

Effects of Bisphenol A on Inflammatory Biomarkers at Low Doses in Female Wistar Rats

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ABSTRACT

Background and Objective: Bisphenol A (BPA) as an endocrine disruptor, is capable of inducing toxic effects on non-reproductive vital organs. The absorption of BPA has caused extensive damage to the liver and kidney. This study investigates the effects of bisphenol A on some inflammatory biomarkers at low exposure doses in female Wistar rats. **Materials and Methods:** Ten experimental groups each containing 10 female rats was 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/kg body weight, respectively for 3 months. The eleventh control group was given water. Animal blood was collected at the end of every week of the studies and the serum sample specimens were analyzed by routine diagnostic procedures for the parameters assayed. **Results:** The result revealed that hydroperoxidase and myeloperoxidase were significantly decreased when compared with the control group. While hydrogen peroxides were significantly higher than the control group. **Conclusion:** The findings suggest that bisphenol A upsets the balance of the cellular system and causes disturbance in the cellular oxidative system, suggesting that BPA perturbation on cells of the system at these doses and levels of exposure were transient and increases cellular oxidative stress which is a known inducer of various forms of inflammation.

KEYWORDS

BPA, hydrogen peroxide, hydrogen peroxidase, myeloperoxidase, inflammatory biomarker

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INTRODUCTION

Bisphenol A (BPA) is a monomer used primarily in the production of Polycarbonate (PC) resins and epoxy resin widely used in consumer products¹, with other uses that include the synthesis of flame retardant, unsaturated polyester resin, Polysulfone (PS) resin and Polyetherimides (PEI)². Another use for bisphenol A is in the plastic and rubber industries, as a plasticizer and as a polymerization inhibitor in Polyvinyl Chloride (PVC). The BPA is a preferred colour developer in thermal paper³. The BPA-based products are also used in foundry casting and for lining water pipes⁴. It is used in many consumer products as lacquers applied as food can linings and coating on metal lids for glass jars and bottle⁵. The BPA is well distributed in a wide range of organs, in the following order, predominantly detected in the lung, followed by kidneys, thyroid, stomach, heart, spleen, testes, liver and brain. Ratios of the organ to serum BPA concentrations for the organs ranged from 2.0-5.8, except for the brain ratio, 0.75⁶.



Environmentally relevant doses of BPA can cause effects on human development and reproduction. Reports have shown that BPA causes, increases in weight and size of the prostate gland, decreases in sperm efficiency and earlier puberty in female offspring of exposed mouse mothers⁷. Low doses of BPA caused abnormalities in the oocytes⁸. Invernizzi⁹ showed that *in utero* exposure to BPA triggers ductal and alveolar structures proliferations, development of ductal hyperplasia¹⁰, modifications of the mammary gland architecture¹¹, mammary carcinogenesis¹², inflammatory cytokine dysregulation¹³ and mitochondrial-mediated apoptosis in the hepatic tissue¹⁴.

Reported health implications associated with BPA exposure include diabetes¹⁵, cardiovascular disease¹⁶, altered liver enzyme activities¹⁷ and obesity-promoting effects¹⁸. The BPA alters glucose homeostasis, increased pancreatic insulin content and induced insulin resistance¹⁹, BPA induces oxidative stress²⁰, coronary artery disease²¹, activates Maxi-K ion channels in coronary smooth muscle cells²², increased BP and decreased Heart Rate (HR)²³, increased the risk of hypertension²⁴, decreased efficiency of sperm production²⁰ and increased ovarian cancer cell proliferation in a dose-dependent manner²⁵.

The BPA is capable of inducing toxic effects on non-reproductive vital organs, several studies have reported that absorption of BPA has caused extensive damage to the liver and kidney^{17,26}, formation of multinucleated giant cells in the liver hepatocytes, DNA adduct formation and induced the production of free radicals in hepatocytes *in vitro*²⁷. Indeed, this chemical compound may be involved in adipose tissue dysfunction, metabolic/endocrine dysfunctions, cancer and fertility problems²⁸⁻³⁰, oxidation of low-density lipoproteins³¹, impaired plasma glucose³², involved in insulin resistance³³, causes permanent chromosomal damage linked to recurrent miscarriage and birth defects³⁴, spur both the formation and growth of fat cells³⁵. This study was aimed at assessing of effects of bisphenol A on some inflammatory biomarkers at low doses in female Wistar rats.

MATERIALS AND METHODS

Study area: The study was carried out at Biochemistry Department, Biochemistry Department Research Laboratory, Faculty of Natural and Applied Sciences, Gregory University, Uturu, Abia State Nigeria, from June to August, 2021.

Animals: Female albino rats aged 5 weeks old were obtained from the animal house, Faculty of Veterinary Medicine, University of Nigeria, Nsukka and used for the study.

Animal experiment: The experimental albino rats were randomly selected and marked for easy identification and kept in their cages at the animal house of Gregory University Uturu (GUU), Abia State for 7 days before dosing to allow for acclimation to the environmental condition at room temperature. Commercial rat pellets and water were provided *ad libitum*. The OECD (2010) guideline for animal care was employed throughout the study.

The animals were randomly divided into 12 groups of 10 rats each, 11 test groups received graded doses of BPA, while 1 group served as the control group.

The eleven test groups of experimental animals received graded doses of bisphenol A in solution. The experimental animals were orally administered 0.5 mL of BPA solution containing 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/kg body weight respectively, daily for 3 months. While the control received water. The animals were fasted before the substance administration but allowed free access to drinking water. Food was withheld overnight before the administration of the test substance. The test substance was administered daily in a single dose to the animals in groups by oral gavage using an intubation cannula. After administration of the test substance, food was withheld further for 3-4 hrs before feeding resumed.

The blood samples of all the animals (control and test groups) were collected at the beginning of the experiment before the administration of the test substance commenced. Afterwards, blood samples were collected weekly for 3 months to monitor the effect of BPA on the desired parameters.

At the end of every week, blood samples were collected from the animals' tails and processed for biochemical assays.

Reagents: All the reagents used were already prepared and commercially obtained as kits and are here listed: Hydrogen Peroxide, Hydrogen Peroxidase and Myeloperoxidase from OXI-Select, Minneapolis, USA. Individual tests were carried out according to the kit specifications as described below.

Hydrogen peroxidase and hydrogen peroxide assay

Principle: The Hydrogen Peroxide/Peroxidase Assay Kit is a sensitive quantitative colourimetric assay for hydrogen peroxide or peroxidase. In the presence of peroxidase, the probe reacts with H₂O₂ in a 1:1 stoichiometry to produce a bright pink-coloured product. This product can be easily read by a standard colourimetric microplate reader with a filter in the 540 nm range. Absorbance values are proportional to the H₂O₂ or peroxidase levels within the samples.

Kit reagent:

- Colorimetric probe (100×)
- HRP
- Hydrogen peroxide
- 10× assay buffer

Procedure

Hydrogen peroxide:

- Number of microwell strips required was placed in the holder
- 50 µL of each sample of H₂O₂ standard, control and unknown were added into an individual microtiter plate well
- 50 µL of hydrogen peroxide working solution was dispensed to each well. The good contents were mixed thoroughly and incubated for 30 min at 25°C in the dark
- Concentration was measured at 540 nm

Peroxidase:

- Number of microwell strips required was placed in the holder
- Add 50 µL of each sample of HRP standard, control and unknown were added into an individual microtiter plate well
- 50 µL of peroxidase working solution was pipetted to each well. The good contents were mixed thoroughly and incubated for 30 min at 25°C in the dark
- Concentration was measured at 540 nm

Myeloperoxidase assay

Principle: The Myeloperoxidase Kit is for measuring the myeloperoxidase activity within a sample. The reaction of Hydrogen Peroxide (H₂O₂) with chloride ions to create Hypochlorous Acid (HOCl) is catalyzed by the MPO enzyme. The Hypochlorous Acid (HOCl) rapidly reacts with taurines to produce taurine chloramine product. This step neutralizes the HOCl and inactivates MPO. A stop solution is added to stop MPO catalysis by eliminating hydrogen peroxide. Finally, taurine chloramine reacts with the chromogen probe. Absorbance is measured at 405 nm. The rate of chromophore reduction is proportional to the concentration of myeloperoxidase activity.

Kit reagent:

- Hydrogen peroxide
- Chromogen probe (100×)
- Assay buffer (5×)
- TCEP reagent (100×)
- Stop solution (500×)
- HTAB extraction reagent

Procedure:

- Number of microwell strips required was placed in the holder
- 25 μ L of each unknown sample and control was added to a 96-well plate
- 25 μ L of the 1 mM hydrogen peroxide solution was added to each well, mixed thoroughly on a horizontal shaker, the plate covered and incubated at 25°C for 30 min in the dark
- 1× stop solution was vortexed and 50 μ L of the vortexed stop solution was added to each sample well, mixed briefly and incubated at 25°C for 15 min
- Then 50 μ L of the 1 mM chromogen working solution was added to each well to which 1× stop solution was added in step 4 and mixed briefly. The plate was covered and incubated at 25°C for 15 min in the dark
- Concentration was measured using 405 nm as the primary wavelength

Statistical analysis: The data obtained from each set of the study were subjected to statistical analysis using the IBM Statistics Software, Version 20. Each attribute was subjected to ANOVA analysis, *post hoc* analysis was carried out using Tukey's and $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Hydrogen peroxidase: There is a significant decrease in the hydrogen peroxidase level when compared with the control from week 1-13 (12.065 ± 0.01 to 12.875 ± 0.03) at $p \leq 0.05$ (Fig. 1a-c). Following the 1st month of BPA administration (weeks 1-4) in Fig. 1a, it was observed that in the first 4 weeks, the group that received 0.6 and 0.9 mg kg^{-1} b.wt., of BPA, revealed a significant decrease in hydrogen peroxidase level that is relatively constant of 2.0 ± 0.2 and 1.0 ± 0.03 , respectively at $p \leq 0.05$ in Fig. 1a while, the group that received 0.05 mg kg^{-1} b.wt., of BPA revealed a relatively constant in hydrogen peroxidase level of 5.2 ± 0.01 from week 1-6 that is significant at $p \leq 0.05$ (Fig. 1a and b). the group that 1.0 mg kg^{-1} b.wt., of BPA showed 8.375 ± 0.00 per week 1, following continued administration of BPA for weeks 2 to 4 the hydrogen peroxidase level declined from 1.015 ± 0.01 to 1.395 ± 0.01 (Fig.1a).

As the BPA administration continued to the 2nd month (weeks 5-8) in Fig. 1b, the groups that received 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg kg^{-1} b.wt., of BPA showed a relatively constant hydrogen peroxidase level of 5.4 ± 0.00 , 4.8 ± 0.03 , 3.1 ± 0.01 , 1.6 ± 0.01 , 8.2 ± 0.02 and 1.2 ± 0.01 , respectively which are significant at $p \leq 0.05$ (Fig. 1b). It was also observed that the group that was administered 0.05 and 0.1 mg kg^{-1} showed a significant decrease in hydrogen peroxidase levels of 0.69 ± 0.03 and 1.57 ± 0.01 $p \leq 0.05$, respectively at weeks 7-9 (Fig. 1b and c).

During the 3rd month of continued BPA administration (weeks 9-13) in Fig. 1c, it was observed that at weeks 11-13, all the experimental dose groups revealed a relatively constant decrease in hydrogen peroxidase level of 0.05 mg kg^{-1} (4.6 ± 0.02), 0.1 mg kg^{-1} (2.5 ± 0.00), 0.2 mg kg^{-1} (1.10 ± 0.04), 0.3 mg kg^{-1} (7.8 ± 0.01), 0.4 mg kg^{-1} (1.1 ± 0.03), 0.5 mg kg^{-1} (0.8 ± 0.00), 0.6 mg kg^{-1} (2.1 ± 0.03), 0.7 mg kg^{-1} (0.5 ± 0.02), 0.8 mg kg^{-1} (0.86 ± 0.00), 0.9 mg kg^{-1} (1.1 ± 0.00) and 1.0 mg kg^{-1} (0.8 ± 0.03) which are significant at $p \leq 0.05$.

The highest concentration was observed in group 10 (0.9 mg kg^{-1} b.wt., of BPA), from weeks 5-10 (Fig.1b and c), although, all the values obtained still fall below that of the control values at all the weeks of exposure (Fig. 1a-c).

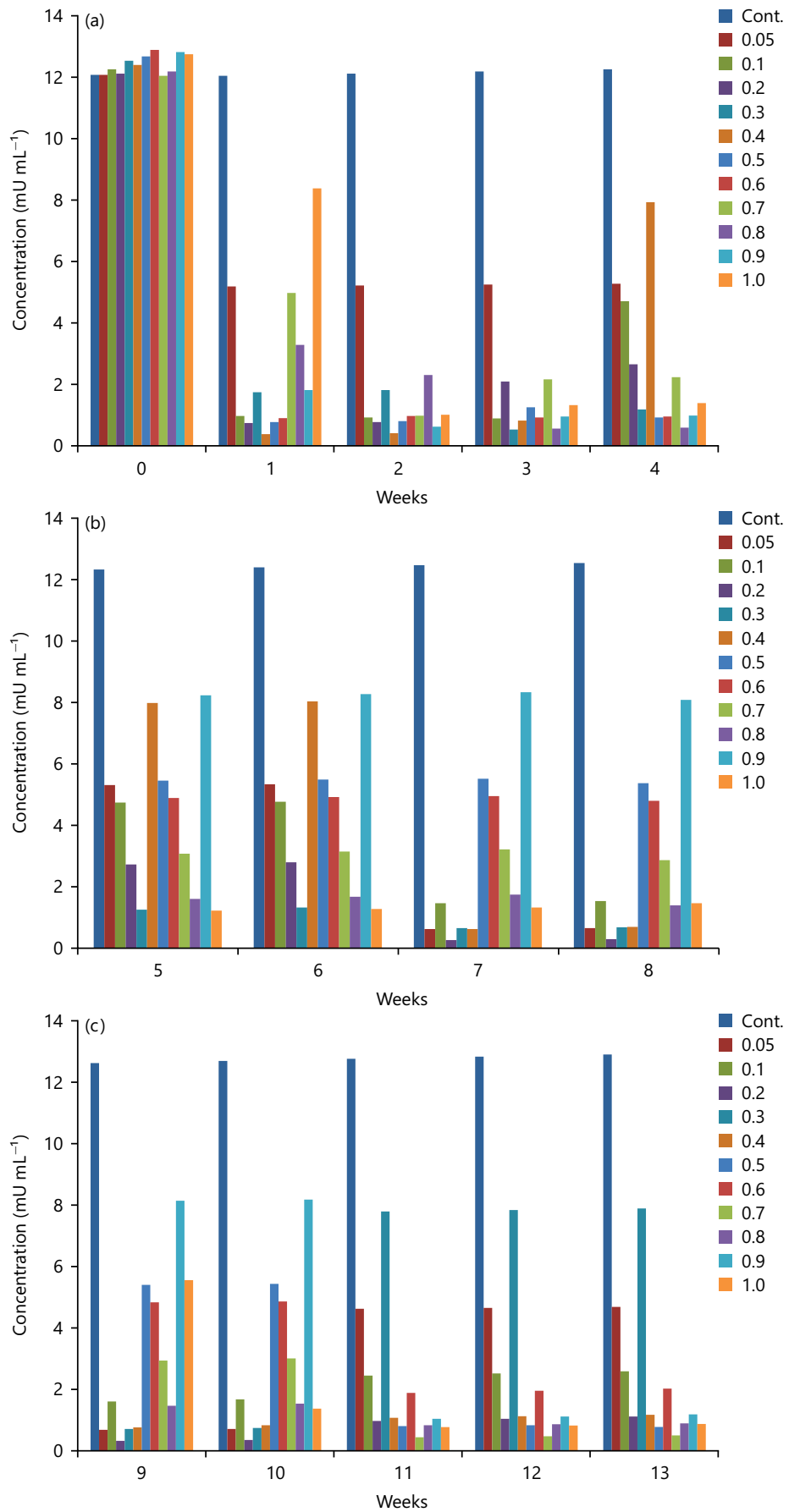


Fig. 1(a-c):Hydrogen peroxidase level, (a) In 1st month, (b) In 2nd month and (c) In 3rd month

Hydrogen peroxide: There is a significant dose-dependent increase in the hydrogen peroxide level when compared with the control from weeks 1-13 (12.385 ± 0.02 , 12.495 ± 0.02) at $p \leq 0.05$ (Fig. 2a-c). Following the continued administration of BPA at the 1st month in Fig. 2a, 2nd month in Fig. 2b and the 3rd month in Fig. 2c, it was observed that throughout the period of exposure, all the experimental groups showed a significant increase in hydrogen peroxide concentration at $p \leq 0.05$, that appeared to be relatively constant over time, 0.05 mg kg^{-1} (59.9 ± 0.04), 0.1 mg kg^{-1} (69.9 ± 0.01), 0.2 mg kg^{-1} (85.3 ± 0.01), 0.3 mg kg^{-1} (91.9 ± 0.02), 0.4 mg kg^{-1} (109.7 ± 0.00), 0.5 mg kg^{-1} (121.1 ± 0.03), 0.6 mg kg^{-1} (136.7 ± 0.02), 0.7 mg kg^{-1} (147.4 ± 0.00), 0.8 mg kg^{-1} (163.9 ± 0.01), 0.9 mg kg^{-1} (184.4 ± 0.02), except for the group that were administered 1.0 mg kg^{-1} where it was observed that the hydrogen peroxide concentration increases with time such as in week 1 (227.85 ± 0.03), week 2 (233.68 ± 0.01), week 3 (239.53 ± 0.00) and week 4 (245.36 ± 0.03). The highest weekly effect was consistently observed in group 11 (1.0 mg kg^{-1} b.wt., of BPA) in Fig. 2a-c.

Myeloperoxidase: There is a significant decrease in the myeloperoxidase level in all the test groups when compared with the control between week 1-13 (99.954 ± 0.02 to 100.442 ± 0.00) at $p \leq 0.05$ (Fig. 3a-c) in the 1st month (week 1-4) of the experiment in Fig. 3a, the experimental group that received 0.05 and 0.1 mg kg^{-1} b.wt., of BPA decreases the level of myeloperoxidase levels that is relatively constant at 96.8 ± 0.03 and 85.7 ± 0.01 , respectively Fig. 3a. For 0.2 mg kg^{-1} b.wt., of the BPA group, the myeloperoxidase level was maintained at 71.85 ± 0.01 except at week 3 where it was 96.997 ± 0.00 whereas, the treated with 0.3 and 1.0 mg kg^{-1} b.wt., of BPA, showed a severe decline in myeloperoxidase levels at week 1 (17.74 ± 0.02 , 14.27 ± 0.02) and week 2 (18.31 ± 0.01 , 16.02 ± 0.03), respectively.

Continued administration of BPA to week 5 in Fig. 3b, the group treated with 0.05 and 0.1 showed a significant decrease in myeloperoxidase levels of 22.91 and 27.04 at $p \leq 0.05$. The groups treated with 0.3 , 0.4 , 0.5 , 0.6 and 0.7 mg kg^{-1} b.wt., of BPA, revealed a significant decrease in myeloperoxidase level at week 6 (20.61 ± 0.01 , 27.41 ± 0.00 , 38.97 ± 0.00 , 47.13 ± 0.01 , 46.45 ± 0.00) and week 7 (27.3 ± 0.2 , 26.52 ± 0.00 , 37.66 ± 0.001 , 44.98 ± 0.001 , 44.37 ± 0.00), respectively at $p \leq 0.05$ when compared with the control.

At weeks 11 and 12 in Fig. 3c, the experimental group that received 0.05 mg kg^{-1} b.wt., of BPA and 0.1 mg kg^{-1} b.wt., of BPA showed a myeloperoxidase level of 97.05 ± 0.01 and 86.30 ± 0.00 , respectively. Also at week 12, it was observed that the group treated with 0.8 and 0.9 mg kg^{-1} b.wt., of BPA, show a significant decrease in myeloperoxidase levels of 8.27 ± 0.02 and 11.27 ± 0.01 at $p \leq 0.05$, which further decrease at week 13 to 5.44 ± 0.00 and 8.98 ± 0.01 , respectively.

The findings of this research showed that hydroperoxidase and myeloperoxidase were decreased when compared with the control group. While, hydrogen peroxides were higher than the control group. Present study results are following Chen and Schopfer³⁶, who reported that BPA shows its toxicity by increasing H_2O_2 . Chitra *et al.*³⁷, showed increased levels of H_2O_2 . Sravani *et al.*³⁸ also revealed an increase in H_2O_2 . Kabuto *et al.*³⁹, observed overproduction of H_2O_2 and Sravani *et al.*³⁸ showed excess production of H_2O_2 radicals which can only be effectively detoxified by increased glutathione peroxidase activity. Circulating myeloperoxidase levels were low in animal studies⁴⁰. Previous studies in rats⁴¹ and mice⁴² were able to show a low measure of myeloperoxidase.

This could also be due to enzyme inactivation caused by excess ROS production in mitochondria and microsomes⁴³. It was found that BPA disturbs the balance of the mitochondrial antioxidant-pro oxidant status through reduction of the activities of mitochondrial respiratory chain enzymes, which can cause mitochondrial dysfunction and increased ROS generation⁴⁴. Additionally, it could be mediated through the ability of BPA to stimulate the polymorphism of oxidative stress-related genes⁴⁵.

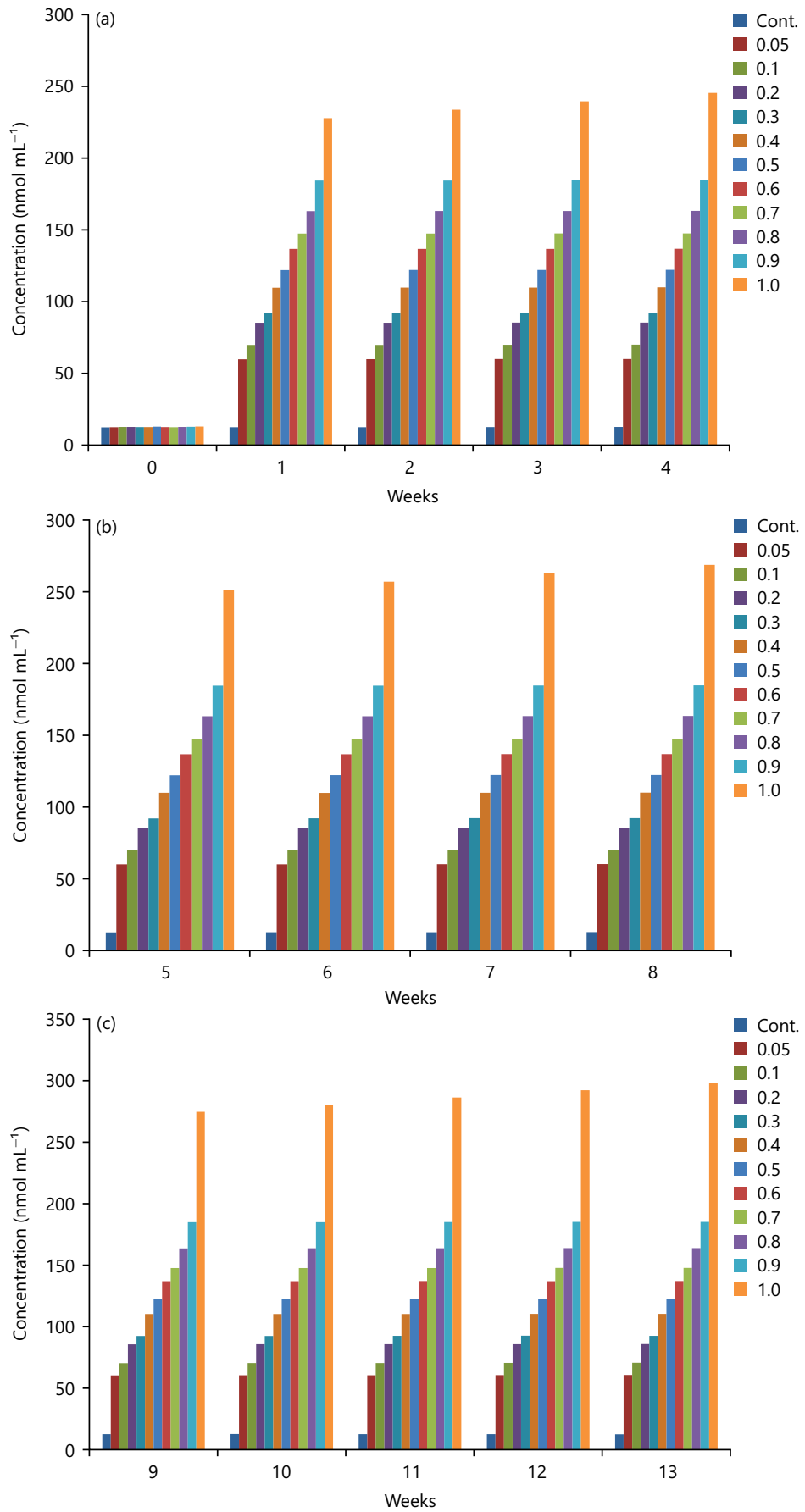


Fig. 2(a-c): Hydrogen peroxide level measurement, (a) In 1st month, (b) In 2nd month and (c) In 3rd month

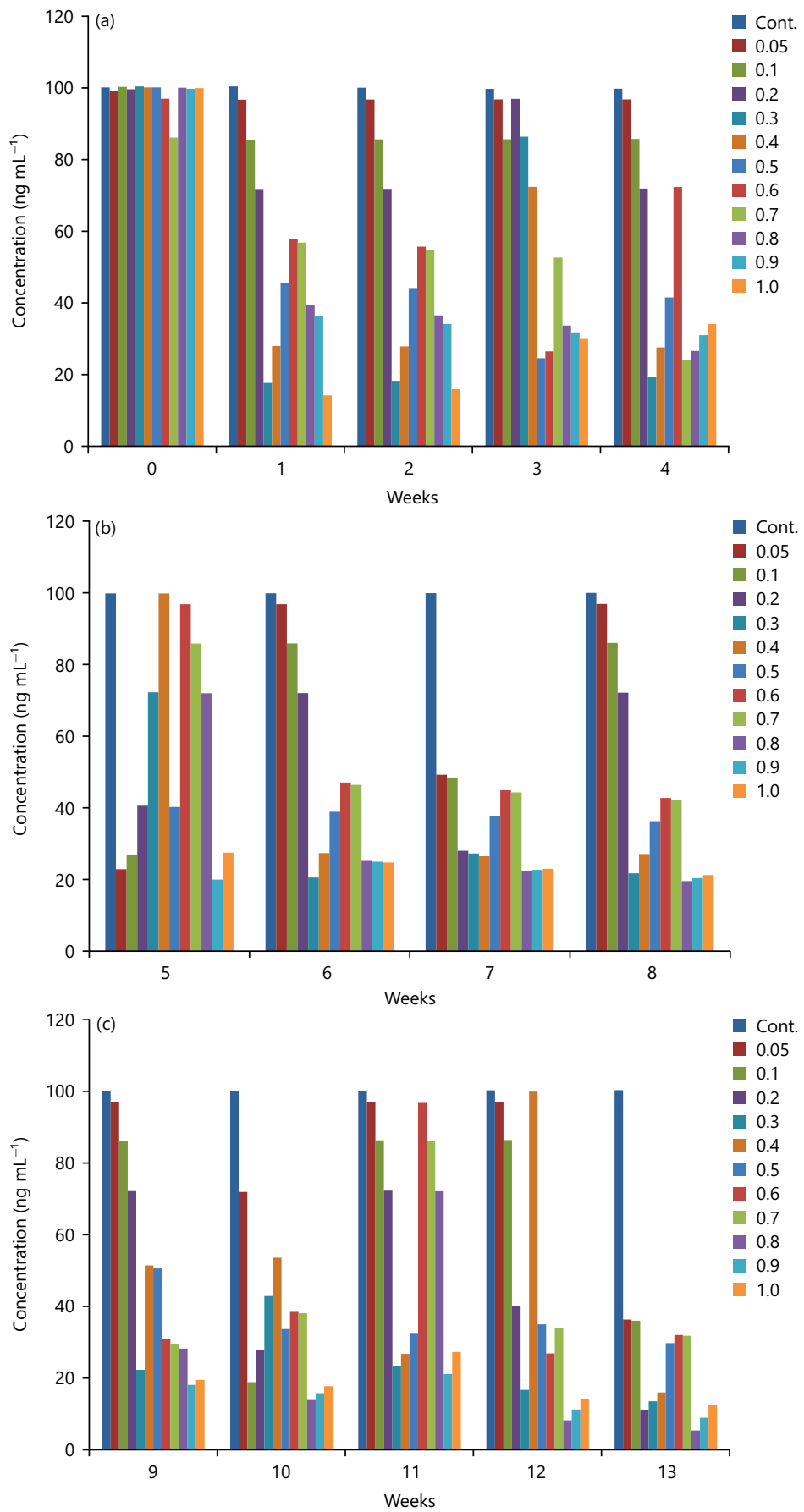


Fig. 3(a-c): Myeloperoxidase level, (a) In weeks 1-5, (b) In weeks 5-10 and (c) In weeks 11 and 12

CONCLUSION

Living organisms are constantly exposed to BPA which triggers the formation of free radicals and reactive oxygen species that led to oxidative stress capable of modifying biomolecules and exerting an important role in the development of tissue damage. An increase in free radicals levels such as the one observed in this study, where H_2O_2 is increased, may lead to decreases in different cellular defence systems and if the elicited damage is irreversible, it may lead to cell death. The observed decrease in the hydroperoxidase and myeloperoxidase assayed can increase cellular oxidative stress which is a known inducer of various forms of inflammation.

SIGNIFICANCE STATEMENT

This study discovered that BPA augments the inflammatory response and is associated with oxidative stress, promoting inflammatory responses and the development of immune upregulation, thereby exacerbating preexisting immune diseases and that continued exposure to BPA may potentiate the incidence and severity of the immune diseases. This study will help the researchers to uncover the critical areas of Reactive Oxygen Species (ROS) production which compromises mitochondrial function from the BPA effect. Thus a new theory on the BPA effect on the activities of the antioxidant system and generation of ROS that causes oxidative damage in organs and tissues may be arrived at.

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