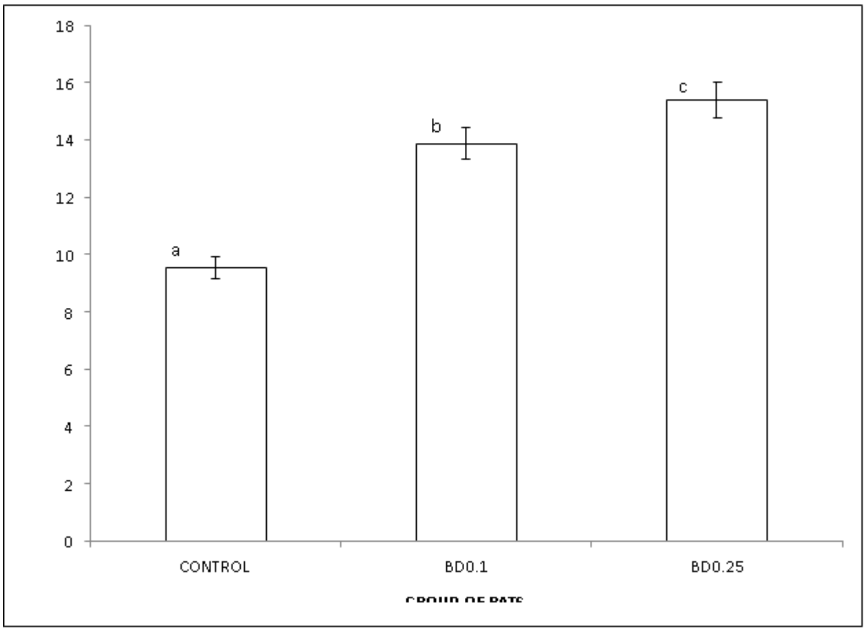

Figure 1 Effects of catfish exposed to PKO biodiesel on liver MDA. Plotted values are means ± SEM for 10 rats. ^{a,b,c} Column values with different superscripts are significantly different (p<0.05)

Table 2 Specific activity (U/mg protein) of selected enzymes of the liver of rats placed on diet formulated with catfish exposed to biodiesel polluted water over a period of 30 days

Group of Rats	**AST**	**ALT**	**GGT**	**CAT**	**SOD**	**GST**
CONTROL	24.9±1.19^{a}	14.6±1.64^{a}	67.9±4.36^{a}	0.82±0.03^{a}	1.21±0.02^{a}	1.32±0.02^{a}
BD0.1	18.8±1.94^{b}	10.4±1.35^{b}	53.2±4.26^{b}	0.61±0.02^{b}	0.92±0.01^{b}	0.88±0.02^{b}
BD0.25	15.2±1.69^{b}	10.0±1.89^{b}	47.3±4.90^{b}	0.55±0.03^{c}	0.81±0.02^{c}	0.56±0.04^{c}

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

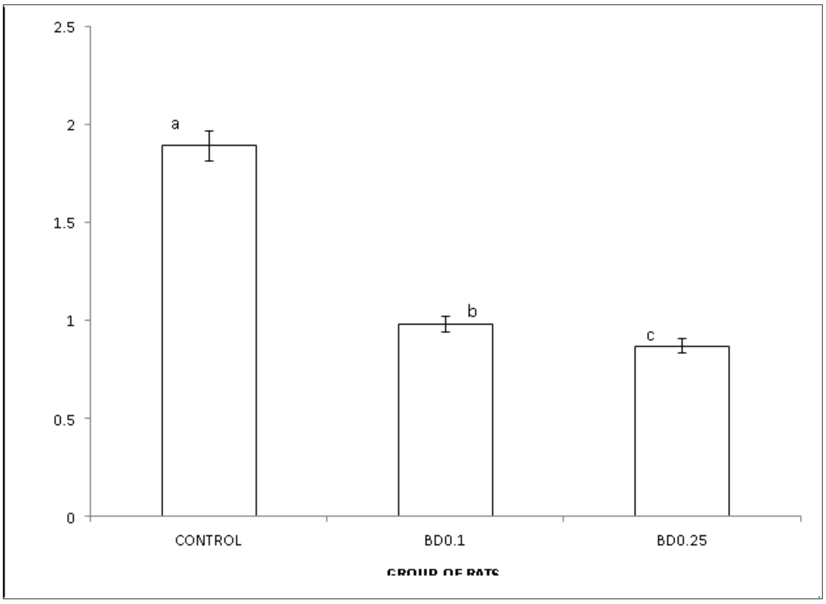

Figure 2 Effects of catfish exposed to PKO biodiesel on relative liver GSH. Plotted values are means ± SEM for 10 rats. a,b,c Column values with different superscripts are significantly different ($p<0.05$).

4. Discussion

The current study demonstrated that catfish exposed to biodiesel from PKO significantly affects function and oxidative status of liver of albino rats. This is the first investigation to report that catfish exposed to biodiesel from PKO decreased liver antioxidant status of albino rats. In addition, these data are the first to demonstrate liver dysfunction of albino rats fed with catfish exposed to environmentally relevant concentration of biodiesel from PKO.

Since organ weight evaluation is an essential part of the toxicologic and risk assessment of drugs, chemicals, biologics, food additives, and medical devices, the relative liver weights of the experimental rats were determined and results were presented in Figure 2. These results revealed that consumption of fish exposed to biodiesel form PKO did not significantly affect relative liver weights of albino rats. However, Haley et al. (2005) reported that organ weight changes themselves were not necessarily toxic effects and emphasized that the organ weight data should be assessed in the context of the entire study. Therefore, the data on relative liver weight may not imply non-toxic effect.

Bilirubin is an endogenous compound that can be toxic (Tiribelli and Ostrow, 2005), especially in neonates. Bilirubin is the ultimate breakdown product of haemoglobin and serves as a diagnostic marker of liver and blood disorders. Elevated serum bilirubin of rats observed in this study (Table 3) suggested liver dysfunction which may not be unconnected with catfish exposed to biodiesel from PKO. 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 water soluble) 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 (Adeyemi et al., 2010). In this study, rats fed with catfish exposed to PKO biodiesel presented elevated serum concentrations of both total and direct bilirubin suggesting that the liver is not able to excrete bilirubin which is an evidence of liver dysfunction.

Serum albumin and globulin concentrations are some biochemical indices for monitoring liver function in the blood (Table 3). Abnormal levels of these proteins have been reported to be associated with haemolysis or increased breakdown of RBC and/ or liver damage (Islam et al., 2004). Decreased serum concentrations of albumin and globulin as observed in this study lend credence to the submission that the liver function may be impaired. Both globulin and albumin are produced by the liver. If the liver is damaged, it may no longer be able to 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 fish.

Hepatic ALT, AST, GGT, GST, CAT, SOD, GSH and lipid peroxidation product (malondialdehyde, MDA) are commonly used as biomarkers of hepatotoxicity (Saura et al., 2005). Abnormal values of serum GGT, ALT and AST have been observed for different diseased conditions, but most especially in hepatic diseases (Adeyemi et al., 2009). The experimental data (Tables 3) revealed that the catfish exposed to PKO biodiesel might have altered protein metabolism, among others, at the subcellular level and this may be indicative of impairment of the function of the tissues.

Ecological stressor such as biodiesel generate reactive oxygen species (ROS) such as O2 , H2O2 and OH radicals have potential to interact with many cellular components, causing significant damage to membrane and other cellular structures (Adeyemi et al., 2008). Some of the ROS are highly toxic and must be detoxified by cellular responses (Adeyemi et al., 2008). The ROS scavenging depends on the detoxification mechanism, which may occur as a result of sequential and simultaneous action of a number of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). The observed decreased activity of SOD, CAT and GST in the liver of rats fed with catfish exposed to PKO biodiesel was indicative of oxidative stress.

Malondialdehyde (MDA) is a lipid peroxidation product generated from reactive oxygen species (ROS), and as such is assayed in vivo as a bio-marker of oxidative stress (Day et al., 1999). Malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, malondialdehyde is reactive and potentially mutagenic. It has been found in heated edible oils such as sunflower and palm oils. The significant increase in the levels of MDA of liver of experimental rats (Figure 3) lend credence to the view that biodiesel caused a reduction in the total antioxidant status by reactive oxygen species.

GSH plays a very important role in the detoxification of xenobiotics. In vitro examinations proved that the free thiol group of glutathione reacts with xenobiotics to form conjugates. These conjugates reveal toxic properties (Flora, 2009). In this study, catfish exposed to PKO biodiesel quickly depletes hepatocyte glutathione levels (Figure 4). It is therefore, a potential agent to which can lead to further lipid peroxidation (Adeyemi, 2014).

5. Conclusion

In conclusion, the author has shown evidence that consumption of catfish exposure to PKO biodiesel alters growth and performance of albino rats. The indices of liver function observed, including serum levels of bilirubin, albumin, globulin, and activities of AST, ALT, GGT have not been reported previously in investigations of the effects of catfish exposed to PKO biodiesel on albino rats. The data on MDA, GSH and antioxidant enzymes (CAT, SOD, GST) of the liver of experimental rats suggests that consumption of catfish exposed to PKO biodiesel may predispose the organ to a condition of oxidative stress. Future study should consider light micrograph of the liver.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors confirm that neither of them has any personal or business interest in or potential gain from any of the part or whole body of this article.

Statement of ethical approval

All animal experiments were conducted in accordance with standard guidelines 22 on use of animals for experimental toxicology study.

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