Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science Applied and Health Science, Anchor University Lagos
URL: journal.aul.edu.ng
In AJOL: https://www.ajol.info/index.php/aujst
Vol. 7 No 1, January 2026, Pp. 1-8
ISSN: 2736-0059 (Print); 2736-0067 (Online)

A mixture of Bisphenol-A analogues elicits neuro-hepatic dysfunction in female rat model

Okoh Olayinka Sunday[*1,2], Akinbode Otitololuwa[1], Adebamiji Eunice T[1], Oluwajembola Abimbola Mary[3,4], Akamo Adio Jamiu[5], Ubong Akpan[6], Akintunde Jacob Kehinde[5]

[1]Department of Chemical Sciences, Anchor University Lagos, Nigeria.

[2]Anchor University Centre for Global Health, Anchor University, Lagos, Nigeria.

[3]Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria

[4]Covenant Applied Informatics and Communications – Africa Center of Excellence

[5]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

[6]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

***Corresponding author Email**: ookoh@aul.edu.ng, yinkaokoh@gmail.com

Submitted 16 November, 2025
Accepted 30 November, 2025

Competing Interests.
The authors declare no competing interests

ABSTRACT

Background: Bisphenol-A is important in the industry; it enhances rigidity of products. However, its numerous toxicities have been established, hence a shift to its analogues. **Objectives:** The impact of varying concentrations of Bisphenols-B, F and S mixture on hepatic function and neuronal cell integrity was assessed. **Methods:** Forty-two female rats were randomly divided into seven groups (n = 6). Group 1 served as control and received olive oil only. Groups 2, 3, 4, 5, 6, and 7 were administered 12, 24, 48, 96, 192 and 384 µg of Bisphenol-B, Bisphenol-F and Bisphenol-S mixture per kg body weight respectively. Exposure lasted for forty-nine days. **Results:** Exposure to the Bisphenol-A analogues mixture for forty-nine days increased hepatic oxidative stress in dose- dependent manner via elevation of MDA (53% - 232%), depletion of GSH (27.3% to 63.6%), and inhibition of the activities of GST (75% to 87.5%), SOD (0.05%) and CAT (up to 90%). These culminated in liver dysfunction evident by increase in serum ALT (53.6% - 783.5%) and AST (241% - 1,013%) coupled with hepatic energy deficiency as a result of decreased LDH activity (14.9% - 58.2%). Histological examination of the liver corroborates our findings with signs of degeneration at 192 and 384 µg/kg body weight. Histopathological examinations of the hippocampus, cortex and striatum reveal degeneration at 24 µg for striatum, 48 µg for cortex and 96 µg for hippocampus. **Conclusion:** Our results suggest that increasing exposure to Bisphenol-A analogues mixture increases risks of liver dysfunction and neuronal impairment.

Keywords. Bisphenol-A analogues, oxidative stress, neuronal damage, hepatic dysfunction, Bisphenol-B, Bisphenol-F, Bisphenol-S

1. Introduction

Bisphenol-A (BPA) is widely used in plastics, resins, food containers, electronics besides numerous industrial and domestic products (Cimmino et al., 2020; Haripriya & Sendhilvadivu, 2021; Pistollato & others, 2020; Samad et al., 2021; Sharma et al., 2023; Vasiljevic & Harner, 2021). Despite its industrial utility, BPA is an endocrine disruptor associated with reproductive, cardiovascular, and neurological toxicity (Abraham & Chakraborty, 2020; Rebolledo-Solleiro, 2021; Shirani et al., 2019; Xing et al., 2022). The toxicity of BPA is majorly consequent of the fact that it can imitate the structure of estrogen 2 (Khamphaya et al., 2021; Santoro et al., 2019), hence it can bind the estrogen receptors thereby altering developmental processes like growth, energy levels, cell repair and foetal development. Oxidative stress, neurodegeneration and cognitive dysfunction are also said to be included in the toxicity path of BPA (Meli et al., 2020; Ni & others, 2021).

Owing to these risks, BPA is being replaced by analogues such as Bisphenol-B (BPB), Bisphenol-F (BPF), and Bisphenol-S (BPS) (Chen et al., 2016). In response to growing concerns over the health risks associated with Bisphenol A (BPA), many manufacturers have substituted BPA with structurally similar compounds, leading to the widespread marketing of 'BPA-free' products. However, these BPA analogues, such as Bisphenol S and Bisphenol F, also leach from consumer products into food, water, and the environment, resulting in continued human and animal exposure.(Chen et al., 2016; Thoene et al., 2017). In real-world scenarios, exposure to BPA analogues often arises from multiple consumer products such as food packaging,

https://dx.doi.org/10.4314/aujst.v7i1.1

thermal receipt paper, dental materials, and household plastics. These compounds co-occur in food, water, and indoor dust, leading to mixed exposure rather than isolated contact with individual bisphenols. Thus, this study evaluated the toxicological impact of a mixture of selected BPA analogues, specifically BPB, BPF, and BPS (at low to relatively high concentration) on hepatic and neuronal health in female rats.

2. Materials and Methods

Forty-two female Wistar rats (7 weeks, ~150 g) were randomly assigned into 7 groups (n=6). Group 1 received olive oil (control). Groups 2–7 received 12, 24, 48, 96, 192, or 384 µg/kg of a BPB + BPF + BPS mixture daily by oral gavage for 49 days. These doses were selected to span a sub-toxic range based on published studies demonstrating biological effects at comparable levels, and to serve as a pilot screen for identifying appropriate concentrations for a subsequent chronic exposure study. After exposure, rats were sacrificed via cervical dislocation, and blood, liver, and brain regions (hippocampus, cortex, striatum) were collected. Liver homogenates were prepared for oxidative stress markers (MDA, GSH) and antioxidant enzyme activities (GST, SOD, CAT). Published protocols were followed in assessing MDA (Varshney & Kale, 1990), GSH (Jollow et al., 1974), GST (Habig et al., 1974), CAT (Sinha, 1972) and SOD (Misra & Fridovich, 1972). Serum ALT and AST were measured, and LDH activity assessed using commercial kit (Fortress Chemicals Ltd, England). Histology of liver and brain tissues was performed using H&E staining. All data analyses were performed in R (version 4.1; R Core Team, 2021) using standard statistical test, ANOVA and Tukey's post hoc tests for multiple comparisons.

3. Results

Body weight and liver weight were unaffected by treatments (Fig. 1A, B). Oxidative stress increased dose-dependently: A dose-dependent increase in oxidative stress was observed, evidenced by elevated MDA levels and reduced GSH levels. (Fig. 1C, D). Antioxidant enzymes GST, SOD, and CAT activities were reduced at higher doses (Fig. 2A – C).

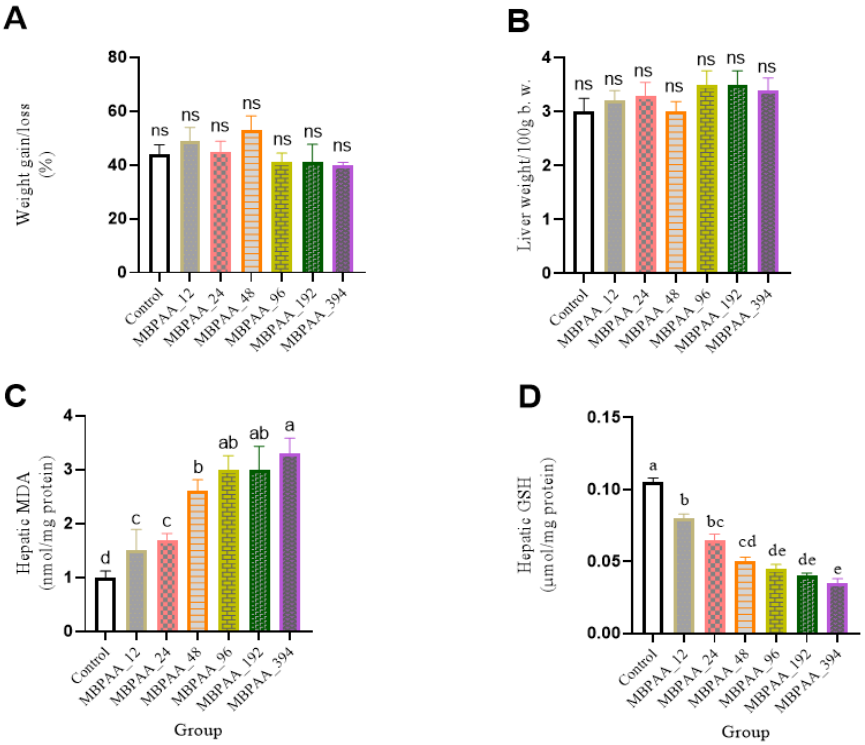

Figure 1: Effect of increasing doses of mixture of Bisphenols-B, F, and S on **A**: body weight of female rats; **B**: relative weight of liver per 100 g body weight; and **C**: malondialdehyde level; and **D**: reduced glutathione concentration in the liver homogenates of rats exposed to varying concentration of Bisphenol-A analogues mixture. Results expressed as mean ± SEM. Superscripts with same alphabets on the bar show insignificant difference at $p < 0.05$ level. Dotted line represents the mean value of the control group.

https://dx.doi.org/10.4314/aujst.v7i1.1

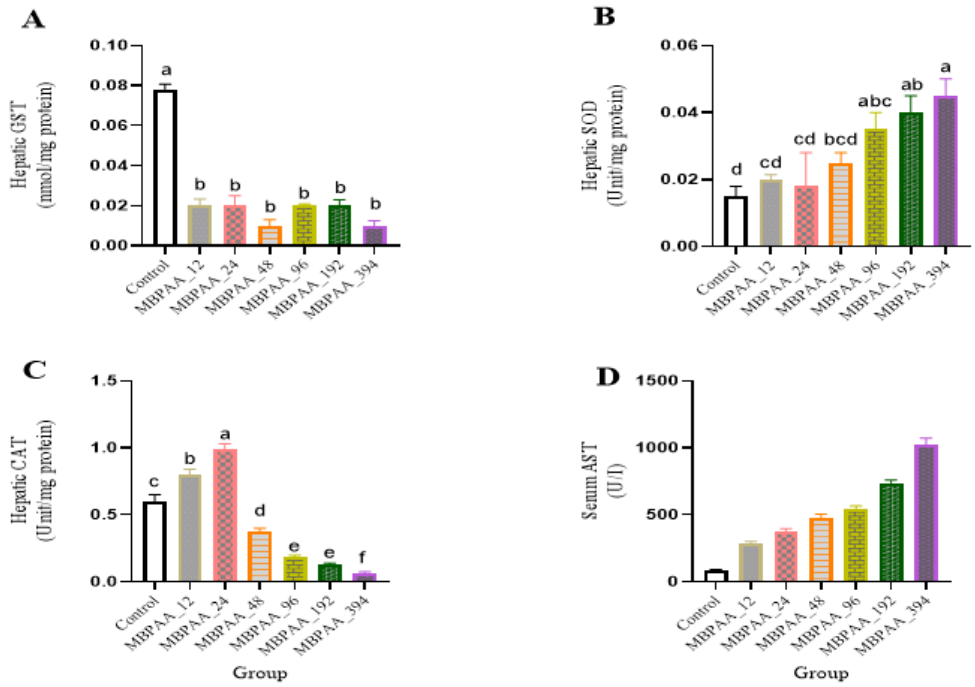

Figure 2: Effect of increasing doses of mixture of Bisphenols – B, F, and S on selected antioxidative enzymes **A**: glutathione transferase (GST), **B**: superoxide dismutase (SOD) inhibition, and **C**: Catalase, and **D**: serum amino transferase (AST). Results presented as mean ± SEM. Superscripts with same alphabets on the bar show insignificant difference at $p < 0.05$ level.

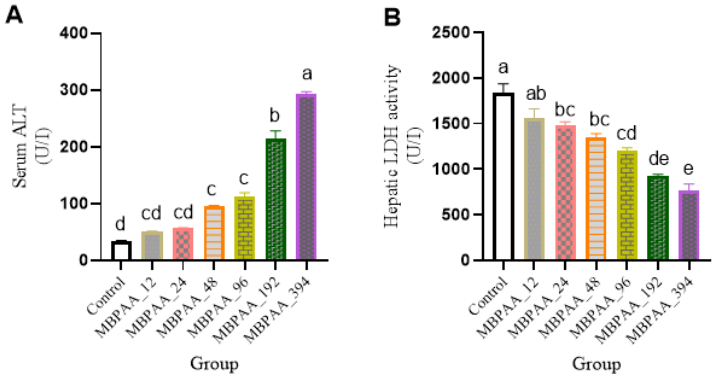

Figure 3: Effect of increasing doses of mixture of Bisphenols-B, F, and S on **A**: serum alanine transferase (ALT); and **B**: lactate dehydrogenase (LDH) in the liver. Result is presented as mean ± SEM. Superscripts with same alphabets on the bar show insignificant difference at $p < 0.05$ level.

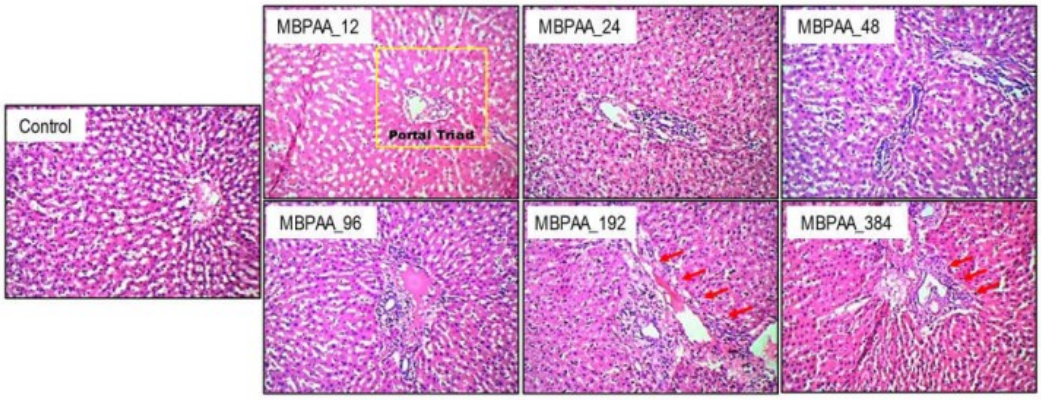

Plate 1: Micromorphological sections of liver in female rats administered increasing doses of mixture of Bisphenols B, F, and S. H&E staining (x100). The hepatocytes, sinusoids, and portal triad are all visible.

https://dx.doi.org/10.4314/aujst.v7i1.1

Serum AST and ALT increased significantly, while hepatic LDH decreased (Fig. 2D, Fig. 3A, B) in a dose dependent manner. Histology revealed dose-related hepatic degeneration (Plates 1 – 2), hippocampal alterations (Plates 3 – 4), cortical damage (Plates 5 – 6), and striatal degeneration (Plates 7 – 8).

4. Discussion

Given that different manufacturers substitute BPA with various analogues, human exposure is unlikely to occur from a single compound but rather from simultaneous contact with multiple BPA analogues, typically at low concentrations (Sellinger & others, 2021). This study therefore seeks to investigate the effect of chronic exposure to mixture of common Bisphenol-A analogues in low concentrations.

In this study, exposure to BPA analogues mixture, irrespective of the concentration administered, had no significant impact on body weight which can be used as growth index. Similarly, the analogues did not impact negatively on the relative weight of the liver per kg body weight. This shows that BPA analogues mixture has seemingly no negative impact on growth. Exposure to the BPA analogue mixture significantly elevated lipid peroxidation levels, which are widely recognised as markers of oxidative degradation of membrane lipids. This oxidative damage disrupts the structural integrity of hepatocyte membranes, thereby increasing cellular susceptibility to injury and enhancing free radical generation during xenobiotic metabolism. Increasing exposure to BPA analogues mixture therefore increases the vulnerability of the system to the various health maladies associated increased generation of free radicals as previously reported.

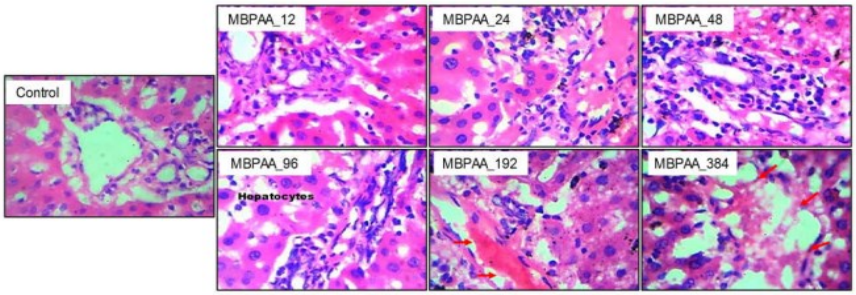

Plate 2: Micromorphological presentations of liver in female rats administered increasing doses of mixture of Bisphenols B, F, and S. H&E (x400). The hepatocytes, sinusoids, and portal triad are all visible.

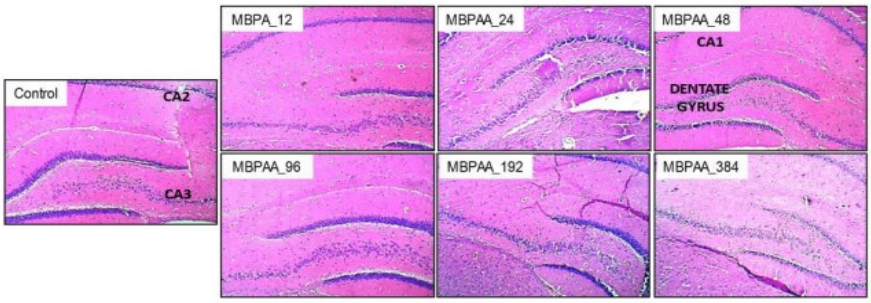

Plate 3: General micromorphological presentations of hippocampus in female rats administered increasing doses of Bisphenols B, F, and S. H&E stain (*X40*). The Dentate gyrus (DG) composed of granule cells, Cornu amonus (CA1-3) containing pyramidal cells, are well demonstrated.

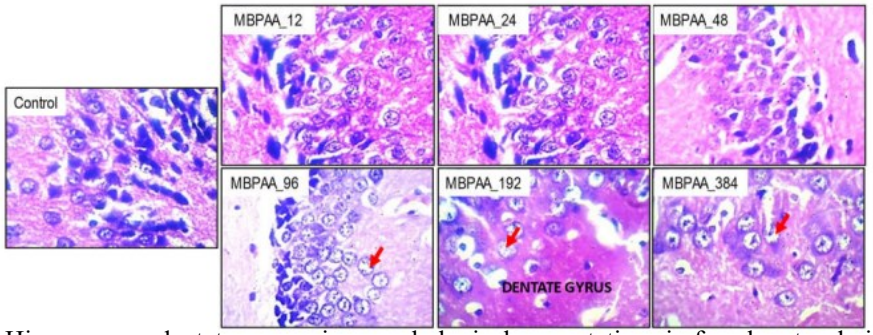

Plate 4: Hippocampus dentate gyrus micromorphological presentations in female rats administered increasing doses of mixture of Bisphenols B, F, and S. H&Eosin stain (*X400*).

https://dx.doi.org/10.4314/aujst.v7i1.1

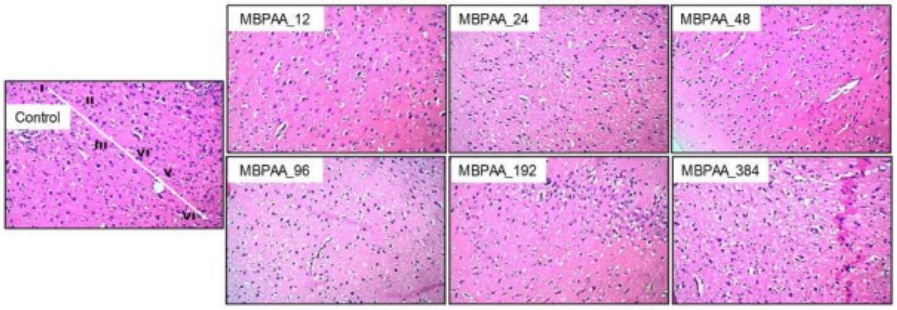

Plate 5: Panoramic views of the cortex in female Wistar rats administered increasing doses of mixture of Bisphenols B, F, and S. H&E stain (*X100 magnification*). The molecular layer (I), External granular layer (II), External pyramidal layer (III), Internal granular layer (IV), Internal pyramidal layer (V) and the multiform layer (VI) are demonstrated across study groups (white line).

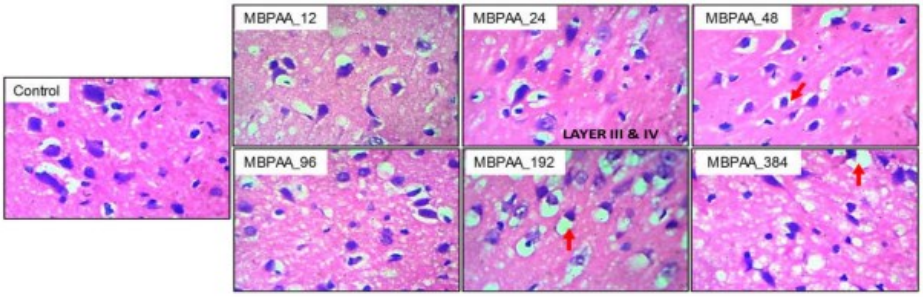

Plate 6: Photomicrographs showing cytoarchitectural arrangement of cortex in female Wistar rats administered increasing doses of mixture of Bisphenols B, F, and S. H&E stain (X400 magnification). External pyramidal layer (III), Internal granular layer (IV) is focused in this magnification across study groups. Red arrow- degenerating and unhealthy cells with loss of cytoplasmic and nuclear materials (MBPAA_48 to MBPAA_384).

Reduced glutathione (GSH); a non-enzymatic antioxidant, is critical for cell survival due to inevitable exposure to oxidants and pro-oxidants. It therefore plays critical role in cellular protection against oxidative damage besides being a substrate for GST. The dose-dependent decrease in GSH concentration in this study might be partly due to progressive increase in its utilization in combating the increase reactive oxygen species produced by exposure to BPA analogues mixture. This is in a tandem with previous report (Akintunde et al., 2015) that over utilization of GSH in mopping up increased ROS culminates in decreased GSH in oxidative stressed systems.

GST is an enzymatic antioxidant involved in detoxifying harmful redox species and eliminating harmful substances. It has GSH as its substrate. From our results, the BPAA mixture at the least doses used in this study decreased GST activity in a dose-dependent manner. This might be a consequence of depleted GSH, as previously reported (Akintunde et al., 2015), increasing the vulnerability of the liver.

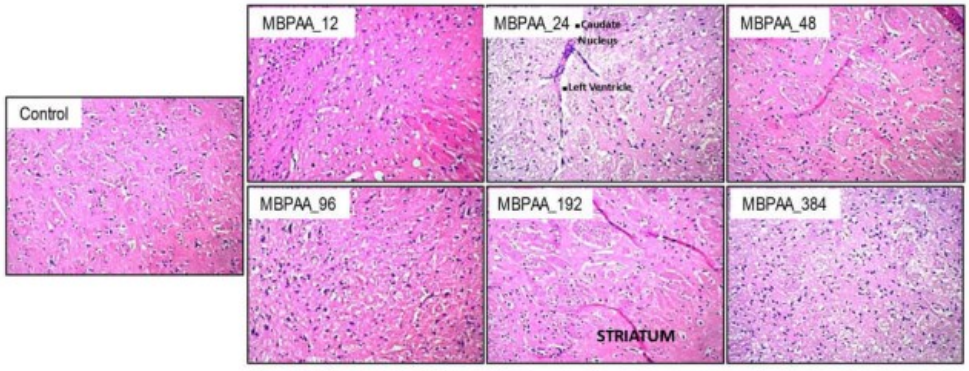

Plate 7: Panoramic views of the striatum (caudate nucleus, putamen and ventral striatum) in female Wistar rats administered increasing doses of mixture of Bisphenols B, F and S. H&E stain (*X100 magnification*).

https://dx.doi.org/10.4314/aujst.v7i1.1

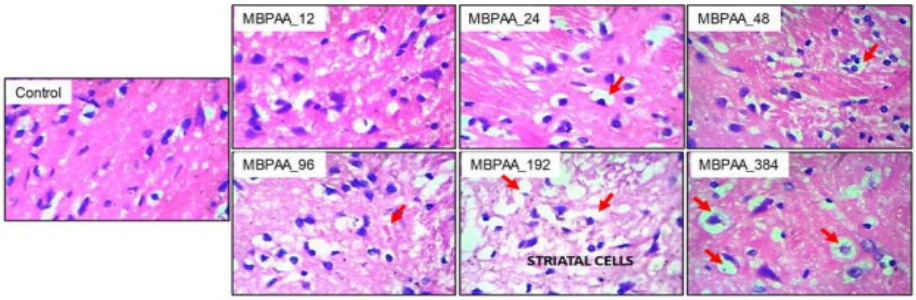

Plate 8: Photomicrographs showing cytoarchitectural arrangement of corpus striatum micromorphological presentations in female Wistar rats administered increasing doses of mixture of Bisphenols B, F and S. H&E stain (X400 magnification).

CAT is also an enzymatic antioxidant acting in concert with GST an SOD in the detoxification of ROS. The initial upregulation of CAT could be as a result of adaptive response to increase production of ROS with view to suppress oxidative stress as previously reported. However, its decreased activity at higher dose of BPA analogues mixtures suggests that continual exposure to relatively high dose of BPA analogues can overcome the antioxidative potential of the system. This is in line with published findings that exposure to BPAA inhibited CAT and SOD (Li et al., 2023); and the inhibition of SOD will no doubt culminate in increase superoxide radicals (Oseni & Okoh, 2020) and ultimately overwhelm the antioxidative system.

ALT and AST are localized in the liver, hence their increase in the blood indicates compromise in the cellular membrane of hepatocytes culminating in leakage. Increased serum AST and ALT have been used as biomarkers of hepatic damage and dysfunction (Oseni & Okoh, 2020). In this study, exposure to BPA analogues, which is inevitable, increases the vulnerability of the liver to damage and dysfunction. Energy is critical for maintenance of normal cellular activities and LDH, an enzyme at the bottom of the glycolytic pathway, plays critical role in generation of ATP (energy). LDH interconverts pyruvate and lactate; and its activity is used to estimate cellular ATP in anaerobic condition. Decreasing LDH activity associated with increasing concentration of BPA analogues suggests that exposure to BPA analogues can culminate in energy deficit in the liver cells to the level of the hepatocytes being unable to carry out their normal metabolic activities.

Dysregulation at the molecular level always culminates in structural deformity of various cells. From the results of this study, BPAA compromised the integrity of the neurons in the hippocampus, prefrontal cortex and striatum in a dose dependent manner. The compromise in the integrity of the neurons would no doubt culminate in disruption in neuronal signaling and central nervous system. This shows that BPAA are capable of dysregulating the neurological system as reported for BPA (Akintunde et al., 2020a, 2020b; Akintunde & Woleola, 2019).

Conclusion

This study demonstrates that mixtures of BPA analogues induce oxidative stress, impair antioxidant defences, and cause hepatic and neuronal damage. Unchanged body weight suggests no effect on growth, but biochemical and histological changes confirm internal toxicity. Reduced GSH and inhibited GST, SOD, and CAT indicate compromised antioxidant defence, while increased ALT and AST reflect hepatocellular injury. Decreased LDH suggests impaired energy metabolism. Neuronal degeneration in hippocampus, cortex, and striatum aligns with reports of BPA-induced neurotoxicity. Thus, chronic exposure to mixtures of BPB, BPF, and BPS may contribute to liver dysfunction and neurological disorders.

References

Abraham, A., & Chakraborty, P. (2020). A review on sources and health impacts of bisphenol A. *Reviews on Environmental Health*, *35*(2), 201–210. https://doi.org/10.1515/reveh-2019-0034

Akintunde, J. K., Akintola, T. E., Adenuga, G. O., Odugbemi, Z. A., Adetoye, R. O., & Akintunde, O. G. (2020a). Naringin attenuates Bisphenol-A mediated

https://dx.doi.org/10.4314/aujst.v7i1.1

neurotoxicity in hypertensive rats by abrogation of cerebral nucleotide depletion, oxidative damage and neuroinflammation. *NeuroToxicology*, *81*, 18–33. https://doi.org/10.1016/j.neuro.2020.08.001

Akintunde, J. K., Akintola, T. E., Adenuga, G. O., Odugbemi, Z. A., Adetoye, R. O., & Akintunde, O. G. (2020b). Naringin attenuates Bisphenol-A mediated neurotoxicity in hypertensive rats by abrogation of cerebral nucleotide depletion, oxidative damage and neuroinflammation. *Neurotoxicology*, *81*(April), 18–33. https://doi.org/10.1016/j.neuro.2020.08.001

Akintunde, J. K., Oboh, G., & Akindahunsi, A. A. (2015). Inhibition of key markers linked with spermatogenesis and cellular ATP by sub-chronic exposure to leachate in a rat model. *Archive of Environmental Contamination and Toxicology*, *68*(1), 159–168.

Akintunde, J. K., & Woleola, M. T. (2019). Impairment of neuro-renal cells on exposure to cosmopolitan polluted river water followed by differential protection of Launea taraxacifolia in male rats. *Comp. Clinical Pathol.*, *28*, 1245 – 1257.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y. L., Wu, Y., & Widelka, M. (2016). Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity - A Review. *Environmental Science and Technology*, *50* (11), 5438–5453. https://doi.org/10.1021/acs.est.5b05387

Cimmino, I., Fiory, F., Perruolo, G., Miele, C., Beguinot, F., Formisano, P., & Oriente, F. (2020). Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease. *International Journal of Molecular Sciences*, *21*(16), 5761. https://doi.org/10.3390/ijms21165761

Habig, W. H., Pabst, M. S., & Jekpoly, W. B. (1974). Glutathione transferase: A first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, *249*, 7130–7139.

Haripriya, P. , & Sendhilvadivu, M. (2021). Review on Impacts of Bisphenol A. Retrieved from https://www.ijsr.net/getabstract.php?paperid=SR21326132044. *International Journal of Science and Research* , *10*(3), 1624–1626.

Jollow, D. J., Michell, J. R., & Gillete, J. R. (1974). Bromobenzene-induced Liver necrosis: Protective role of glutathione and evidence for 3,4- Bromobenzene oxide as hepatotoxic metabolite. *Pharmacology*, *11*, 151–169.

Khamphaya, T., Pouyfung, P., Kuraeiad, S., Vattanasit, U., & Yimthiang, S. (2021). Current Aspect of Bisphenol A Toxicology and Its Health Effects. *Trends in Sciences*, *18* (21), 408. https://doi.org/10.48048/tis.2021.408

Li, Y., Li, F., Liu, X., Zu, J., Zhang, W., Zhou, S., Zhu, J., Zhang, T., Cui, G., & Xu, C. (2023). Association between serum neurofilament light chain levels and sleep disorders in patients with Parkinson's disease. *Neuroscience Letters*, *812*. https://doi.org/10.1016/j.neulet.2023.137394

Meli, R., Monnolo, A., Annunziata, C., Pirozzi, C., & Ferrante, M. C. (2020). Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. *Antioxidants*, *9*(5), 405.

Misra, H., & Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephryne and a simple assai for superoxide dismutase. *Journal of Biological Chemistry*, *247*, 3170-3175.

Ni, Y., & others. (2021). Bisphenol A impairs cognitive function and 5-HT metabolism in adult male mice by modulating the microbiota-gut-brain axis. *Chemosphere*, *282*, 130952. https://doi.org/10.1016/j.chemosphere.2021.130952.

Oseni, O. A., & Okoh, O. S. (2020). Doxorubicin - Induced Heart and Kidney Toxicities in Female Wistar Rats: Effect of Aqueous Extract of the Sprout of Phaseolus vulgaris (L) 1,2. *Anchor University Journal of Science and Technology (AUJST)*, *1*(1), 116–125.

Pistollato, F., & others. (2020). Assessment of developmental neurotoxicity induced by chemical mixtures using an adverse outcome pathway concept. *Environmental Health*, *19* (1), 1–26. https://doi.org/10.1186/s12940-020-00578-x.

R Core Team. (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://

//www.r-project.org/

Rebolledo-Solleiro, D. (2021). Impact of BPA on behavior, neurodevelopment and neurodegeneration Daniela. *Frontiers in Bioscience*, *26*(2), 4898. https://doi.org/10.2741/4898

Samad, N., Manzoor, N., Muneer, Z., Bhatti, S. A., & Imran, I. (2021). Reserpine-induced altered neuro-behavioral, biochemical and histopathological assessments prevent by enhanced antioxidant defence system of thymoquinone in mice. *Metabolic Brain Disease*, *36*, 2535–2552.

Santoro, A., Chianese, R., Troisi, J., Richards, S., Nori, S. L., Fasano, S., Guida, M., Plunk, E., Viggiano, A., Pierantoni, R., & Meccariello, R. (2019). Neuro-toxic and Reproductive Effects of BPA. *Current Neuropharmacology*, *17*(12), 1109–1132. https://doi.org/10.2174/1570159X17666190726112101

Sellinger, E. P., & others. (2021). Perinatal phthalate exposure increases developmental apoptosis in the rat medial prefrontal cortex. *Neurotoxicology*, *87*, 167–173. https://doi.org/10.1016/j.neuro.2021.09.007

Sharma, V., Jain, D., Rai, A. R., Kumari, P., Nagar, V., Kaur, A., Singh, A., Verma, R. K., Pandey, H., & Sankhla, M. S. (2023). Toxicological assessment and concentration analysis of Bisphenol A in food grade plastics: A systematic review. *Materials Today: Proceedings*, *95*, 18–25. https://doi.org/10.1016/j.matpr.2023.06.336

Shirani, M., Alizadeh, S., Mahdavinia, M., & Dehghani, M. A. (2019). The ameliorative effect of quercetin on bisphenol A-induced toxicity in mitochondria isolated from rats. *Environmental Science and Pollution Research*, *26*(8), 7688–7696. https://doi.org/10.1007/s11356-018-04119-5

Sinha, A. K. (1972). Colorimetric assay of catalase. *Anal. Biochem.*, *47*, 389–394.

Thoene, M., Rytel, L., Dzika, E., Włodarczyk, A., & Id, E. K. (2017). *Bisphenol A Causes Liver Damage and Selectively Alters the Neurochemical Coding of Intrahepatic Parasympathetic Nerves in Juvenile Porcine Models under Physiological Conditions*. 1–17. https://doi.org/10.3390/ijms18122726

Varshney, R., & Kale, R. K. (1990). 1990. Effects of Camolin Antagonist. *Int. J Rad. Biol.*, *58*, 733–743.

Vasiljevic, T., & Harner, T. (2021). Bisphenol A and its analogues in outdoor and indoor air: Properties, sources and global levels. *Science of The Total Environment*, *789* (148013). https://doi.org/10.1016/j.scitotenv.2021.148013

Xing, J., Zhang, S., Zhang, M., & Hou, J. (2022). A critical review of presence, removal and potential impacts of endocrine disruptors bisphenol A. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *254*, 109275. https://doi.org/10.1016/j.cbpc.2022.109275

Acknowledgement

We acknowledge Anchor University for laboratory and animal facilities. We thank Imoniakemu P. O. and Ogunleye O. for technical assistance, and Prof. Oseni A. O. for his expertise support.