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Research Article

Effects of Biodiesel from PKO on Selected Phosphatases and Transaminases of Some Tissues of African Catfish (*Clarias gariepinus*)

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Abstract

This study evaluates the effect of water contaminated with 0.1%v/v and 0.25%v/v of biodiesel from PKO (palm kernel oil) on the cellular system of African catfish, *Clarias gariepinus.* Generally, a significant decrease in the activity of the enzymes of the tissues of *C. gariepinus* cultivated in the contaminated water was observed relative to the control ($p < 0.05$). Particularly, activity of acid phosphatase of liver of *C. gariepinus* cultivated in 0.25%v/v concentration of biodiesel contaminated water was found to be 30.31 ± 1.54 nmol/mim/mg protein while that of control was 42.65 ± 1.49 nmol/mim/mg protein. It could be inferred that biodiesel from PKO could trigger biochemical responses in fish, at the experimental conditions tested, indicating that this fuel can also represent a risk to the aquatic biota.

Keywords: Transaminases, phosphatases, biodiesel, catfish, palm kernel oil, tissues

Introduction

The increased demand for alternative energy sources has created interest in biodiesel; biodiesel is promoted as a diesel substitute that is safer, produces less harmful combustion emissions, and biodegrades more easily. All these have increased its commercialization and use, making its fate in the environment a matter of concern. In the context of environmental contamination by petroleum special attention in the scientific community has been given to the impacts of accidental spills of oil and its derivatives. There are several scientific studies that address biological treatment of soils contaminated by petroleum products (Vieira et al.. 2009ab). The search for alternative sources of energy and sustainable processes in order to reduce environmental pollution and global warming has spurred the global market for clean fuels such as biodiesel, which is renewable and environmentally safe relative to fossil fuels. Biodiesel in its pure form or mixed with diesel can possibly generate accidental spills in the environment (soil and water) causing a potential risk of contamination. Although biodiesel is considered to be easily biodegradable, it is a foreign compound in natural environment like conventional diesel fuel.

Palm kernel oil (PKO) is one lauric vegetable oil in Nigeria, which had hitherto been underutilized as edible oil. Available records however ranked Nigeria as one of the world producers of palm kernel. Between 1995 and 1998, Nigeria's share in the world production of palm kernel were 0.27, 0.26 and 0.25 million metric ton for 1995/96, 1996/97 and 1997/98 production seasons respectively. This record placed Nigeria next to Malaysia and Indonesia, and ahead of PKO producing countries like Cote d'ivore, Colombia, Thailand, Zaire and Equador (Alamu et al. 2007a, b). Investments for the production of biodiesel from PKO in Nigeria have grown and increases concerns about possible contamination of water.

Animal models represent a major tool for the study of mechanisms in virtually all of biomedical research (Uetrecht 2006). Exposure to crude oil and its derivatives triggers some biochemical changes in aquatic animals in general and *Clarias gariepinus*, in particular (Achuba and Osakwe 2003). Effects of xenobiotics, such as biodiesel, may involve different mechanisms of cytolethality (Kedderis 1996; Kaplowitz 2004). These mechanisms may have either direct effect on organelles, or indirect effect on cellular organelles through the activation and inhibition of enzymes. Certain biochemical markers like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) can be used to diagnose cellular injury (Reuben 2004). Levels of these enzymes in tissues and/or serum are clinicobiochemical changes considered more specific and sensitive for measuring cellular injury (Kedderis 1996). In Nigeria, studies in this sector are scarce and effects of biodiesel from PKO on aquatic animals are still unknown. This study examines some clinicobiochemical changes considered more specific and sensitive for measuring cellular injury to evaluate the effect of biodiesel from PKO on *Clarias gariepinus.*

Materials and Methods

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^{o}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.

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 60^{o}C 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

ASTM standard fuel tests were conducted on the PKO biodiesel. Specific gravity and viscosity measurements were made using the Thermal-Hydrometer apparatus and Viscometer (Canon Fenske Calibrated, 15cSt max. range), following ASTM standards D1298 and D445 respectively. The biodiesel was analyzed for cloud point and pour point using Baskeyl Setapoint cloud and pour point apparatus following ASTM standards D25100-8 and D97 respectively.

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 borehole 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. The cat fish were sacrificed at the end of the experiment and were quickly dissected and the whole liver, kidney, brain and heart were excised, freed of fat, blotted with clean tissue paper and weighed. A portion of each organ was homogenized for biochemical studies and enzyme assays. The activity of ALP and ACP in the tissues of experimental animals was determined following the method described by Bessey *et al.* (1946) as modified by Wright *et al.*(1972). In this method, the amount of phosphate ester that is split within a given period of time is a measure of the phosphatase enzyme activity. The activity of ALT and AST in the serum and tissues of experimental animals was determined following the procedure reported by Schmidt and Schmidt (1963).

Results and Discussion

Analysis of the physicochemical characteristics of the biodiesel produced from PKO as compared with petrol-diesel and the ASTM standard for biodiesel is shown in Table 1. Generally, from Table 1, it can be said that this biodiesel produced from PKO meet with international standard comparing favourably with that of the ASTM standard. The characterization results as revealed that biodiesel compared favourably with ASTM D6751 (US), EN 14214 (Europe) and BIS (India) as well as other plant-oil based biodiesel (Peterson et al. 1990; Graboski and McCormick 1998; Abigor et al. 2000). The higher viscosity of PKO biodiesel will enhance it's fluidity in diesel engines. From the results of this study, the PKO biodiesel is in very good agreement with global biodiesel standards (Abigor et al. 2000; Alamu et al. 2007a; 2008).

Table 1: Comparison between Biodiesel produced from PKO, petrol-diesel and ASTM (D6751) Standards

Properties	**Experimental biodiesel B100**	**Fuel diesel**	**ASTM D6751 (USA)**
KinematicViscosity (mm^2/s)	4.921	1.3-4.1	1.9 - 6.0
Density (Kg/m^3)	0.8801	0.8368	0.8833
Flash Point (^oC)	129	60-80	100-170
Cloud Point (^oC)	7.5	-15-5	-3-12
Cetane No	65	40-53	48-65

Alkaline phosphate (ALP) activity of selected tissues of *C. gariepinus* cultivated in biodiesel contaminated water is presented in Table 2. There was generally no significant difference ($p>0.05$) in the liver, heart and brain ALP activities of the 3 groups of experimental fish. However, the activity of kidney ALP of group C fish was significantly lower ($p<0.05$) than that of group A (control) A. Contamination of water by petroleum products had been linked to decreased ALP activities in tissues of cat fish as a result of damaged plasma membrane (Wang et al. 2006; Sunmonu and Oloyede 2009).

Table 2: Specific activity of alkaline phosphatase (nmol/mim/mg protein) of selected tissues of *Clarias gariepinus* cultivated in water contaminated with bio-fuel from PKO

Group	**Liver**	**Kidney**	**Heart**	**Brain**
A	9.42 ± 2.09^a	86.57 ± 4.56^a	3.45 ± 0.91^a	6.01 ± 0.69^a
B	7.89 ± 1.12^a	79.85 ± 4.44^{ab}	3.12 ± 0.88^a	5.59 ± 0.66^a
C	7.64 ± 1.09^a	77.39 ± 4.34^b	3.20 ± 0.79^a	5.54 ± 0.56^a

Values in the same column bearing different superscripts are significantly different ($P<0.05$). Tabulated data are means of three (3) determinations ± SEM.

Acid phosphate (ACP) activity of selected tissues of *C. gariepinus* cultivated in biodiesel contaminated water is presented in Table 3. Liver ACP activities of the three groups of experimental fish were found to be significantly different ($p<0.05$), thus the activities of ACP in the liver of rats in the Test Groups (B and C) decreased significantly relative to Group A (control) C. Conversely, ACP activities of the brain of the three (3) groups of experimental fish showed no significant difference ($p>0.05$). Decreased ACP activity had been associated with cytotoxicity which may predispose to cell death (Oloyede et al. 2003; Adeyemi et al. 2009). It could also be inferred that biodiesel could possibly cause severe damage to cells of the liver, kidney and heart of the experimental fish.

Table 3: Specific activity of acid phosphatase (nmol/mim/mg protein) of selected tissues of *Clarias gariepinus* cultivated in water contaminated with bio-fuel from PKO

Group	Liver	Kidney	Heart	Brain
A	42.65±1.49[a]	28.46±2.41[a]	5.39±0.89[a]	5.55±0.59[a]
B	34.01±1.67[b]	23.48±1.89[b]	4.89±0.77[b]	5.01±0.45[a]
C	30.31±1.54[c]	21.16±1.76[b]	4.23±0.65[b]	4.98±0.67[a]

Values in the same column bearing different superscripts are significantly different (P<0.05). Tabulated data are means of three (3) determinations ± SEM.

Alanine transaminase (ALT) activity of selected tissues of *C. gariepinus* cultivated in biodiesel contaminated water is presented in Table 4. Liver and kidney ALT activities of rats in groups B and C were significantly (*p<0.05*) lower than that of Control. Aspartate transaminase (AST) activity of selected tissues of *C. gariepinus* cultivated in biodiesel contaminated water is presented in Table 5. The AST activity in the liver and kidney of groups B and C fish showed no significant difference (*p>0.05*), but AST activity in both group B and C is significantly lower (*p<0.05*) than that of group A. However, AST activities of the brain and heart in the 3 groups of fish shows no significant difference (*p>0.05*). The depressed AST and ALT activities, as earlier reported, suggests impaired cellular amino acids metabolism (Adeyemi et al. 2009; Bakde and Poddar 2011). The result of this study indicated that the contaminated water might have altered protein metabolism, among others, at the subcellular level and this may be indicative of impairment of the function of the tissues.

Table 4: Specific activity of alanine transaminase (nmol/mim/mg protein) of selected tissues of *Clarias gariepinus* cultivated in water contaminated with bio-fuel from PKO

Group	Liver	Kidney	Heart	Brain
A	3.54±0.21[a]	3.21±0.16[a]	0.57±0.01[a]	0.60±0.03[a]
B	2.76±0.23[b]	2.56±0.22[b]	0.54±0.02[a]	0.61±0.02[a]
C	2.54±0.19[b]	2.48±0.18[b]	0.55±0.02[a]	0.61±0.01[a]

Values in the same column bearing different superscripts are significantly different (P<0.05). Tabulated data are means of three (3) determinations ± SEM.

Table 5: Specific activity of aspartate transaminase (nmol/mim/mg protein) of selected tissues of *Clarias gariepinus* cultivated in water contaminated with bio-fuel from PKO

Group	Liver	Kidney	Heart	Brain
A	3.89±0.27[a]	3.88±0.25[a]	1.87±0.03[a]	0.82±0.05[a]
B	2.92±0.43[b]	3.01±0.19[b]	1.88±0.05[a]	0.79±0.08[a]
C	2.88±0.56b	2.97±0.22b	1.85±0.04a	0.77±0.06a

Values in the same column bearing different superscripts are significantly different (P<0.05). Tabulated data are means of three (3) determinations ± SEM.

Conclusion

Conclusively, the risk of biodiesel to aquatic organisms is still quite substantial. Thus, biodiesel must be carefully handled during transportation, storage or usage to avoid spill and discharge into the aquatic environment.

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