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In Vitro Profiling Of The Glutathione Reductase And Glutathione Peroxidase To Determine Low Dose Effects Of Bisphenol A On Glutathione System And Oxidative Stress.

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Abstract:
Background*: Bisphenol-A (BPA) is an environmental xenoestrogen that has been studied for its impact on fertility in animals and humans. Growing evidence showed that Bisphenol-A can disrupt the cytochrome P450 enzymes activities, leading to generation of reactive oxygen species with endocrine Interference effect, and can affect multiple organs. The objective of the study was to investigate the effects of varying concentrations of BPA (0.05 - 1μM for 13 weeks) on glutathione reductase and on glutathione peroxidase in female rats.* ***Method****: The eleven experimental groups 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/kg bw/day for 13weeks respectively and blood samples collected weekly. The glutathione reductase and glutathione peroxidase were assayed using ready-to-use reagent kits and autochemicalanalyser. Individual tests were carried out according to the kit specifications.* ***Results****: the results showed significant increase in glutathione reductase, but glutathione peroxidase and were decreases after BPA exposure. The findings suggest that BPA induce oxidative stress.* ***Conclusion****: BPA-induced changes in the oxidative state in rats and may be a potential cue of BPA-mediated disease conditions.*

Keywords: ***Bisphenol A, glutathione reductase, glutathione peroxidase, oxidative stress.***

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I. Introduction

Bisphenol A (BPA) is a monomer used primarily in the production of polycarbonate (PC) resins and epoxy resin widely used in consumer products[1]. Mourad and Khadrawy[2] reported increased urinary BPA (total BPA) levels in epoxy resin sprayers. other uses that include the synthesis of flame retardant, unsaturated polyester resin, polysulfone resin and polyetherimides[3]. BPA absorption from the gastrointestinal tract and dermal absorption is relatively high. Zalko*et al* [4], on the other hand, observed absorption of 46 % via the human skin. A penetration of 8.6 % with a maximum penetration rate of 0.022 μg/cm2/hour was measured[5]. BPA accumulates in adipose tissue[6], body fluids, human placenta[7], cord blood[8], amniotic fluid[9,10], fetal liver[11] and breast milk[12,13], and in fetus[10]. BPA is well distributed in a wide range of organs, predominantly, the lung, kidneys, thyroid, stomach, heart, spleen, testes, liver, and brain[14].

There are evidences indicating that developmental exposure to BPA perturb the neurotransmitter; BPA alter the dopaminergic system at presynaptic and post synaptic level[15], changes the gene expression of dopamine transporter[16], altered dopamine metabolism[17], and activation of dopamine receptor mediated G-proteins[18]. BPA affect gamma-amino butyric acid type A receptor[19], inhibits the expression of ERβ protein and increases aromatase activity[20], suggesting BPA exposure perturb the neurotransmitter system[21]

Another use for bisphenol A is in the plastic and rubber industries. it is used as a colour developer in thermal paper[22], in foundry casting and for lining water pipes[23]. It was reported that female and male health has been seriously threatened, and the environmental pollution was thought to be the main reason of this phenomenon[24]. BPA has been in use commercially for over 50 years, and workers producing this compound and its products have been exposed to time-weighted average air levels to about 10 mg/m3 over decades[13]. it

has been found that continous exposures to BPA are irritating to the eye and respiratory tract, and may cause skin lesions and photosensitization of the skin[13]. BPA has the potential to cause Slight and transient nasal tract epithelial damage[25]. BPA appears to have a respiratory irritation potential.

Also, BPA exposure has been associated with autoimmune pathophysiology. BPA disruption of cytochrome P450 enzymes by downregulation, and alterations in immune function, is a potential mechanism for autoimmune pathophysiology induced by BPA exposure[26]. BPA is a common environmental estrogen with endocrine Interference effect, and can affect multiple organs of human[27,28,29]. research revealed that BPA can affect the function of thyroid gland and disrupt the function of nuclear hormone receptors and their cofactors to disturb the internal hormonal environment[30]. Braun *et al*.[31], suggested that prenatal exposure of BPA is associated with externalizing behaviors in children. Gestational BPA exposure affects behavioral and emotional regulation domains in children[32]. BPA exposure promotes a podocytopathy with proteinuria, glomerular hyperfiltration and podocytopenia[33], raises the levels of cytokines and induce developmental immunotoxicity[29]. As an environmental estrogen, BPA can affect the functions of reproductive system and lead to infertility. Fujimoto *et al*. [34], suggested that BPA exposure interfere with oocyte quality, influence semen quality parameters[35], decrease sperm count and quality, and DNA damage in somatic and germ cells[36]. BPA up regulated cluster in expression in atrophic prostate epithelial cells and induced lipid peroxidation and DNA fragmentation in spermatozoa[37]. The aim of this study is to unveil/establish the possible effects and physiological disposition of Bisphenol A on oxidative stress markers in female wistar albino rats by carryingout analysis of glutathione enzymes.

II. Materials And Methods

Study area: The study was carried out at Applied Biochemistry Lab, NnamdiAzikiwe University, Awka, Nigeria and Biochemistry Lab, Gregory University Uturu, Abia state, Nigeria from January 2018 to April, 2018.

Methodology:

Total 60 non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for 7 days and randomly divided into 11 experimental groups of 5 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/kg bw/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 glutathione reductase and glutathione peroxidase were assayed using Autochemicalanalyser (Lx 20 pro Autoanalyser, Beckman Coulter, Woerden, Netherland andChemwell chemical Analyzer, Manufacturer: Roche Hitachi, GMI.). All reagents were commercially obtained as already prepared kits. The kits for glutathionereductase and glutathione peroxidase were purchased from Sigma-Aldrich, St. Louis, USA. Individual tests were carried out according to the kit specifications.

Statistical analysis:

Differences between obtained values (mean±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. At $p \leq 0.05$ was taken as a criterion for a statistically significant difference.

III. Results

- **GLUTATHIONE REDUCTASE**

During the first four weeks of exposure to BPA (fig 1a), it was observed there was no significant difference between the glutathione reductase level of the control (CONT.) and the baseline (0). In fig 1a, the groups that received 0.2 – 0.7mg/kg BPA and 0.9 mg/kg BPA, when compared with the control group, showed significant increase in glutathione reductase level which decrease over time. The 0.1mg/kg BPA test group showed an increase in glutathione reductase level which was relatively constant over time. A relatively slight nonsignificant increase in glutathione reductase was observed in the experimental group that were administered 0.05 and 0.8 mg/kg BPA. Only 0.1 mg/kg BPA test group showed a nonsignificant decrease in glutathione reductase level when compared with the control group.

As the orally administration of BPA continued to the second month (week 5-8) in fig 1b, it was observed that there was a significant increase in the level of glutathione reductase when compared with the control group, for the groups that were exposed to 0.05, 0.6, 0.9 and 1 mg/kg BPA. The glutathione reductase level for 0.05mg/kg BPA test group tends to increase over time, those of 0.6 and 0.9 mg/kg BPA tend to

decrease with time, while that of 1 mg/kg BPA was relatively constant. Interestingly, the test group that were administered with 0.3 mg/kg BPA showed a decreased level of the enzyme over time. A characteristic behaviour was observed in the experimental group that were given 0.5 and 0.7 mg/kg BPA where the enzyme level were high at weeks 5 and 6 but decreased in weeks 7 and 8. The groups that were exposed to 0.1 and 0.8 mg/kg BPA showed a slight nonsignificant increase in the enzyme level over time, while 0.2 mg/kg BPA experimental group showed a noncharasteristic fluntuation in the enzyme level during the time of exposure.

In fig 1c,the eefect of continued BPA exposure for the third month was monitored. There is a significant decrease in glutathione reductaes for the test groups that were administered 0.2, 0.4-0.7 mg/kg BPA over time of exposure but a charasteristic significant increase in the enzyme level was observed in week 13 for 0.4 and 0.6 mg/kg BPA test group. The 0.3 mg/kg BPA experimental group showed a noncharacteristic decrease in the enzyme level. The test group of 0.05, 0.1, 0.8, 0.9 and 1 mg/kg BPA showed an increase in the enzyme level; 0.05 mg/kg BPA test group showed a significant increase over time, those of 0.1 and 0.8 mg/kg BPA test group showed a slight nonsignificant increase in the enzyme level over time, while 0.9 mg/kg BPA test group tends to decrease over the period of exposure but 1 mg/kg BPA test group remained relatively constant throughout the last five weeks of exposure.

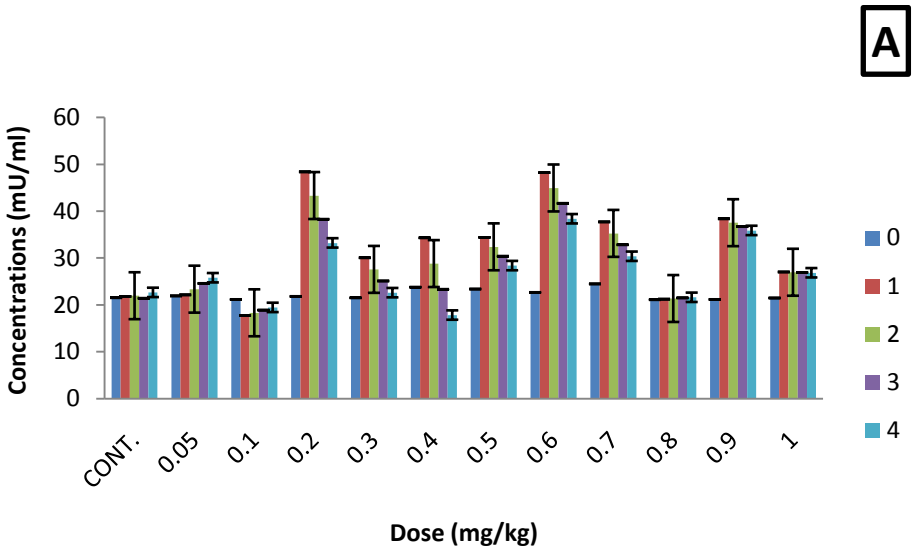

Fig. 1a; Glutathione reductase level after first month (week 1-4) of exposure to BPA.

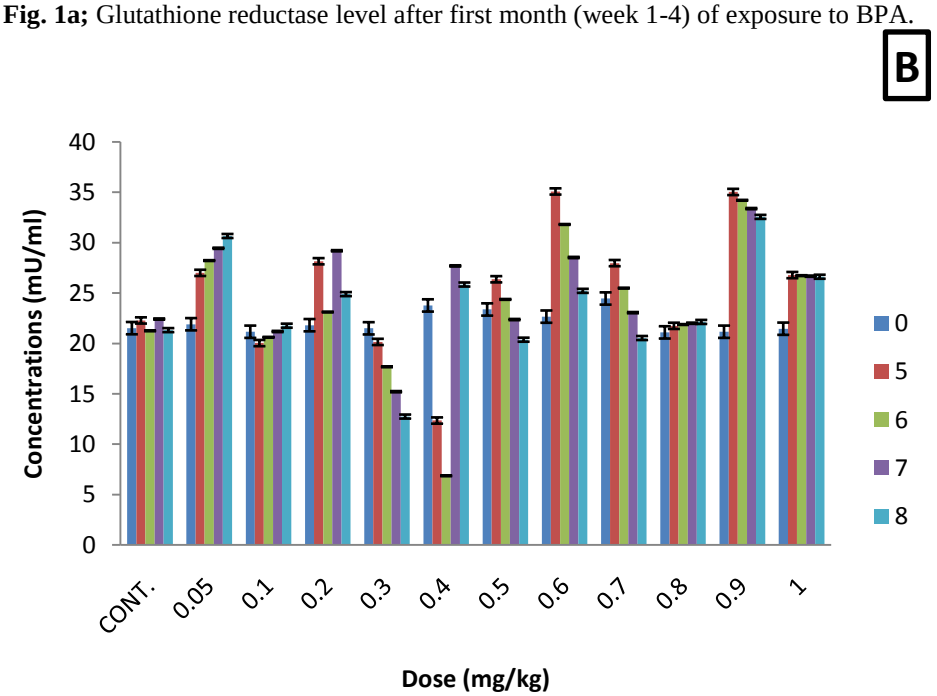

Fig. 1b;Glutathione reductase level after the second month (week 5-8) of exposure to BPA

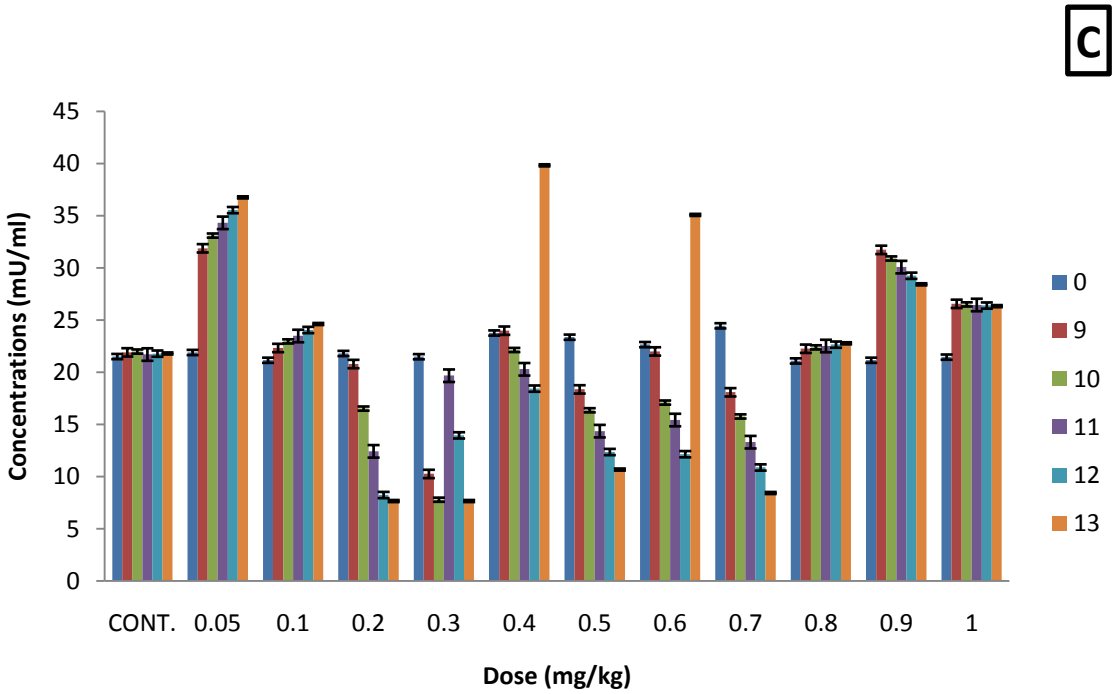

Fig. 1c;Glutathione reductase level after the third month (week 9-13) of exposure to BPA

- **GLUTATHIONE PEROXIDASE**

Generally, there is a significant dose dependent decrease in the glutathione peroxidase level when compared with the control and week 0 at $p \leq 0.05$. Fig. 2a to 2c showed the result for glutathione peroxidase after the 13 weeks (1 to 3 month) of exposure to graded doses of BPA. Glutathione peroxidase was significantly decrease in all the experimental groups over time, with 0.6, 0.8 and 0.9 mg/kg.BPA test groups showing a relatively constatnt glutathione peroxidase levels. Fig.2b, further revealed that the enzyme level was significantly decreased following the continued exposure to BPA. The last phase of the experiment (fig 2c), showed a more significant decrease in the glutathione peroxidase level in the experimental group. The 0.2mg/kg BPA test group showed a relatively high glutathione peroxidase level when compared with the past months results (fig 2a and 2b)

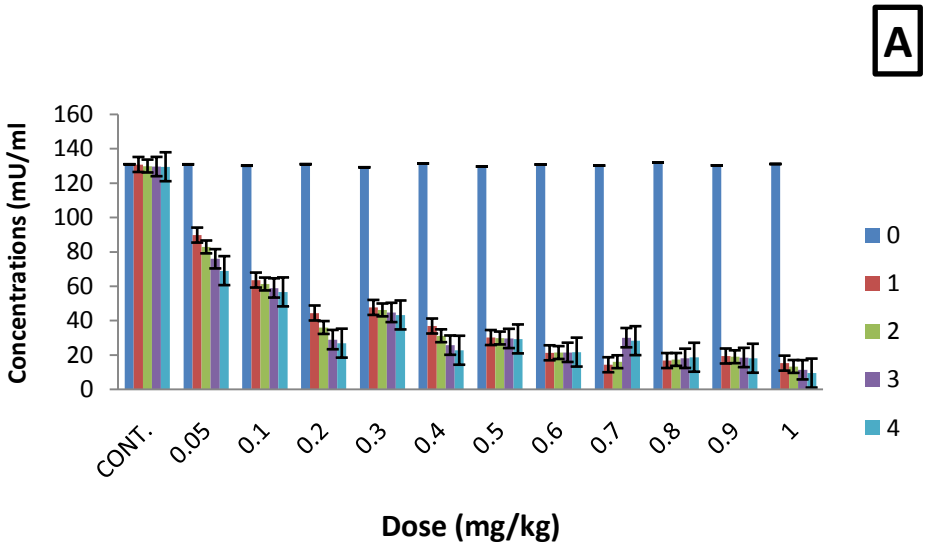

Fig 2a; Glutathione peroxidase level after the first month (week 1- 4) of exposure to BPA

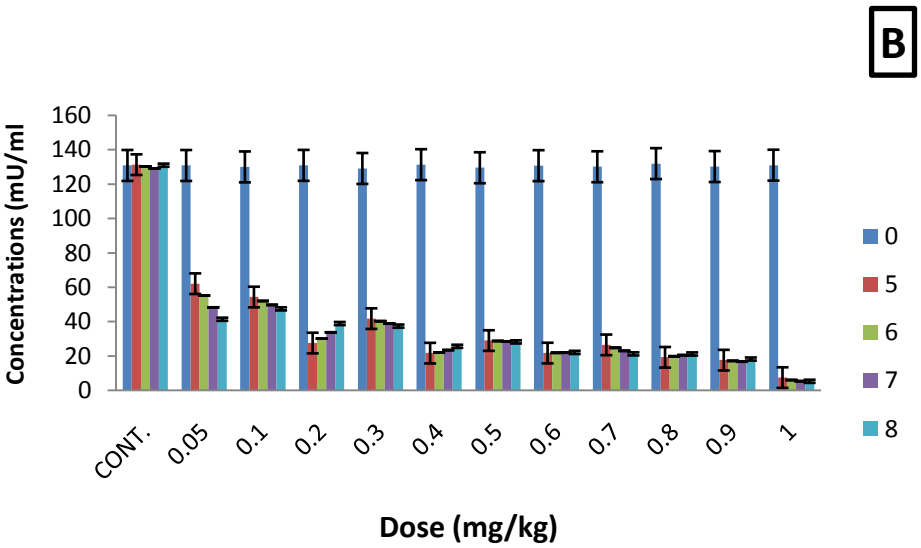

Fig 2b;Glutathione peroxidase level after the second month (week 5-8) of exposure to BPA

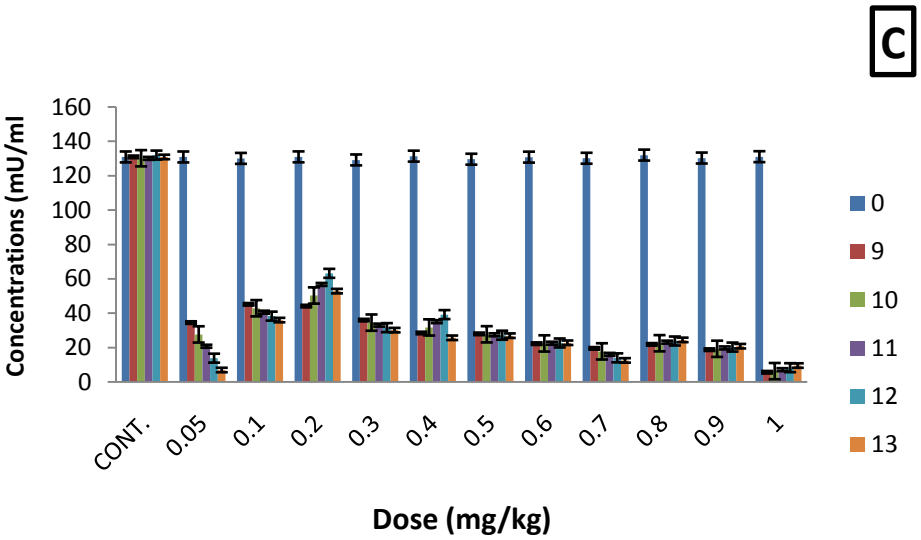

Fig 2c;Glutathione peroxidase level after the third month (week 9-13) of exposure to BPA

IV. Discussion

The findings of these results showed an increase in glutathione reductase, but glutathione peroxidase and were decreases after BPA exposure. In concordance with our results, Hassan *et al*, [38] shows a significant decreases in reduced glutathione (GSH), Wu *et al* [39], showed significant decrease in the levels of GSH in BPA group. Ansoumane*et al* [40], showed that BPA administration causes a decrease in the activities of antioxidant enzymes, namely, glutathione peroxidase. Korkmaz*et al* [41], reported the decrease in GSH. Hassan *et al* [38], showed decreased in glutathione peroxidase. Also, Chitra*et al* [42],, showed that the activities of glutathione peroxidase were decreased significantly, Eid*et al* [43],demonstrated that BPA caused decrease in the activities of antioxidant enzymes glutathione peroxidase. AboulEzz*et al* [44], revealed that BPA administration induced a state of oxidative stress by decrease in GSH levels.

A growing body of evidence shows higher BPA concentrations were associated with increased abnormal liver function tests [45]. BPA has the ability to generate reactive oxygen species (ROS) and reduce antioxidant reserves and enzymes that are critical for hepatic phase I and II biotransformation, including glutathione peroxidase and glutathione reductaseactivity[38]. Similarly, others demonstrated that BPA generates ROS that causes oxidative damage in organs and tissues such as the brain, reproductive tract, and kidney of rats[41,46].The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage, because glutathione peroxidase have been considered the primary scavengers of H_2O_2[47];

However, in absence of adequate glutathione peroxidase activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and may contribute to the oxidative stress of BPA.

CONCLUSION

Glutathione provides defence against reactive oxygen species, as it can scavenge free radicals. It acts directly as an antioxidant and also participates in catalytic cycles of antioxidant enzymes such as glutathione peroxidase and glutathione reductase. These enzymes (Glutathione peroxidase and reductase) protects cells and tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes. Prolong exposure to BPA causes oxidative stress by disturbing the balance between ROS and antioxidant defenses system and significantly compromise the liver integrity.

SIGNIFICANCE STATEMENT

Studies show that BPA is toxic to laboratory animals at doses overlapping with or very near to human exposures, and that the chemical causes toxic effects that are on the rise and very common in people. These disturbing facts raise questions about the extent to which current, widespread exposures to BPA are contributing to the burden of human disease. This study will help the researcher to uncover the critical areas of prolonged Bisphenol A exposure that many researchers were not able to explore. Thus a new theory on BPA contribution to the burden of human disease may be arrived at.

DECLARATIONS

Ethical Approval and Consent to participate: Not Applicable
Consent for publication: Not Applicable
Availability of supporting data: Not Applicable
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Authors' contributions:

Chinenye E. Oguazu –analysis of glutathione reductase, glutathione peroxidase and result.
Francis C. Ezeonu – supervisor, proof reading
Anajekwu B. Azuka and Ani N. Onuabuchi- Animal experiment which includes feeding, administration of graded doses of BPA.
Charles G. Ikimi and Chinenye E. Oguazu – statistical analysis and result presentation
Charles G. Ikimi and Charles C. Dike- blood sample collection and processing.

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