NISEB Journal Vol. 19, No. 2. June, 2019 1595-6938/2019
Printed in Nigeria (2019) Society for Experimental Biology of Nigeria
http://www.ojs.klobexjournals.com/index.php/nisebj

# Toxicological Evaluation of Biodiesel Emission Particles on Growth and Haematological Properties of Albino Rat

**Adeyemi O and Isukuru EJ*
*Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria*

## Abstract
*This study evaluates the effect of combustible flames of biodiesel on growth and haematological properties of rats exposed to it over a period of ten days. Biodiesel were categorized into FD, 100BD, 75BD, 50BD and 25BD depending on the blend. After the exposure, rats were sacrificed by jugular puncture and blood was collected for haematological analysis. Rats in the Control group had a body weight gain of 10.0±0.51 g while a body weight gain 0f 4.01±0.10, 2.00±0.10, 0.00±0.00, 6.05±0.20, 4.05±0.20 g was recorded for rats in FD, 100BD, 75BD, 50BD and 25BD group of rats respectively. No significant difference (p>0.05) was observed for RBC and Hb of all rat groups. The MCV, MCH, MCHC, PCV and monocytes of groups FD and 100BD rats showed no significant difference (p>0.05). This study has demonstrated that exposure to biodiesel flames can also lead to growth defect and haematological abnormalities. The mechanism through which biodiesel flame was able to achieve these health conditions is still a serious concern and yet to be unraveled. Experimental evidence from this study has also shown that threat posed by biodiesel is milder in effect relative to fuel diesel.*
**Keywords**: Toxicological, Biodiesel, Emission particles, Haematological, Rat

## Introduction
The world is presently confronted with the twin crises of fossil fuel depletion and environmental degradation (1). The search for alternative fuels, which promise a harmonious correlation with sustainable development, energy conservation, efficiency and environmental preservation, has become highly pronounced in the present context. The fuels of bio-origin can provide a feasible solution to this worldwide petroleum crisis (2).
The fact that price of diesel and petroleum products is increasing, they are non-renewable, their combustion emits greenhouse gases and toxic materials which are harmful to living organisms, coupled with the fact that they cause depletion of the ozone layer which results in global warming and climate change. Scientist, governmental and non-governmental organizations are calling for cleaner forms of energy which are effective and environmentally friendly as well as a reduction in the release of toxic materials (3). This has led to the realization of biofuels; a fuel produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter. Biofuels include biogas, bioethanol and biodiesel, the focus of this research.
Biodiesel is an animal or vegetable oil based diesel fuel that burns without the emission of much soot, carbon IV oxide and particulate matter (2). Biodiesel can be produced from animal or plant oil. It is formed from the reaction of alcohols with the triglycerides in vegetable oil to form an alkyl ester (biodiesel) and glycerin (4).
The cytotoxic effects of biodiesel exhaust emissions have been evaluated and compared to petroleum diesel fuel emissions (5). Exhaust from rapeseed biodiesel was 4-fold more potent than petroleum diesel exhaust in inducing cytotoxicity (measured as the median effective dose; ED50). Cytotoxicity of biodiesel emissions increased with extract collected with the engine "idling." Although it can sometimes be difficult to place the results of animal investigation into the context of potential human health hazard, animal models are viewed to be superior to *in vitro* studies for establishing pulmonary and extrapulmonary responses to potentially toxic exposures. Subchronic exposure of rats to emissions from a diesel engine burning soybean oil–derived biodiesel fuel induced a dose-related increase in particle containing alveolar macrophages—a consistent observation in rats subchronically exposed to petroleum diesel exhaust (6). The vast majority of exposed rats had little or no evidence of lung neutrophilia and centriacinar fibrosis. To date, only very few epidemiologic studies have been conducted in which the acute health effects from exposure to biodiesel exhaust fumes were assessed by a questionnaire given to workers who are typically exposed to diesel fumes (e.g., delivery truck drivers, road-maintenance workers, and industrial fork lift truck drivers) (7).
Currently there is a strong desire and need for alternative fuels in Nigeria (8). Employment of biodiesel fuel is favorably viewed, and there are suggestions that its exhaust emissions are less likely to present any risk to human health relative to petroleum diesel emissions. However, the speculative nature of a reduction in health effects based on chemical composition of biodiesel exhaust needs to be followed up with investigations using

**Corresponding Author's E-mail: lexicoa@yahoo.com*

newer biologic approaches gained from years of diesel research. This study is therefore, initiated to evaluate the health effects of exposure to biodiesel exhaust using growth and haematological responses of rats.

## Materials and Methods

### Materials

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

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

***Preparation of Bio-diesel from Vegetable oil:*** Biodiesel was prepared from vegetable oil in accordance with the method described by Adeyemi (4). 100g vegetable oil was used for the transesterification process.

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

Blend 1: 100% fuel diesel (100FD)
Blend 2: 100% biodiesel (100BD)
Blend 3: 75% biodiesel and 25% fuel diesel (75BD)
Blend 4: 50% biodiesel and 50% fuel diesel (50BD)
Blend 5: 25% biodiesel and 75% fuel diesel (25BD)

### Methods

***Experimental Rat Treatment***

Thirty six (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 - 30ºC, 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 (30cm x 30cm x 30cm) 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.

***Anaesthetization of Animals and Collection of Blood Samples***

The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform. The blood was obtained through cardiac puncture. A portion of the blood was collected in heparinized bottles for determination of haematological properties.

***Analysis of Blood Samples***

Blood samples were analysed using automated haematology cell counter (BC-2800 Mindray auto haematology analyzer, China) for the estimation of haematological parameters: Red blood cell (RBC) count, Haematocrit (PCV), Haemoglobin (Hb) concentration, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH), White blood cell (WBC) count; (Total WBC count, Differential WBC), Platelet count, using standard methods (9-11).

### Statistical Analyses

All numerical results were obtained from the five (5) groups (control and treated). Data were presented as mean±SEM and analysed based on one way analysis of variance (ANOVA) and Duncan Multiple Range Test using SPSS-18.0 (Statistical packages for social Scientists – version 18.0) statistical program. P values<0.05 were considered significant (12).

## Results

As shown in Figure 1, all rat groups showed positive growth response curve. Rats in the Control group had a body weight gain of 10.0±0.51 g while body weight gain 0f 4.01±0.10, 2.00±0.10, 0.00±0.00, 6.05±0.20, 4.05±0.20 g were recorded for rats in FD, 100BD, 75BD, 50BD and 25BD groups of rats respectively. Generally, all rat groups showed similar growth pattern in that there was an initial weight loss at day 2 found to be most significant ($p<0.05$) in 75BD group of rats. Afterwards, the rats began to gain weight until the end of the experiment.

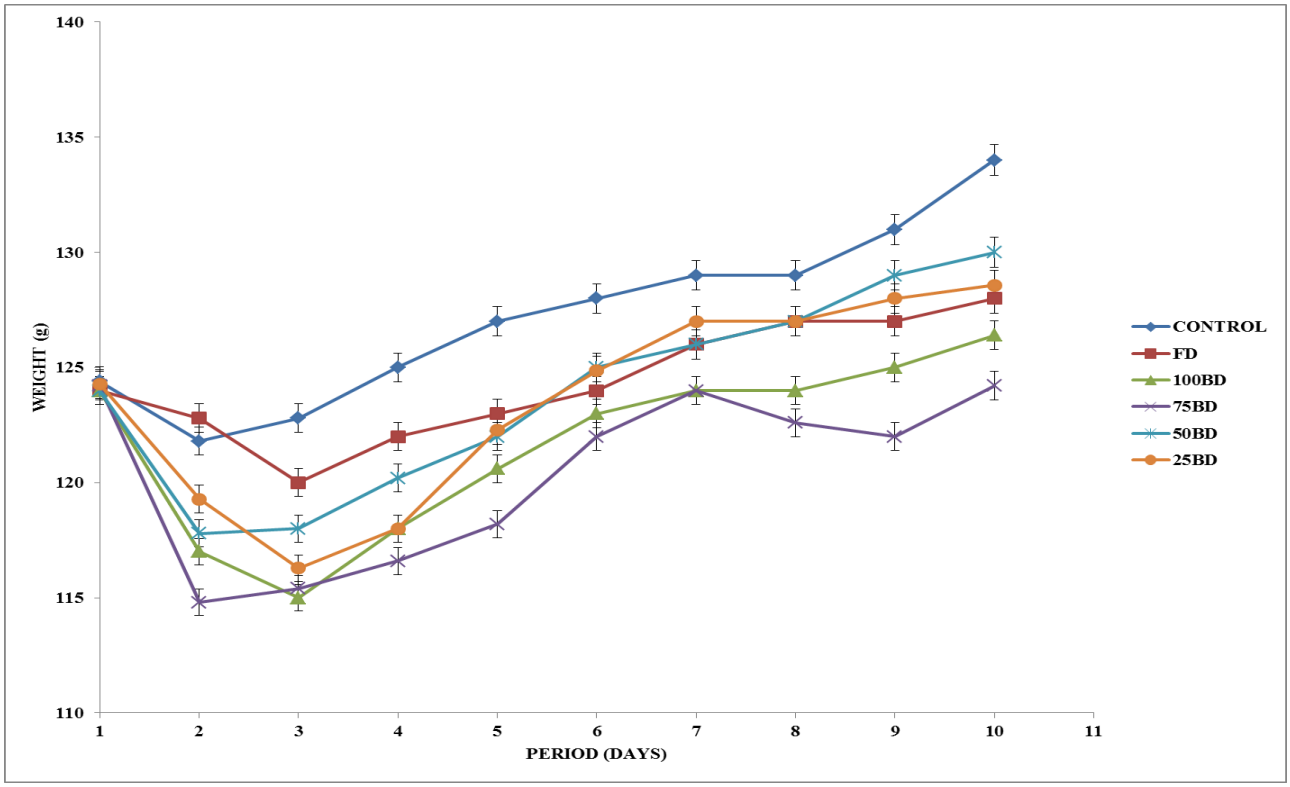


Figure 1: Growth curve of rats exposed to combustible flame of biodiesel over a period of ten days

Table 1: Haematological properties of rats exposed to combustible flame of biodiesel over a period of ten **days**

| **Haematological parameters** | **Control** | **FD** | **100BD** | **75BD** | **50BD** | **25BD** |
|---|---|---|---|---|---|---|
| RBC ($x10^6/mm^3$) | $4.88\pm0.30^{a}$ | $4.38\pm0.30^{a}$ | $4.67\pm0.42^{a}$ | $4.70\pm0.32^{a}$ | $4.75\pm0.28^{a}$ | $4.71\pm0.29^{a}$ |
| Hb (g/dL) | $7.45\pm0.66^{a}$ | $6.91\pm0.78^{a}$ | $7.02\pm0.74^{a}$ | $7.10\pm0.57^{a}$ | $7.15\pm0.69^{a}$ | $7.00\pm0.58^{a}$ |
| MCV ($\mu^3$) | $76.64\pm1.77^{a}$ | $67.52\pm2.32^{b}$ | $66.78\pm2.38^{b}$ | $69.23\pm1.38^{cb}$ | $71.45\pm1.44^{c}$ | $70.15\pm2.18^{c}$ |
| MCH (µµg) | $17.50\pm0.82^{a}$ | $15.76\pm0.57^{b}$ | $16.06\pm0.96^{b}$ | $16.45\pm0.72^{b}$ | $16.77\pm0.74^{b}$ | $16.67\pm0.88^{b}$ |
| MCHC (%) | $19.32\pm0.86^{a}$ | $17.69\pm0.75^{b}$ | $17.82\pm1.01^{bc}$ | $18.02\pm0.76^{c}$ | $18.75\pm0.65^{c}$ | $18.44\pm0.94^{c}$ |
| PCV (%) | $26.47\pm0.55^{a}$ | $24.85\pm0.48^{b}$ | $25.05\pm0.56^{b}$ | $25.50\pm0.48^{b}$ | $25.79\pm0.44^{b}$ | $25.00\pm0.77^{b}$ |
| WBC ($x10^3/mm^3$) | $29.54\pm0.95^{a}$ | $55.67\pm2.63^{b}$ | $64.22\pm3.14^{c}$ | $35.72\pm1.12^{d}$ | $32.01\pm0.98^{e}$ | $33.24\pm1.00^{e}$ |
| Neutrophils (%) | $4.66\pm0.25^{a}$ | $6.25\pm0.32^{b}$ | $7.24\pm0.25^{c}$ | $5.34\pm0.27^{de}$ | $5.05\pm0.27^{d}$ | $5.84\pm0.31^{e}$ |
| Eosinophils (%) | $0.00\pm0.00^{a}$ | $0.40\pm0.01^{b}$ | $0.67\pm0.01^{c}$ | $0.15\pm0.01^{d}$ | $0.05\pm0.01^{e}$ | $0.20\pm0.01^{f}$ |
| Basophils (%) | $0.36\pm0.01^{a}$ | $1.25\pm0.01^{b}$ | $1.44\pm0.01^{c}$ | $0.87\pm0.01^{d}$ | $0.56\pm0.01^{e}$ | $1.06\pm0.01^{f}$ |
| Lymphocytes (%) | $25.48\pm1.04^{a}$ | $44.12\pm1.58^{b}$ | $53.35\pm1.88^{c}$ | $37.36\pm1.33^{d}$ | $34.29\pm1.01^{e}$ | $40.22\pm1.02^{f}$ |
| Monocytes (%) | $14.50\pm0.84^{a}$ | $23.26\pm1.19^{b}$ | $24.05\pm1.25^{b}$ | $19.23\pm1.02^{c}$ | $17.12\pm0.89^{c}$ | $21.64\pm0.92^{d}$ |

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

Table 1 presents haematological parameters of rats exposed to combustible flame of biodiesel over a period of ten days. No significant difference ($p>0.05$) was observed for RBC and Hb of all groups relative to Control. The MCV, MCH, MCHC, PCV and monocytes of treatment groups were significantly ($p<0.05$) different relative to Control while those of FD and 100BD rats showed no significant difference ($p>0.05$). The neutrophils, eosinophils, basophils and lymphocytes differ significantly ($p<0.05$) from one another. The highest neutrophils, eosinophils, basophils and lymphocytes were observed in 100BD group.

## Discussion

The present study has delved into impact of biodiesel combustible flame on the growth and haematological properties of rats exposed to it over a period of ten days. This study is significant as little or no information is available in literature addressing this subject. As presented in Figure 1, the growth increased in this order 75BD<100BD<FD<25BD<50BD<Control. It is not clear what could be responsible for the growth pattern, however, it could be inferred that the combustible flame did have adverse effects on the growth of rat because the rats in the Control group had a growth superior to rats in the other treatment groups. The authors suggest that

the particulate matter (PM) of biodiesel may be responsible for the adverse effect of biodiesel on growth of the rats.

PM emitted from diesel engines is considered to be the most harmful substance among all of its exhaust emissions (13-15). Considering its harmful effect on human health, the International Agency for Research on Cancer (IARC) recently included this substance as a carcinogen under group-I (16). Despite all of the developments in diesel engine technology or in the exhaust after treatment systems of modern diesel engines, PM emissions from diesel engines still remain a considerable hazard, for both human health and the environment, and pose a huge challenge to the scientific community and automotive engineers. The growth pattern observed in this study may be due to the presence of PM from the biodiesel.

It is evident from this study that combustible flame of biodiesel had effect on haematological properties of rats exposed to it. It should be mentioned that RBC and haemoglobin were not affected by the flame of biodiesel but PCV was significantly reduced. Further investigation will be done to unravel the reason for the result, although many authors claimed that the results of PCV are often unreliable due to laboratory errors (17-18).

The three cardinal RBC measurements (hemoglobin, hematocrit, and RBC count) are used to arithmetically derive the erythrocyte indices – mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. When the MCV is low, the blood is said to be microcytic, and when high, it is macrocytic. Normocytic refers to blood with a normal MCV. The MCV can be normal while the individual red cells of the population vary wildly in volume from one to the next. Such an abnormal variation in cell volume is called anisocytosis. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV (19). The MCH is something of a minor leaguer among the indices in that it adds little information independent of the MCV. MCHC is the mean concentration of hemoglobin in the red cell. Cells with normal, high, and low MCHC are referred to as normochromic, hyperchromic, and hypochromic, respectively (20). In this study, rats exposed to biodiesel emission particles were found to have significantly lower MCV, MCH and MCHC relative to the Control.

Neutrophils are most populous of the circulating white cells; they are also the most short-lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, infarction, trauma, emotional distress, or other noxious stimuli. These large cells are actually more closely related to neutrophils than are the other "granulocytes," the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Eosinophils are capable of ameboid motion (in response to chemotactic substances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses (21). Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The life span of eosinophil the peripheral blood is about the same as that of neutrophils. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises. In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase (22). In the immune/inflammatory response, if the neutrophils and monocytes are the brutes, the lymphocytes are the brains. Results obtained from this study are suggestive of stress condition induced by the flame of biodiesel that has resulted into abnormalities in haematological properties reported in Table 1.

***Conclusion***

This study has demonstrated that exposure to biodiesel flames may lead to growth defect and haematological abnormalities. The mechanism through which biodiesel flame was able to achieve these health conditions is still a serious concern which is yet to be unraveled, however, it is the view of the authors that particulate matter of biodiesel may be responsible. Experimental evidence from this study has also been able to show that threat posed by biodiesel is less in effect relative to fuel diesel.

## References


1. Adeyemi O: Toxicological Evaluation of Palm Kernel Oil (PKO) Biodiesel-Contaminated Catfish on Kidney of Albino Rats. *International Journal of Biochemistry Research & Review* 6(2): 82-91. 2014.
2. Oliveira LE, Da Silva MLCP: Comparative study of calorific value of rapeseed, soybean, jatropha curcas and crambe biodiesel. *Renewable Energy and Power Quality Journal*, 11, 1-4. 2013.
3. Adeyemi O, Olayebi OO: Optimization of Pre-treatment Conditions of Water Hyacinth Leaf, Stem and Root for Bioethanol Production. *American Chemical Science Journal* 15(4): 1-10. 2016.
4. Adeyemi O: Biodiesel causes oxidative damage in tissues of *Clarias gariepinus*. *Advances in Research* 4(5): 329-335. 2015a.

5. Bünger J, Krahl J, Baum, K, Schröder O, Muller M, Westphal G: Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel. *Arch Toxicol* 74(8):490–498. 2000.
6. Mauderly JL: Health issues concerning inhalation of petroleum diesel and biodiesel exhaust. In: Plant Oils as Fuels: Present State of Science and Future Developments (Martini N, Schell J, eds). 92–103. 1997.
7. Hasford B, Wimbauer M, Höppe P, Fruhmann G, Weyermann M: Respiratory symptoms and lung function after exposure to exhaust fumes from rapeseed oil in comparison to regular diesel fuel. In: Proceedings of the 9th International Conference on Occupational Respiratory Diseases*: Advances in Prevention of Occupational Respiratory Diseases. Kyoto, Japan:Elsevier*,131–135. 1997.
8. Adeyemi O: Effects of Biodiesel from PKO on selected phosphatases and transaminases of some tissues of African catfish (*Clarias gariepinus*). *International Journal of Advanced Research in Biological Sciences* 2(3): 224-228. 2015b.
9. Mitruka BM, Rawnsley M: Materials and methods in hematology and clinical biochemistry. In: *Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals. Masson Publishing Inc USA*, 41-58. 1977.
10. Muthaya NM: Blood In: Human Physiology, *3rd Edition. Jaypee Brothers Medical Publishers (P) Ltd New Delhi India*, 51 – 91. 2002.
11. Islam MS, Lucky NS, Islam MR, Ahad A, Das BR, Raham MM, Siddiui MSI: Haematological parameters of Fayoumi, Assil and local chickens reared in sylhet region in Bangladesh. *Int J Poult Sci*. 3(2): 144-147. 2002.
12. Duncan DB: Multiple range and multiple F test. *Biomet*. 11:1-10.1955.
13. Cassee FR, Héroux ME, Gerlofs Nijland FJ: Particulate matter beyond mass: Recent health evidence on the role of fractions, chemical constituents and sources of emission. *Inhalation Toxicology*. 25: 802-812. 2013.
14. Gangwar JN, Gupta T, Agarwal AK: Composition and comparative toxicity of particulate matter emitted from a diesel and biodiesel fuelled CRDI engine. *Atmospheric Environment*. 46: 472-481. 2012.
15. Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DL, Samaras Z, Låg, M, et al: Cell toxicity and oxidative potential of engine exhaust particles: Impact of using particulate filter or biodiesel fuel blend, *Environmental Science and Technology*. 47: 5931-5938. 2013.
16. International Agency for Research on Cancer (IARC): Diesel engine exhausts carcinogenic. *press release N 213*. 2012.
17. Bull BS, Houwen B, Koepke JA, Simson EA: Reference and selected procedures for the quantitative determination of hemoglobin in blood, 2nd edition, 14(6):14-20. 1994.
18. Zwart A, van Assendelft OW, Bull BS, England JM, Lewis SM, Zijlstra-WG: Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemiglobinocyanide standard (4th edition). *J Clin Pathol*. 49:271-274. 1996.
19. Adeyemi O, Oloyede OB, Oladiji AT: Effect of Leachate-Contaminated Groundwater on the Growth and Blood of Albino Rats. *The Internet Journal of Hematology* 3(2): 1-7. 2007.
20. Adeyemi O, Osubor CC: Effects of water hyacinth leaf protein concentrate on growth and haematological properties of rats. *European Journal of Biomedical and Pharmaceutical Sciences*. 4 (6), 116-123. 2017.
21. Adeyemi O, Osubor CC, Adeyemi O: Growth and Haematological Properties of Rats Fed with Clarias gariepinus Cultivated in Contaminated Water. *J. Env. Prot. Sci.* 3: 97 – 101. 2009.
22. Adeyemi O, Adenitire GS, Oloyede OB, Oladiji AT, Oluba OM, Adeyemi O, et al: Biochemical assessment of lead, phenol, and benzene-contaminated water on the heart and blood of Albino rats. *Toxicological & Environmental Chemistry*, 92: 7, 1347 — 1356. 2010.