



# Bisphenol a Exposure Causes Increased Oxidation of Low Density Lipoprotein (LDL) and Its Abduct in Rats

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Background and Objectives:** Living organisms are exposed to oxidant agents constantly from both endogenous and exogenous sources. One of such oxidant agent is Bisphenol A (BPA) and its exposure is capable to modify biomolecules and induce damages. Bisphenol A (BPA) is a contaminant with increasing exposure. It exerts toxic effects on cells. This study investigates the possibility of BPA exposure on Low Density Lipoprotein (LDL) perturbations at prevailing low exposure doses in female albino Wistar rats, following exposure for the period of three (3) month. **Materials and Methods:** Total 12 groups were formed; out of which 11 experimental groups, each containing 10 non-pregnant female rats were administered; 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg of BPA/kgbw/day. To the 12<sup>th</sup> control group was given water. Blood was collected from animals at the end of every week of the study and serum sample specimens analyzed by routine diagnostic procedures for oxidized LDL such as *malondialdehyde* modified- LDL (MDA-LDL), oxidized phospholipids LDL (OX-PL LDL), N (epsilon) (carboxymethyl) lysine-modified-LDL (CML LDL) and 4-hydroxynonenal-LDL (HNE-LDL) using Autochemical Analyzer.

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**Results:** Significantly increased concentrations of serum oxidized LDL such as MDA-LDL, OX-PL LDL, CML LDL and HNE-LDL were observed at all concentrations of BPA exposure.

**Conclusion:** Bisphenol A alters oxidized LDL such as MDA-LDL, OX-PL LDL, CML LDL and HNE-LDL balance and causes disturbance of internal oxidative statuses.

**Keywords:** BPA; oxidized LDL; MDA-LDL; CML-LDL; HNE-LDL; Ox-PI LDL; exposure.

## 1. INTRODUCTION

Bisphenol A (BPA) is a known endocrine disruptor that is ubiquity in our environment. BPA enters the body by the ingestion of contaminated food and beverages. It leaks from polycarbonate plastics, and penetrates into the body through the skin[1,2]. or inhalation[3,4]. Because BPA is well absorbed into the body by ingestion, pregnant women, infants and young children are particularly vulnerable to BPA. The risk of adverse health effects may be due to the increased absorption and decreased excretion of BPA from the body[5] and also due to several factors, such as e.g., body weight, metabolic rate. BPA provoked an increase in body weight[6] and adipose tissue weight [7] alteration in adipogenesis and an increase in white adipose tissue and over expression of some adipogenic genes[8] and increase lipid accumulation in the differentiating adipocytes and upregulates the expression of adipocyte proteins through the activation of glucocorticoid receptor[9]. BPA suppresses low glucose-induced intracellular calcium oscillation on  $\alpha$ -cells *ex vivo*[10], increase the activation of the transcription factor cAMP (cyclic adenosine monophosphate) response element binding protein (CREB) [3], abnormal levels of the liver enzyme  $\gamma$ -glutamyl-transferases, alkaline phosphatase and lactate dehydrogenase[11,12].

BPA has been reported to have adverse health effects on the developing reproductive organs, fetal BPA exposure has been shown to decrease the efficiency of sperm production in male mice offspring[8]. Recent data of Park et al.[13] showed that BPA increased human ovarian cancer cell proliferation. BPA is capable of inducing toxic effect on non-reproductive vital organs; *several studies have reported* that absorption of BPA causes extensive damage to the liver and kidney[11,14]. BPA has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes, DNA adduct and induce the production of free radicals in rat hepatocytes *In vitro* [15]. The aim of this study is to unveil the possible effects of Bisphenol A on oxidation of lipid in female wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out at Applied Biochemistry Lab, Nnamdi Azikiwe University, Awka, Nigeria, Biochemistry Lab, Gregory University Uturu, Abia state, Nigeria from November 2017 to March, 2018.

### 2.2 Methodology

Total 110 non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for 7 days and randomly divided into 11 experimental groups of 10 rats each and respectively administered; 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg of BPA/kgbw/day. The first group which served as control did not receive any treatment but distilled water instead. The graded doses of BPA were dissolved in distilled water and administered by oral gavage using intubation cannula (Lars Medicare Pvt. Ltd, new delhi, india). Blood were obtained from the tail of the various groups by capillary action weekly, after BPA administration for 13 weeks. Blood samples were processed for clinical assay.

Animals were housed in aluminum wire-mesh cages in a well-ventilated animal house with a 12 h dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from Vital group of Company, Nigeria) and water *ad libitum*.

At the end of the experiments serum CML-LDL, MDA-LDL, HNE-LDL and OXPL-LDL were assayed using Autochemical analyser (DxC 800 and LX20 auto-analyzer chemistry analyser Beckman coulter, USA). All reagents were commercially obtained as already prepared kits. The kits for CML-LDL, MDA-LDL, HNE-LDL and OXPL-LDL were purchased from OXI- select (cell Biolabs, inc USA). Individual tests were carried out according to the kit specifications.

### 2.3 Statistical Analysis

Differences between obtained values (mean $\pm$ SD) were carried out by one-way analysis of variance

(ANOVA) using SPSS software version 20.0 followed by the Tukey-Kramer multiple comparison test. A  $p \leq 0.05$  was taken as a criterion for a statistically significant difference.

### 3. RESULTS

#### 3.1 Oxidised Phospholipid (OXPL-LDL)

There is a significant increase in the OXPL-LDL level when compared with the control  $p \leq 0.05$  (Fig 1a). The significance were observed at all weeks in all test groups except for group 6 (0.5mg/kg) at week 1-5; then group 1(0.05mg/kg)-4(0.3mg/kg) shows significant increase only at week 9-13(Fig 1b). The first four (1-4) weeks of exposure to BPA, the groups that were administered 0.05mg/kg to 0.4mg/kg of BPA showed a dose dependent increase of the oxidised phospholipid level (Fig. 1a). The group that was exposed to 0.9mg/kg of BPA showed lower oxidised phospholipid level relative to that 1mg/kg at all time of exposure and also, The group that was exposed to 0.6mg/kg of BPA showed lower oxidised phospholipid level relative to that 0.7mg/kg at all time of exposure at all time of exposure (Fig.1a). The group that received 1mg/kg showed the highest level of oxidised phospholipid at all time of exposure (Fig. 1a and 1b). At the week 1<sup>st</sup> and 2<sup>nd</sup> week of exposure, the serum level of oxidised phospholipid declined

below that of control and week-0 for the groups exposed to 0.05mg/kg, 0.1mg/kg and 0.5mg/kg of BPA. (Fig. 1b). The time sensitive effect observed in Fig.1b, was mild at 0.4mg/kg and relatively time dependent effect was observed (Fig. 1b).

#### 3.2 MDA-LDL

There is a significant increase in the MDA-LDL level when compared with the control at  $p \leq 0.05$  (fig 2a), in groups 6 (0.5mg/kg) and 11(1mg/kg); group 7 (0.6mg/kg)- 10 (0.9mg/kg) shows significant increase only at week 8-13. There is no significant difference observed in group 1 (0.05mg/kg)- 4 (0.3mg/kg) (Fig 2b). The group that received 1mg/kg of BPA showed the highest level of MDA-LDL, which consistently rise at week 1 to 5, 6 to 11 and week 12 to 13 with its peak at week-5 of exposure (Fig.2a). Another group that showed high level of MDA-LDL was the group that received 0.5mg/kg of BPA (Fig. 2a). At all exposure time, the groups exposed 0.6mg/kg of BPA showed a gradual dose dependent increase except that in all instance the group that received 0.8mg/kg of BPA showed a decline in MDA-LDL relative to that of 0.7mg/kg. The study reveale a time sensitive effect (Fig. 2b). The observed effect was time dependent except at the test group of 0.2mg/kg and 1mg/kg (Fig.2b).

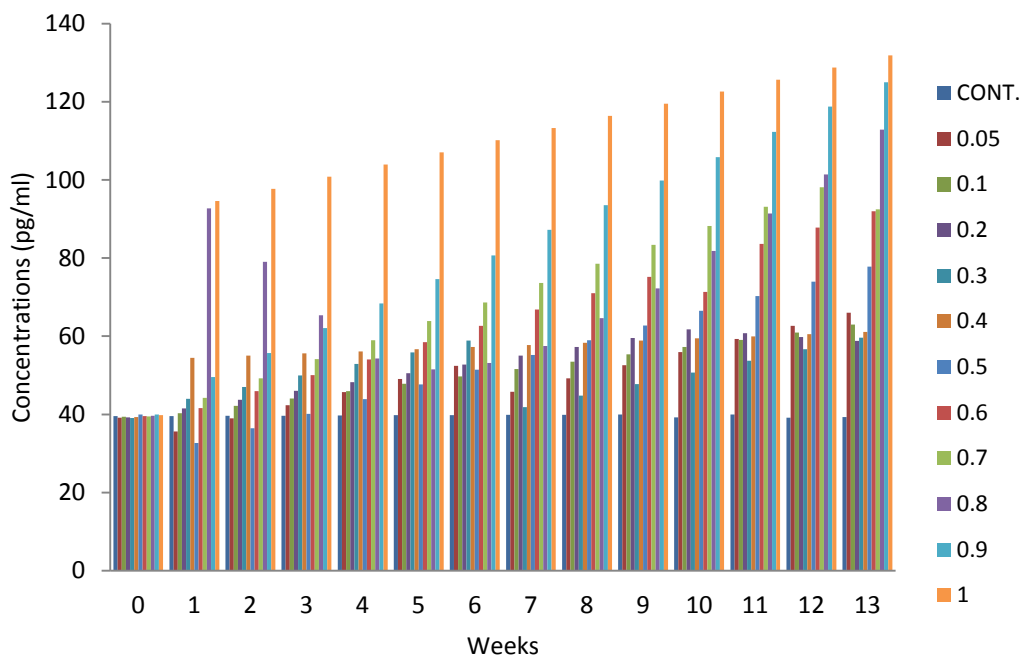


Fig. 1a. Chart of concentration against weeks (durations) for OXPL-LDL level

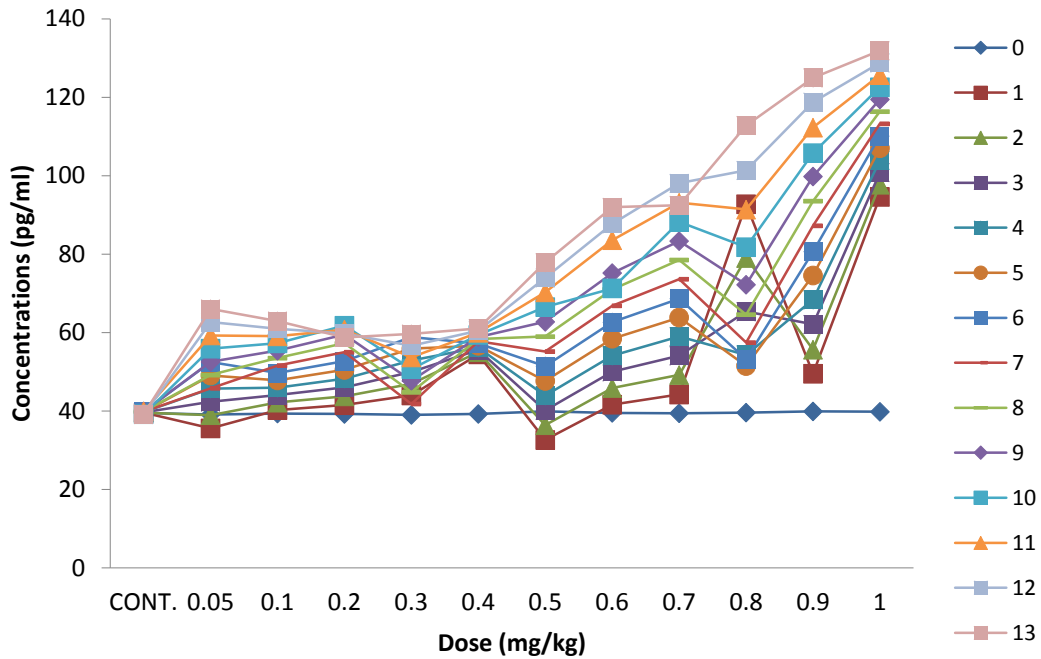


Fig. 1b. Graph of concentration against dose for OXPL-LDL level

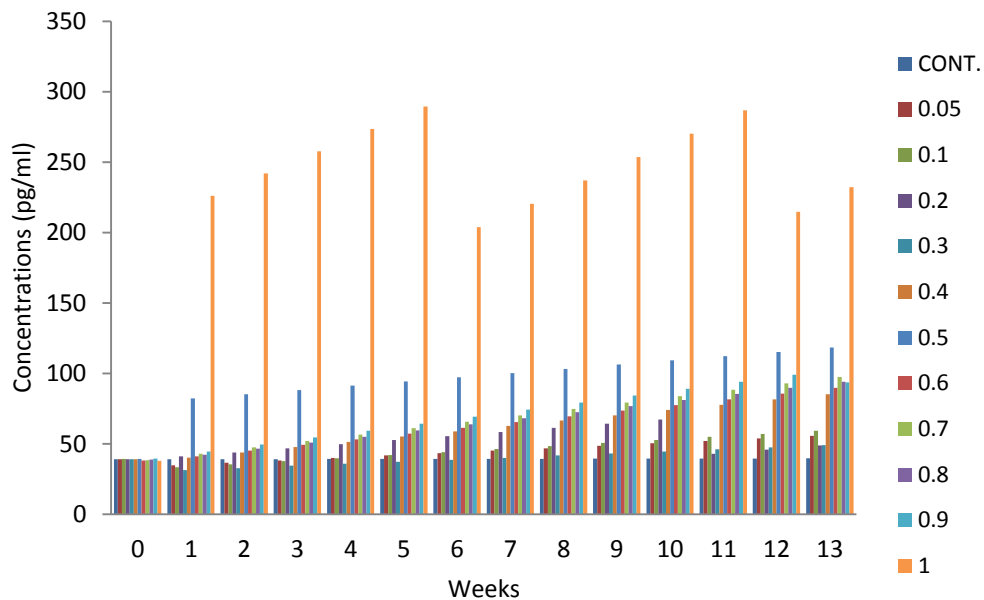


Fig. 2a. Chart of concentration against weeks (durations) for MDA-LDL level

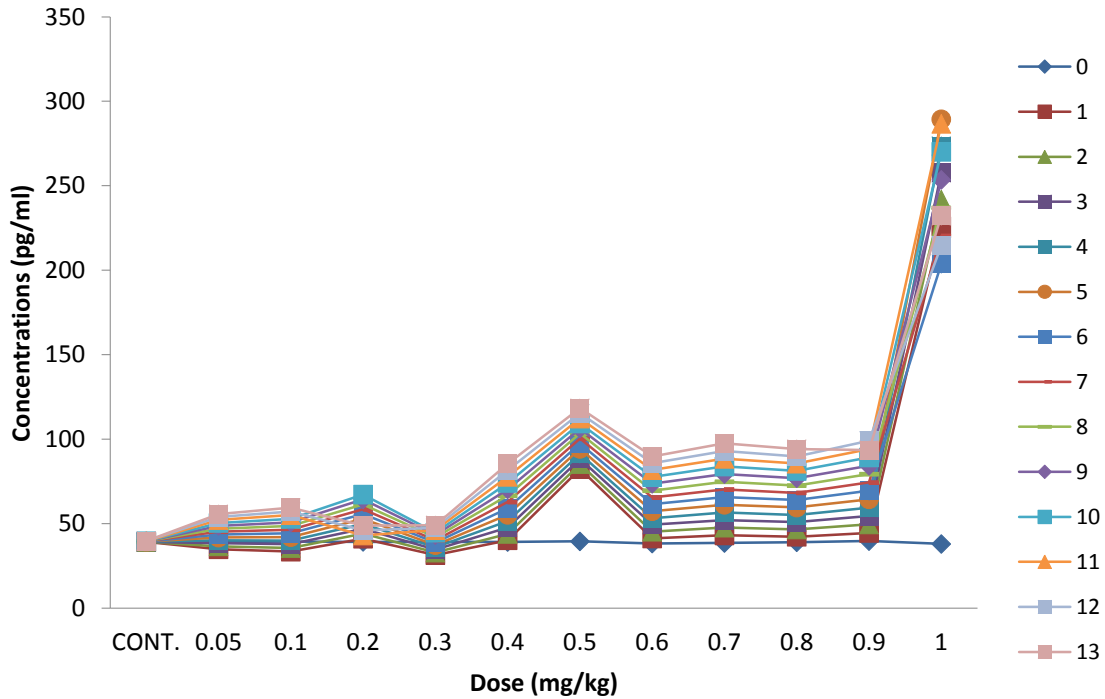
### 3.3 Carboxymethyllysine Modified LDL (CML-LDL)

There is a significant increase in the CML-LDL level in groups 2(0.1mg/kg), 6(0.5mg/kg), 9(0.8mg/kg) -11 (1mg/kg), when compared with the control at  $p \leq 0.05$  (Fig 3a). At week 5 to 13 of

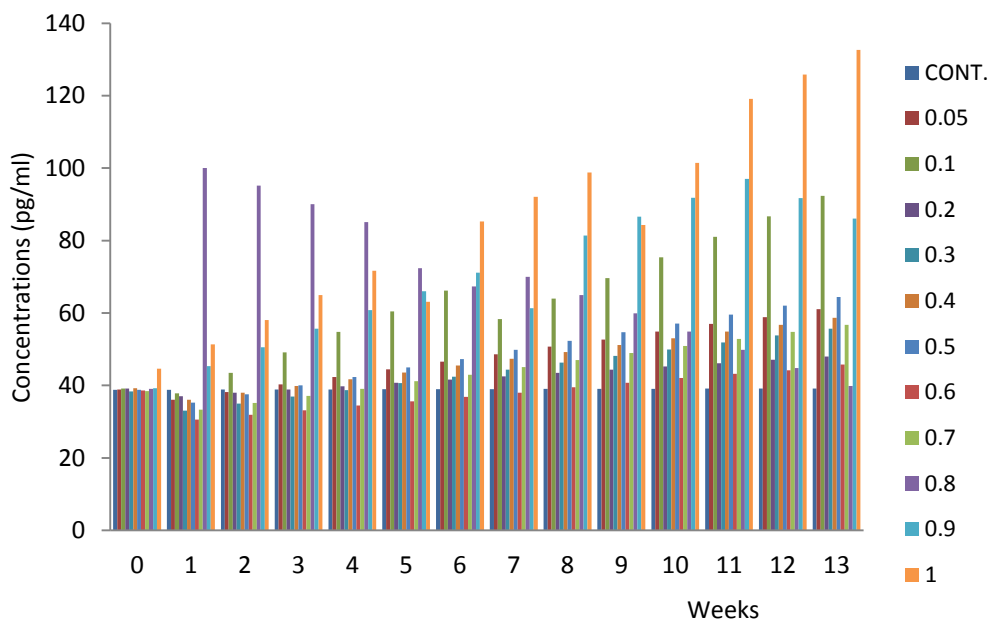
exposure to BPA, the groups exposed to 0.2mg/kg to 0.5mg/kg showed dose dependent increase in carboxymethyllysine modified LDL concentration (Fig. 3a). At group 3 (0.2mg/kg) - 8(0.7mg/kg) at week 1 to 3, showed a non significant decrease compared to the control and week 0; while weeks 7-13 shows a significant

increase (Fig. 3b). At group 9 (0.8mg/kg), the concentration of carboxymethyllysine modified LDL decreases with time of exposure while other exposure groups showed increases in the carboxymethyllysine modified LDL level with time

of exposure (Fig. 3b) except at group 11 (1mg/kg) where there is a non systematic response to the effect of BPA (Fig 3b). The observed effect was time sensitive (Fig. 3b).



**Fig. 2b. Graph of concentration against dose for MDA-LDL level**



**Fig. 3a. Chart of concentration against weeks (durations) for CML-LDL level**

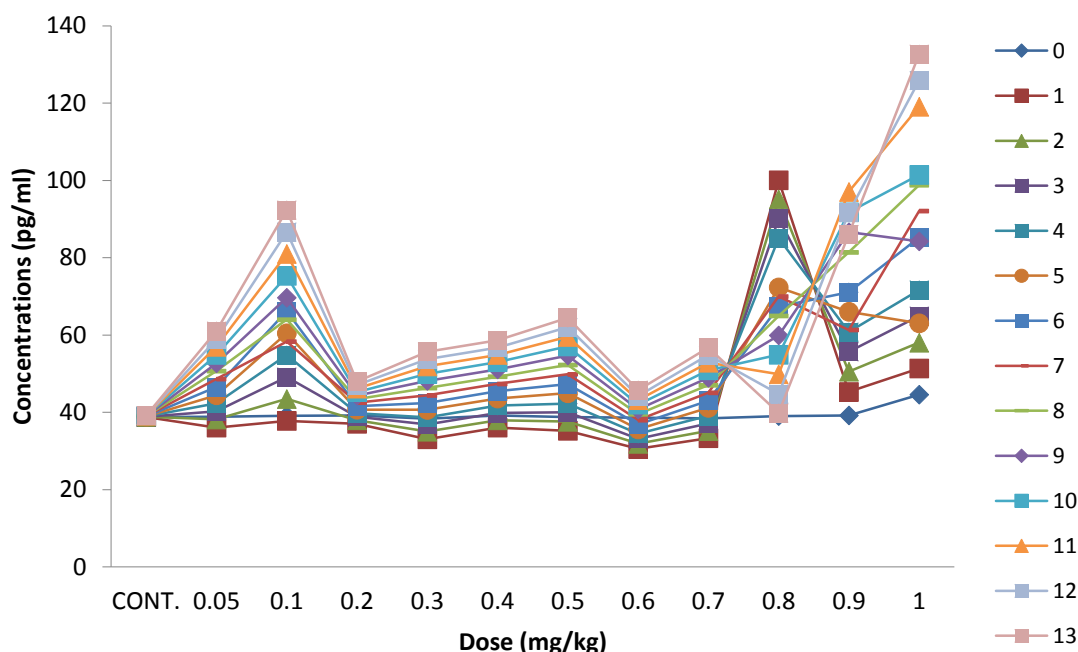


Fig. 3b. Graph of concentration against dose for CML-LDL level

### 3.4 4-HYDROXYNONENAL MODIFIED LDL (HNE – LDL)

There is a significant increase in the HNE-LDL level when compared with the control at  $p \leq 0.05$ . Initially, there was no significant observed for group 1 (0.05mg/kg) to 6 (0.5mg/kg) at week 1 to 4. The rise was more pronounced in week 13 for the entire exposure groups (Fig 4a). At week-1 to 11, the test groups that received 0.4mg/kg to 0.8mg/kg of BPA showed a dose dependent increase in the serum concentration of HNE-LDL, except at week-1 to 6, where the that received 0.8mg/kg showed a decline in the HNE-LDL level relative to that of 0.7mg/kg (Fig. 4a); also, it was observed that the test group exposed to 0.9mg/kg of BPA showed lower level of HNE-LDL relative to that of 1mg/kg group (Fig. 4a). At week-12 to 13, the observed dose dependent effect extends to 1mg/kg with a slight decline by the group that received 0.9mg/kg relative to that of 1mg/kg and in all the group that received 0.9mg/kg of BPA showed lower HNE-LDL level relative to that of 1mg/kg (Fig.4a). The observed effect was time dependent except at test group 0.2mg/kg and time sensitive except at 0.7mg/kg of BPA exposure group (Fig. 4b).

## 4. DISCUSSION

In the current study, BPA increased Reactive Oxygen Species (ROS) production as assessed

by the measurement of the end product of lipid peroxidation such as the oxidized lipids such as OXPL-LDL, MDA-LDL, CML-LDL, and HNE-LDL were high. These results are in agreement with the previous studies Abdelhaffeza *et al.*[16] showed an increase in lipid peroxidation. Ansoumane *et al.*[17] reported, an increased in lipid peroxidation (LPO). BPA administration induced oxidative stress in the heart of rats as evident from the increase in MDA levels, hepatic damage and mitochondrial dysfunction [18,19]. BPA enhances generation of ROS and reduction of antioxidant defenses leading to aggravated state of oxidative stress. Prolonged exposure to BPA can trigger pathological conditions at extremely low levels thus the demand for its prohibition in the use of consumers products. Increased lipid peroxidation indicates an increased oxygen free radical generation which compromises the integrity of mitochondrial function [20]. It has been reported that BPA exposure results in augmentation of oxidative stress, with increased levels of lipid peroxidation and reactive oxygen species and depletion of the antioxidant defense system[21]. Lipids such as phospholipid and other types of lipids found in cells especially as components of plasma membrane and organelle membranes are another major target for the generated ROS, free radicals and Oxidative stress[22]. Lipid oxidation derived products assayed as oxidized lipid, the

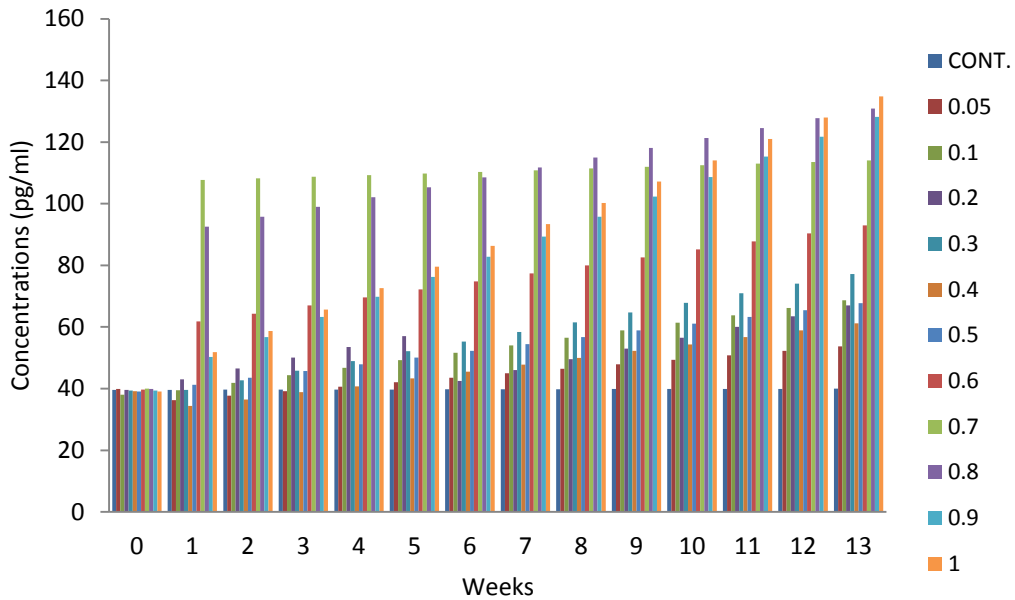


Fig. 4a. Chart of concentration against weeks (durations) for HNE-LDL level

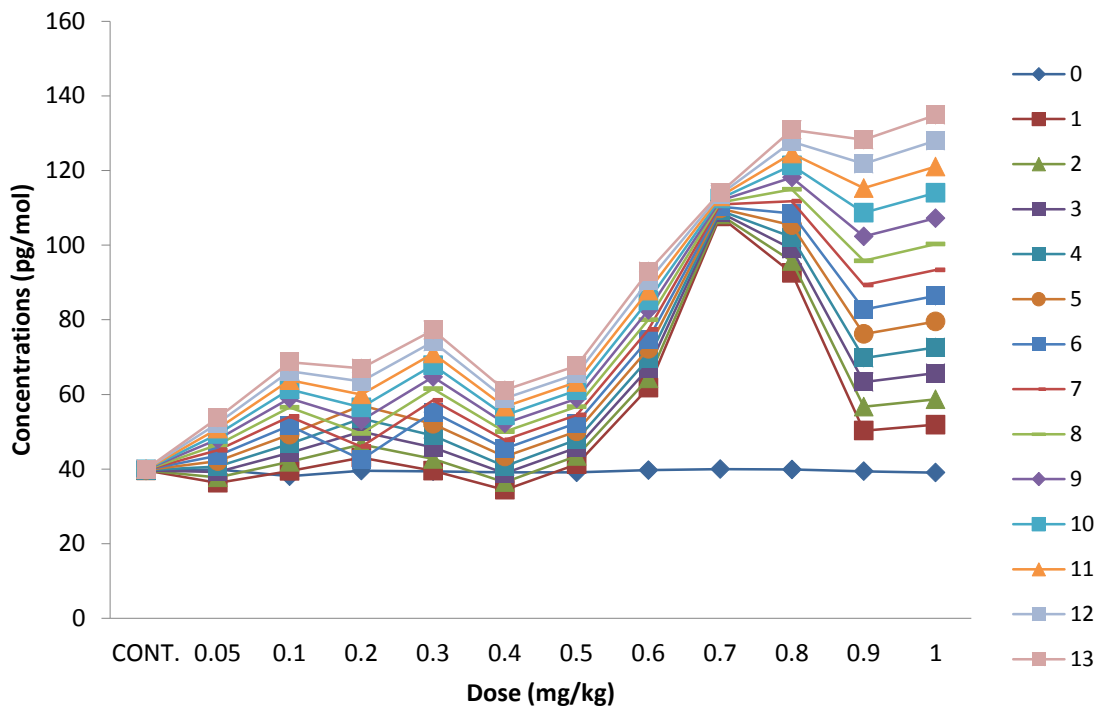


Fig. 4b. Graph of concentration against dose for HNE-LDL level

study showed that the serum levels of the 4 oxidized lipid products were high. They includes oxidized phospholipid LDL, MDA-LDL, CML-LDL and HNE- LDL. The consequence of these findings are impaired membrane functions,

inactivate membrane bound receptors and enzyme and increase cellular membrane permeability, leading to the opening of the mitochondrial permeability transition pore that may cause the release of pro apoptotic molecules to

the cytosol[17]. In addition to the oxidized lipids, depletion of reduced glutathione (GSH) also facilitates the opening of the mitochondrial permeability transition pore. These molecules are usually more reactive than the initial molecules they were formed from and present a range of pathological effects, including increasing vascular permeability, Low Density Lipoprotein (LDL) oxidation and enhancing oxidative stress[20]. Oxidation of free radical is the cause of degradation of fatty acids and their esters in cellular membranes and lipoproteins, leading to the development severe pathological conditions. The damage on cellular membrane by lipid peroxidation of membranes fatty acid[22] and the oxidative damage to lipoproteins, specifically LDL, plays a role in some diseases conditions, which includes cardiovascular diseases, arthritis, dementia and the metabolic syndrome. High oxidized LDL concentrations have been linked to an increased cardiovascular disease risk[20], promotes endothelial cell damage, and are chemotactic for leukocytes and endocytosed by macrophages[20].

Oxidative damages to lipids have seriously deleterious and are concomitant with the oxidative damages to proteins which are the vehicle for oxidative damage on cells because they are often catalysts; hence, the effect of damage to one molecule is greater than stoichiometric. ROS leading to protein oxidation include radical species such as superoxide, hydroxyl, hydroperoxyl, and nonradical species such as hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $1O_2$ ), and peroxynitrite ( $ONOO^-$ )[23].

## 5. CONCLUSION

At very low doses, BPA is capable of causing alterations of lipid in the cells that precede cancer, a wide range of metabolic diseases.

## SIGNIFICANCE STATEMENT

BPA is associated with a number of health problems and diseases that are on the rise in the human population, including cancer and infertility. It induces oxidative stress, provokes antioxidant activity and interferes with lipid metabolism and energy balance.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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