



## Toxicological Effect of Biodiesel Emission Particles on Cellular System of Albino Rats

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### Authors' contributions

This work was carried out in collaboration between both authors. Author AO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJRB/2020/v6i230114

#### Editor(s):

(1) Dr. Pratima Tripathi, Dr. Ram Manohar Lohia Institute of Medical Sciences, India.

#### Reviewers:

(1) Oguh, Collins Egwu, University of Nigeria Nsukka, Nigeria.

(2) Arunrat Chaveerach, Khon Kaen University, Thailand.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55848>

Received 27 January 2020

Accepted 02 April 2020

Published 07 April 2020

Original Research Article

### ABSTRACT

**Aim:** This study investigates toxicological effect of biodiesel smokes on cellular system of albino rats.

**Study Design:** Biodiesel was blended with fuel diesel at 100, 75, 50, and 25% v/v. Rats were exposed to each flame for 120 seconds daily over a period of ten days.

**Place and Duration of Study:** Laboratory work was carried out in the Toxicology Laboratory, Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Nigeria.

**Methodology:** Rats were grouped into six each containing six rats designated; Control group (no exposure), FD (exposed to smoke of fuel diesel), 100BD, 75BD, 50BD and 25BD exposed to 100, 75, 50 and 25% blend of biodiesel respectively. Afterwards, rats were sacrificed, liver, lungs and brain was isolated and homogenized. Serum was also separated. Alkaline phosphatase (ALP), acid phosphatase (ACP), choline esterase (CEST) and malondialdehyde (MDA) were assayed for in the selected tissues.

**Results:** ALP activity of liver of rats in Control group was significantly higher ( $p < .05$ ) relative to rats in other treatment groups. Lungs ACP activity of rats in Control groups was 3 folds that of rats in FD group, 2 folds those of rats in 100BD, 75BD and 25BD groups. CEST activity in brain of rats in

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Control group was 3 folds that of FD group and two folds that of 50BD and 25BD group of rats. Conversely, serum CEST activity of rats in Control group was significantly lower ( $p < .05$ ) relative to that of other treatment groups it is about 16% that of FD group in particular. Serum MDA level of Control rats was significantly lower ( $p < .05$ ) relative to each of the other treatment groups.

**Conclusion:** Biochemical evidence from this study indicated loss of membrane integrity, possible inflammation of cells of the brain, lungs, and liver.

*Keywords: Biodiesel; cell; fuel; serum; smoke.*

## 1. INTRODUCTION

Most biodiesel studies have focused on the analysis of exhaust emission material, motor efficiency, and the different processes for obtaining biodiesel with oleaginous microorganisms and palm, soybean, kitchen oil, palm oil, or animal fat [1,2]. Due to leaks in storage, fuels pollute underground waters. In that case, humans and animals are exposed to biodiesel cutaneously and orally. There are numerous studies investigating the effects of inhaled diesel and biodiesel fuels. However, there are very few studies on the fuels taken orally, directly [3,4] or indirectly through the food chain [5]. As a result of paucity of information, the present investigation was embarked upon to study the effect of biodiesel smokes on cellular system of albino rats.

McCormick [6] reviewed biodiesel emission data published through 2001 for heavy-duty engines. It was clear there were substantial reductions in the emissions of particulate matter (PM), carbon monoxide (CO), and hydrocarbon (HC), although there was an increase in nitrogen oxides (NOx) obtained by using B20 (blends of 20% biodiesel with 80% conventional diesel). A more detailed analysis of blends of biodiesel emissions data has indicated that the solid carbon fraction of the PM was reduced and the soluble organic fraction (SOF) was increased [7]. Some studies have compared biodiesel and conventional diesel extracts using mutagenicity assays [8]. The acute toxicity of biodiesel was assayed by the Microtox test, which demonstrated an increased toxicity in the semivolatiles fraction compared with the particulates [9], and was shown to be a potent inflammatory agent [10] compared with exposure of diesel PM extracts. Moreover, the type of motor, fuel, particle size, and chemical composition has all been shown to influence the mechanisms and magnitude of adverse manifestations [1].

Liver is the primary organ for the detoxification, as well as being the target of many of environmental chemicals including

biodiesel. Today, we are all exposed to mixtures of different chemicals at a time because of industrialization and fast life style. Hepatic toxicity of chemical and/or biological mixtures may have serious effects on the functions of liver, which is the non-stopping machine for biotransformation as well as several very important physiological processes.

Toxicity biomarkers, such as malondialdehyde (MDA), have been also proposed to reflect the oxidative status of exposed species. MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation. An increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality. The level of antioxidant enzymes have been extensively used as an early warning indicator of lake pollution [11].

Cellular antioxidant defense systems in biological systems are impaired when exposed to environmental pollutants, but the levels of antioxidants in living organisms can increase in order to restore the imbalance caused by oxidative damage. Levels of antioxidant enzymes can be used as an indicator of the antioxidant status of the organism and can serve as biomarkers of oxidative stress [11]. When antioxidant defenses are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation [12].

Enzymatic and non-enzymatic antioxidants serve as an important biological defense against environmental pollutants. Thus, the purpose of this study is to evaluate the effect of biodiesel smokes on cellular system of albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Reagents

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

## 2.2 Vegetable Oil

Vegetable oil was purchased at the local market in Effurun, Delta State. Nigeria.

## 2.3 Preparation of Bio-diesel From Vegetable Oil

Biodiesel was prepared from vegetable oil in accordance with the method described by Alamu et al. [13]. 100 g vegetable oil was used for the transesterification process.

## 2.4 Biodiesel Blend

The Biodiesel from vegetable oil was blended with fuel diesel and grouped as follows:

- Blend1: 100% fuel diesel (100FD)
- Blend2: 100% biodiesel (100BD)
- Blend3: 75% biodiesel and 25% fuel diesel (75BD)
- Blend4: 50% biodiesel and 50% fuel diesel (50BD)
- Blend 5: 25% biodiesel and 75% fuel diesel (25BD)

## 2.5 Experimental Rat Treatment

Thirtysix (36) albino rats were obtained from an animal house of Department of Anatomy University of Benin, Benin City, Nigeria. The experimental animals were handled in accordance with the principles guiding the use and handling of experimental animals as stipulated by FUPRE animal research ethics committee of the College of Science. The rats were maintained on standard rat feed (growers feed) and tap water available all through the period of experiment. The animals were maintained at an ambient temperature between 28-30°C, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 hours of light (06:30 hour – 18:30 hour) alternating with approximately 12 hours of darkness (18:30 hour - 06:30 hour). The rats were allowed to acclimatize for a period of 14 days before treatment commenced.

The experimental rats were grouped into six (6) of six rats in each group:

- Control: served as control and the rats were not exposed to any fuel smoke/flame
- FD: rats exposed to FD 1minute per day for 10days
- 100BD: rats exposed to 100BD 1minute per day for 10days

75BD: rats exposed to 75BD 1minute per day for 10days

50BD: rats exposed to 50BD 1minute per day for 10days

25BD: rats exposed to 25BD 1minute per day for 10days

A glass chamber (30 cm x 30 cm x 30 cm) was constructed in the Department of Mechanical Engineering which was used to enclose the experimental rats while they were being exposed to the smoke released from the burning of the biodiesel blends over a period of 10 days.

## 2.6 Anaesthetisation of Animals and Isolation of Tissues

The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. And were quickly dissected and the whole liver, lungs, brain were excised, freed of fat, blotted with clean tissue paper and weighed into a beaker containing ice cold 0.25 M sucrose solution. The blood was obtained through cardiac puncture. A portion of the blood was collected in heparinised bottles and others in nonheparinised bottles. Some blood samples were thereafter centrifuged at 3,500 rpm for about 15 min using refrigerated centrifuge RC650s and the serum samples obtained were preserved at -8°C until required for analyses.

## 2.7 Preparation of Homogenate

The isolated tissues were weighed and a portion of each tissue was cut out, chopped into very small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25 M sucrose solution to the tune of 1 in 30 dilutions. A portion of each organ was homogenized for biochemical studies and enzyme assays. The diluted homogenates were stored at temperature of -8°C until required for use.

## 2.8 Biochemical Assays

The MDA concentration in the serum and tissues of rats experimental was determined following the method described by Bird et al. [14]. The activities of ALP and ACP in serum and tissues of experimental rats were determined following the method described by Bessey et al. [15] as modified by Wright et al. [16]. 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. Acetylcholinesterase activity was determined in accordance with standard method [17].

## 2.9 Statistical Analyses

All numerical results were obtained from the three (6) 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 [18]. Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple range test [19].

## 3. RESULTS

Alkaline phosphatase (ALP) activity of tissues of rats exposed to combustible flames of biodiesel over a period of ten days is presented in Table 1. ALP activity of liver of rats in Control group was significantly higher ( $p < .05$ ) relative to rats in other treatment groups. Liver ALP activities of rats in FD and 25BD were not significantly different ( $p > .05$ ), similarly activities of ALP of the liver of rats in 100BD, 75BD and 50BD were not significantly different ( $p > .05$ ) from one another. ALP activity of the lungs of rats in Control group was significantly higher ( $p < .05$ ) relative to the other treatment groups, it was about 2 folds of each of the other treatment groups. In the brain, while the ALP activity of each of the other treatment group was not significantly different ( $p > .05$ ) from one another, it was significantly lower ( $p < .05$ ) relative to that of the rats in the Control group. In serum however, ALP activity of rats in Control group was significantly lower ( $p < .05$ ) relative to the other treatment groups. Particularly, ALP activity of serum of rats in FD group is 3 folds that of Control rats.

Activities of acid phosphatase (ACP) of liver of rats in FD, 100BD, 75BD, 50BD and 25BD groups of rats were not significantly different ( $p > .05$ ) from one another but significantly lower ( $p < .05$ ) relative to that of Control (Table 2). Lungs ACP activity of rats in Control groups was 3 folds that of rats in FD group, 2 folds those of rats in 100BD, 75BD and 25BD groups. In the brain however, ACP activities of rats in 100BD and 25BD were not significantly different and those of rats in 75BD and 50BD were also not significantly different ( $p > .05$ ). Serum ACP activity of rats in Control group was significantly lower ( $p < .05$ ) relative to those of other treatment groups. It was about 5% that of rats in FD group and 17% that of 100BD group.

Choline esterase (CEST) activity of rats exposed to combustible flames of biodiesel over a period of ten days is presented in Table 3 Activity of CEST of liver of rats in Control group was significantly higher ( $p < .05$ ) relative to each of the other treatment groups, it was about 4 folds that of rats in FD group in particular. CEST activities of lungs of rats in 75BD and 50BD were not significantly different ( $p > .05$ ). Activities of CEST of lungs of rats in FD and 25BD were not significantly different ( $p > .05$ ). CEST activity in brain of rats in Control group was 3 folds that of FD group and two folds that of 50BD and 25BD group of rats. Conversely, serum CEST activity of rats in Control group was significantly lower ( $p < .05$ ) relative to that of other treatment groups it is about 16% that of FD group in particular. Notably, serum CEST activities of rats in 100BD and 75BD were not significantly different ( $p > .05$ ).

Table 4 presents the concentration of malondialdehyde (MDA) in tissues of rats exposed to combustible flames biodiesel over a period of ten days. MDA content of liver of rats in Control group was significantly lower ( $p < .05$ ) relative to each of the other treatment groups. Particularly, MDA content of liver of rats in FD group was 2 folds that of Control. MDA levels of liver of rats in 50BD and 25BD were not significantly different ( $p > .05$ ). in the lungs however, MDA of rats in groups 100BD and 75BD were not significantly different ( $p > .05$ ) and the levels in 50BD and 25BD were also not significantly different ( $p > .05$ ). There existed no significant difference ( $p > .05$ ) between the levels of MDA of brain of rats in 100BD relative to the Control. Levels of MDA of brain of rats in 100BD, 75BD, 50BD and 25BD were not significantly different ( $p > .05$ ). Serum MDA level of Control rats was significantly lower ( $p < .05$ ) relative to each of the other treatment groups. Besides the serum MDA level of rats in the FD group, MDA levels of serum of other treatment groups were not significantly different ( $p > .05$ ) but significantly higher ( $p < .05$ ) relative to the Control.

## 4. DISCUSSION

This study presents a documented report on the effect of biodiesel flame on some enzymes of selected tissues of rats. Many studies reported the physicochemical properties of diesel particulate matter (PM) implicating them in respiratory diseases [20-24]. Particles emitted from biodiesel fuel engines were found to be dissimilar to particles emitted from normal diesel fuel engines. Despite significant reductions in

**Table 1. Alkaline phosphatase (ALP) activity of tissues of rats exposed to combustibles flames of biodiesel over a period of ten days**

Tissues	Control	FD	100BD	75BD	50BD	25BD
Liver	7.65±0.78 <sup>a</sup>	4.23±0.67 <sup>b</sup>	6.08±0.55 <sup>c</sup>	5.56±0.64 <sup>c</sup>	5.12±0.65 <sup>c</sup>	4.96±0.56 <sup>b</sup>
Lungs	0.58±0.04 <sup>a</sup>	0.23±0.01 <sup>b</sup>	0.31±0.04 <sup>c</sup>	0.28±0.01 <sup>c</sup>	0.26±0.01 <sup>c</sup>	0.24±0.03 <sup>bc</sup>
Brain	0.64±0.02 <sup>a</sup>	0.51±0.01 <sup>b</sup>	0.58±0.03 <sup>b</sup>	0.55±0.02 <sup>b</sup>	0.55±0.03 <sup>b</sup>	0.53±0.02 <sup>b</sup>
Serum	0.73±0.03 <sup>a</sup>	2.19±0.22 <sup>b</sup>	0.88±0.05 <sup>c</sup>	1.16±0.11 <sup>d</sup>	1.52±0.16 <sup>e</sup>	1.89±0.18 <sup>e</sup>

Values on the same row bearing different superscripts are significantly different ( $P < .05$ ). Tabulated data are means of three (3) determinations ± SEM

**Table 2. Acid phosphatase (ACP) activity of tissues of rats exposed to combustibles flames of biodiesel over a period of ten days**

Tissues	Control	FD	100BD	75BD	50BD	25BD
Liver	156.25±4.17 <sup>a</sup>	136.16±3.88 <sup>b</sup>	144.21±2.68 <sup>b</sup>	140.24±3.12 <sup>b</sup>	139.17±2.33 <sup>b</sup>	138.16±2.19 <sup>b</sup>
Lungs	0.88±0.02 <sup>a</sup>	0.24±0.01 <sup>b</sup>	0.33±0.01 <sup>c</sup>	0.43±0.02 <sup>d</sup>	0.56±0.02 <sup>e</sup>	0.37±0.02 <sup>f</sup>
Brain	0.87±0.03 <sup>a</sup>	0.49±0.02 <sup>b</sup>	0.58±0.03 <sup>c</sup>	0.68±0.03 <sup>d</sup>	0.63±0.03 <sup>d</sup>	0.53±0.02 <sup>c</sup>
Serum	0.45±0.01 <sup>a</sup>	8.44±1.11 <sup>b</sup>	2.67±0.86 <sup>c</sup>	2.98±0.58 <sup>c</sup>	6.25±1.00 <sup>d</sup>	6.83±1.12 <sup>bd</sup>

Values on the same row bearing different superscripts are significantly different ( $P < .05$ ). Tabulated data are means of three (3) determinations ± SEM

**Table 3. Choline esterase (CEST) activity of rats exposed to combustible flames of biodiesel over a period of ten days**

Tissues	Control	FD	100BD	75BD	50BD	25BD
Liver	0.61±0.02 <sup>a</sup>	0.15±0.01 <sup>b</sup>	0.47±0.02 <sup>c</sup>	0.42±0.01 <sup>d</sup>	0.36±0.01 <sup>e</sup>	0.27±0.01 <sup>f</sup>
Lungs	0.23±0.01 <sup>a</sup>	0.11±0.01 <sup>b</sup>	0.20±0.01 <sup>c</sup>	0.16±0.01 <sup>d</sup>	0.14±0.01 <sup>de</sup>	0.12±0.01 <sup>be</sup>
Brain	0.16±0.01 <sup>a</sup>	0.05±0.01 <sup>b</sup>	0.12±0.01 <sup>c</sup>	0.09±0.01 <sup>d</sup>	0.08±0.01 <sup>de</sup>	0.07±0.01 <sup>e</sup>
Serum	0.34±0.01 <sup>a</sup>	2.14±0.25 <sup>b</sup>	0.47±0.01 <sup>c</sup>	0.49±0.01 <sup>c</sup>	0.87±0.01 <sup>d</sup>	0.96±0.01 <sup>e</sup>

Values on the same row bearing different superscripts are significantly different ( $P < .05$ ). Tabulated data are means of three (3) determinations ± SEM

**Table 4. The concentration of malondialdehyde (MDA) in tissues of rats exposed to combustible flames biodiesel over a period of ten days**

Tissues	Control	FD	100BD	75BD	50BD	25BD
Liver	43.67±2.17 <sup>a</sup>	95.12±2.67 <sup>b</sup>	68.42±2.01 <sup>c</sup>	76.05±1.77 <sup>d</sup>	86.23±2.10 <sup>e</sup>	88.17±2.00 <sup>e</sup>
Lungs	1.20±0.01 <sup>a</sup>	7.18±1.04 <sup>b</sup>	4.19±0.64 <sup>c</sup>	4.65±0.72 <sup>c</sup>	5.87±0.54 <sup>d</sup>	6.02±0.88 <sup>bd</sup>
Brain	1.38±0.23 <sup>a</sup>	2.12±0.18 <sup>b</sup>	1.67±0.24 <sup>ac</sup>	1.86±0.25 <sup>c</sup>	1.88±0.05 <sup>c</sup>	1.96±0.23 <sup>c</sup>
Serum	5.60±0.93 <sup>a</sup>	13.16±1.14 <sup>b</sup>	8.99±1.00 <sup>c</sup>	9.01±0.88 <sup>c</sup>	10.23±0.76 <sup>c</sup>	10.87±0.55 <sup>c</sup>

Values on the same row bearing different superscripts are significantly different ( $P < .05$ ). Tabulated data are means of three (3) determinations ± SEM

PM, the mean particle size from biodiesel combustion is much smaller than for diesel combustion and it is composed of higher fraction of organic carbon. Therefore, these particles are expected to be more toxic than diesel emitted particles, which is the main reason behind current scientific debate about the substitution of diesel for biodiesel. Increased PAHs and ROS emissions from biodiesels have been reported by several studies [25,26], while others found a decrease or no significant change [27]. Recently Guarieiro et al. [28] found a reduction in PAHs for B50 blends, however they increased for B100 waste cooking oil (WCO), while the redox activity increased consistently with biodiesel percentage. Limited information is currently available from effect of direct exposure on phosphatases and choline esterase of brain, liver, lungs and serum of rats.

ALP catalyses the hydrolysis of organic phosphates at alkaline pH. ALP is a membrane bound enzyme and is often used as an indicator of monitoring membrane integrity [29]. The reduced ALP activity in the tissues of rats exposed to biodiesel flame as observed in this study (Table 1) is suggestive of damage to plasma membrane of the tissues which may give rise to reduction or loss of membrane integrity. In contrast, however serum ALP was elevated in rats exposed to flame of biodiesel indicating possible leakage of ALP from tissues to serum as a result of membrane damage.

Acid phosphatase on the other hand is an hydrolytic enzyme localized in the lysosomes. It catalyses the removal of phosphoryl group from a phosphate ester in an acidic medium. Several studies associated reduced ACP activity as observed in this study (Table 2) to the early stage of apoptosis [30-32]. Elevated level of serum ACP lends credence to the earlier submission of likelihood of tissue damage as a result of exposure to flame of biodiesel. These findings indicate that, in addition to respiratory diseases, flame of biodiesel is capable of inducing cellular damage or may alter cellular metabolism which may in turn predispose to more complicated health challenge.

Neurosecretion such as choline esterase (ChE) has been reported to regulate animal behavior and that inhibition of its activity is indicative of neurotoxicity [33]. Reduced activity of ChE (Table 3) is suggesting that flame of biodiesel is likely to be neurotoxic. Decreased cholinesterase (ChE) activity decreases the cellular metabolism, induces deformities of cell membrane, and

disturbs metabolic and nervous activity. Conversely, serum levels of ChE was elevated. This finding tends to negate inhibition model put forward initially. Close scrutiny revealed tissue directed leakage of this enzyme in to the extracellular fluid through a mechanism not yet clear. Nevertheless, elevated serum ChE activity could possibly be a condition of oxidative damage.

Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in Malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage [34]. It is also a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content can be related to degradation of an environment due to flames from biodiesel. The significant increase in lipid oxidation (MDA) (Table 4) may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. It could therefore, be inferred that oxidative cell injury is likely to be the cause of leakage of ChE from tissues to the serum, although, this claim needs to be substantiated by experimental evidence.

## 5. CONCLUSION

Biochemical evidence from this study indicated that biodiesel emission particles could cause loss of membrane integrity, possible inflammation of cells of the brain, lungs, and liver. The mechanism involves the development of oxidative stress followed by inflammation which acts as precursor to the development and acute exacerbation of cellular damage. Global attention is shifting towards biodiesel as an alternate to fuel diesel because the former contained no sulfur and no aromatics but it emits particulate matters that are suspected carcinogen and that more of it will have to burn to produce energy thereby producing more PM than fuel diesel. These facts are beginning to raise greater concern of the toxicology of biodiesel flame and mechanism underlying the effect with a view to preventing serious health challenge.

## ETHICAL APPROVAL

The experimental animals were handled in accordance with the principles guiding the use and handling of experimental animals as stipulated by FUPRE animal research ethics committee of the College of Science.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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