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Full length Research Article

Gastroprotective Biochemicals in Wistar Rats Orally Exposed to Bisphenol A and Co-treated with either *Garcinia kola* (Heckel) Seeds or its Biflavonoid, Kolaviron

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Summary: This study evaluated gastroprotective biochemicals in Wistar rats exposed to both Bisphenol A and either *Garcinia kola* or its biflavonoid, kolaviron. Fifty-six rats (140-160g) divided into 7 groups (n=8), and treated orally for 28 days as follows; Group I was the control (distilled water, 1.5mL/kg) while group II (vehicle control) received corn oil (1.5mL/kg), groups III-V were exposed to BPA (50mg/kg) only and treated with distilled water (1.5mL/kg), *Garcinia kola* (200mg/kg) and kolaviron (200mg/kg), respectively. Animals in groups VI and VII received *Garcinia kola* (200mg/kg) and kolaviron (200mg/kg) only, respectively. Thereafter and under anaesthesia, the stomach was dissected out, estimated for mucin (n=3), homogenized (n=5), centrifuged, and the clear supernatant obtained was analyzed for malondialdehyde, superoxide dismutase, catalase, reduced glutathione, glutathione S-transferase, nitrites, myeloperoxidase, interleukin-6 and tumor necrosis factor-α, respectively. Gastroprotective biochemicals were significantly ($p<0.05$) reduced in animals exposed to BPA while values in animals exposed both BPA and either *Garcinia kola* or kolaviron were elevated. Exposure to *Garcinia kola* and kolaviron alone also showed a potentiation of gastric antioxidant and anti-inflammatory activities. This study shows that *Garcinia kola* and especially its biflavonoid, kolaviron, protects the gastric mucosa against Bisphenol A induced impairment by potentiating gastroprotective biochemicals in male Wistar rats.

Keywords*: Bisphenol A, stomach, Garcinia kola, kolaviron, antioxidant, antiinflammation*

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INTRODUCTION

Bisphenol A (BPA) is one of the most produced industrial chemicals globally. It is extensively used in the production of epoxy resins and polycarbonate plastics that are used in food and drink packaging industry (Manzoor *et al*., 2022). Bisphenol A also belongs to a group of compounds that are categorized as endocrine disruptive chemicals (EDCs) that are capable of mimicking or antagonizing the mechanism of action, synthesis, transportation, receptor interaction, storage and disposal of hormones in the body resulting in multi organ toxicities in both animals and human beings (Yoon *et al*., 2014). Studies have shown that in plastic products, not all monomers and additives utilized during production are sufficiently polymerized and covalently bonded. Hence, some of the chemicals used can potentially leach and diffuse through the polymeric matrix of the product and into the foods stored in these plastics (Muzeza *et al*., 2023). Bisphenol A has been reported to leach out into food when plastics there are contained in are exposed to high temperature and abrasive chemicals (Obuzor and Onyedikachi, 2023).

Bisphenol A has been reported to cause multiple organ toxicities after entering the body through the respiratory, dermal, and / or gastrointestinal tract. It has been reported that the main exposure route to BPA in humans is orally and the stomach as well as the intestine have been observed to be especially vulnerable to its (i.e., BPA) adverse effects (Vandenberg *et al*., 2007). Bisphenol A has been reported to promote inflammatory processes in the stomach and intestine, damage gastrointestinal barrier function, and cause changes in the neurochemical characterization of nervous structures supplying the gastrointestinal tract (Szymańska *et al*., 2020). The ulcerative effect of BPA has also been attributed to its ability to induce gastric oxidative stress (Abo-Elsoud *et al*., 2022). The mechanisms adduced to BPA induced gastric impairment suggests that antioxidant substances, especially natural products such as *Garcinia kola* and kolaviron, may exert beneficial effects.

Garcinia kola, which belongs to the genus "Garcinia", family "Clusiaceae" and order "Malpighiales", is found in tropical African countries and all over Asia. In Nigeria, the seed (commonly referred to as bitter kola) is widely consumed for traditional hospitality as well as at

cultural and social ceremonies (Ogwu *et al*., 2024). In folkloric medicine, it is used to manage and treat hypertension, cancer, malaria, diabetes, and numerous other ailments (Emmanuel *et al*., 2021). Investigations for medicinal properties have also revealed that it exerts antioxidant, antidiabetic, antihypertension, anti-analgesic, anti-inflammatory effects and possesses neuro-, cardio- and gastro- protective properties (Dogara *et al*., 2022). Kolaviron, an active complex of at least 3 compounds in *Garcinia kola* seed, has been reported to possess a wide array of pharmacological properties some of which include antidiabetic, cardioprotective, neuroprotective, haematoprotective, nephroprotective, gastroprotective, and hepatoprotective activities (Oyagbemi *et al*., 2016). Despite the presence of various active principles in *Garcinia kola*, studies have reported most of its pharmacological activity could be attributed to that of its principal biflavonoid, kolaviron (Tauchen *et al*., 2023).

Garcinia kola and kolaviron have both been reported to mitigate, ameliorate, or reverse several toxicities in diverse experimental studies (Olatoye and Akindele, 2021). The likely therapeutic effects of either *Garcinia kola* or kolaviron against BPA induced toxicities are sparse with limited information on gastroprotective potentials. One of such is our previous investigation on the neuroprotection offered by *Garcinia kola* and kolaviron against BPA induced behavioural impairment and hippocampal inflammation (Kayinu *et al*., 2024). In the gastrointestinal tract, gastroprotection is said to be proffered through two main mechanisms which include mechanisms that decrease or counter acid/pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defensive factors which include mucin secretion, cellular mucus production, gastric antioxidants, bicarbonate secretion, mucosal blood flow and cell turnover (Goyal and Sairam, 2003, Ige *et al*., 2016).

This study was therefore designed to evaluate gastric cytoprotective biochemicals in male Wistar animals exposed to both Bisphenol A and either *Garcinia kola* or kolaviron.

MATERIALS AND METHODS

Drugs, plant preparation and extraction of kolaviron: Bisphenol A, *Garcinia kola* and kolaviron were obtained as previously described (Kayinu *et al.,* 2024). Briefly, BPA (Sigma-Aldrich, St. Louis, MO, USA) was suspended in corn oil prior to administration. *Garcinia kola* seeds, purchased from a local vendor in Kaduna state, Nigeria, was authenticated at the University of Ibadan Herbarium where a voucher specimen already existed. These seeds were peeled, sliced, air-dried, pulverized and stored in a sterile container until needed. Daily, fresh solutions of *Garcinia kola* suspended in distilled water were prepared and administered at a dose of 200 mg/kg. Kolaviron was extracted from *Garcinia kola* using the method described by Olaleye *et al.,* (2000). Briefly, dried pulverized *Garcinia kola* was defatted with n-hexane (in order to extract nonpolar inactive compounds) using the cold maceration method. The dried marc obtained was subsequently re-defatted twice using n-hexane. Thereafter the defatted filtrate obtained was dried and repeatedly extracted with acetone (for optimal extraction of flavonoids, saponins, phenolic compounds and other extractable solids) thrice to get the kolaviron-rich crude extract. This crude acetone extract was concentrated to about 100mL with a rotary evaporator at 40℃, diluted to twice its volume with distilled water and then partitioned with ethyl acetate (to extract nonpolar flavonoids). The ethyl acetate fraction obtained was concentrated to get a yellow-brown powder known as kolaviron which was administered at a treatment dose of 200 mg/kg (Kayinu *et al.,* 2024).

Animals, groupings and experimental protocol: Fifty-Six (56) rats of Wistar strain (140-160 g), obtained from Central Animal House, College of Medicine, University of Ibadan, were used for this study. The animals were house and acclimatized for 2 weeks prior to experimental procedures in solid bottom plastic cages, under standard environmental conditions at room temperature (approximately 26-30 C), and natural alternating day and nighttime cycles respectively. Animals were maintained under humane conditions in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996). The animals were allowed access to standard rat chow and clean drinking water *ad libitum* throughout the experiment. Thereafter, Animals were divided into seven (7) equal groups and treated as follows. Group I, the control group, received distilled water (1.5 mL/kg), while group II served as vehicle control and was treated with corn oil (1.5 mg/kg). Animals in groups III – V were co-treated with BPA (50mg/kg) (Zhang *et al*., 2013) and either distilled water (1.5 mL/kg), *Garcinia kola* (200 mg/kg), or kolaviron (200 mg/kg), respectively. All treatments were administered via the oral route using an oral cannula, daily for 28 days.

Sample collection, preparation and biochemical assays: At the end of the treatment duration, animals in each group were anaesthetized using an intraperitoneal administration of ketamine (87 mg/kg) and xylazine (13 mg/kg) thereafter a midline incision was carried out and the stomach of each animal (n=5/group) was excised, perfused with cold phosphate buffered saline (PBS) (pH 7.4) to wash away any debris contained therein, weighed, homogenized in PBS (w/v 1:4) and centrifuged at 10,000 rpm for 10min at 4 ^{0}C. The clear supernatant obtained was aliquoted into labeled sample bottles and stored, until use, at -4 ^{0}C. The supernatant obtained was analysed for malondialdehyde (Papastergiadis *et al.,* 2012), superoxide dismutase (Misra and Fridovich, 1972), catalase (Sinha, 1972), reduced glutathione (Jollow *et al*., 1974), glutathione S-transferase (Habig *et al.,* (1974), nitrites (Olaleye *et al*., 2007), myeloperoxidase (Xia and Zweier 1997), interleukin-6 and tumor necrosis factor-α (ELISA**),** respectively. Stomach samples (n=3/group) were also estimated for gastric barrier mucous (as mucin) using the method described by Corne *et al.,* (1974).

Statistical analysis: Data obtained was analyzed using One-way ANOVA, followed by Turkey multiple comparisons with statistical significance between groups at $p < 0.05$.

RESULTS

Gastric biomarkers of oxidative status in control and experimental groups: Gastric malondialdehyde (MDA), a marker of lipid peroxidation, was increased ($p<0.05$) in

group III (96.6%) when compared with control. Values in groups IV and V decreased ($p<0.05$) by 26.3% and 45.7% when compared with group III, while values in groups VI and VII were comparable with controls (Group I). Superoxide dismutase activity (U/mg protein) in group III (0.94±0.04) was significantly reduced ($p<0.05$) compared with groups I (1.21±0.16), IV (1.23±0.03) and V (2.60±0.14) respectively. Compared to controls, SOD values in groups V (2.60±0.14) and VII (1.91±0.11) were increased, while values in group VI (1.50±0.12) were comparable. Catalase, reduced glutathione, glutathione-S-transferase and nitrites were reduced in group III (the BPA only group) compared to controls, while values in groups IV and V increased when compared with the same group (group III), respectively (Table 1).

Gastric mucin and inflammation biomarkers in control and experimental groups: Mucin levels (mg/g protein) reduced in groups III (15.69±5.07), IV (21.81±4.52) and V (27.19±5.91) when compared with controls (55.92±3.48) while values in groups VI and and VII were significantly increased when compared with control and all other treatment groups (Figure 1). Myeloperoxidase (MPO) (a marker of oxidative stress and inflammation), interleukin-6 (Il-6), and tumour necrosis factor – alpha (TNF-α) was significantly increased ($p<0.05$) in group III compared with control and all other experimental groups (Figure 2-4). The values for Il-6 (ρg/mg protein) and TNF-α (ρg/mg protein) were however increased in groups IV (57.36±3.27; 77.59±2.84) and VII (54.55±4.07; 69.15±0.96) compared with control (30.59±1.31; 40.13±3.12). Values for TNF were also elevated in group V (78.66±3.72) when compared with control (40.13±3.12) (Figure 4).

Table 1.
Gastric oxidative status in control and experimental groups

Groups	Antioxidant: Oxidant biomarkers					
	MDA (ηmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μM/mg protein)	GST (U/mg protein)	Nitrites (μmoles/mg protein)
I	0.89 ± 0.03	1.21 ± 0.16	16.18 ± 1.13	8.24 ± 0.27	0.112 ± 0.010	3.02 ± 0.06
II	1.24 ± 0.04	1.32 ± 0.08	12.33 ± 0.64	6.76 ± 0.49	0.077 ± 0.004	3.11 ± 0.23
III	1.75 ± 0.03*	0.94 ± 0.04*	9.75 ± 0.30*	5.83 ± 0.37*	0.048 ± 0.005*	2.24 ± 0.09*
IV	1.29 ± 0.05*#	1.23 ± 0.03#	13.45 ± 0.27#	10.70 ± 0.50#	0.111 ± 0.008#	4.18 ± 0.29#
V	0.95 ± 0.08#	2.60 ±0.14*#	17.18 ± 0.58#	14.86 ± 0.85#	0.147 ± 0.015#	3.25 ± 0.21#
VI	0.86 ± 0.04	1.50 ± 0.12	12.32 ± 0.50	7.82 ± 0.24	0.122 ± 0.008	2.36 ± 0.16
VII	0.81 ± 0.05	1.91 ± 0.11*	18.11 ± 0.46	9.97 ± 0.32	0.142 ± 0.012	2.57 ± 0.11

*Values are mean±SEM, * indicates values that are significantly different from controls, while # indicates values that are significantly different from group III, the BPA only treatment group.*
I – Control, II – Vehicle group, III – BPA only, IV – BPA+G. kola, V – BPA+kolaviron, VI – G. kola only, VII – kolaviron only. MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, GST = glutathione-S-transferase.

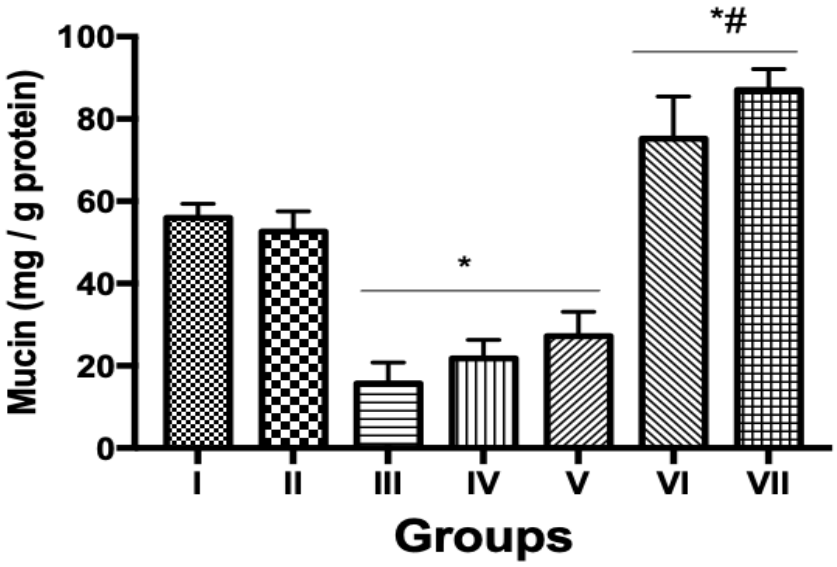

Figure 1.
Gastric mucin levels in control and experimental groups exposed to Bisphenol A and either *Garcinia kola* or kolaviron.
Values are mean±SEM, * indicates values that are significantly different from controls, while # indicates values that are significantly different from group III, the BPA only treatment group. I – Control, II – Vehicle group, III – BPA only, IV – BPA+*G. kola*, V – BPA+kolaviron, VI – *G. kola only*, VII – kolaviron only. MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, GST = glutathione-S-transferase.

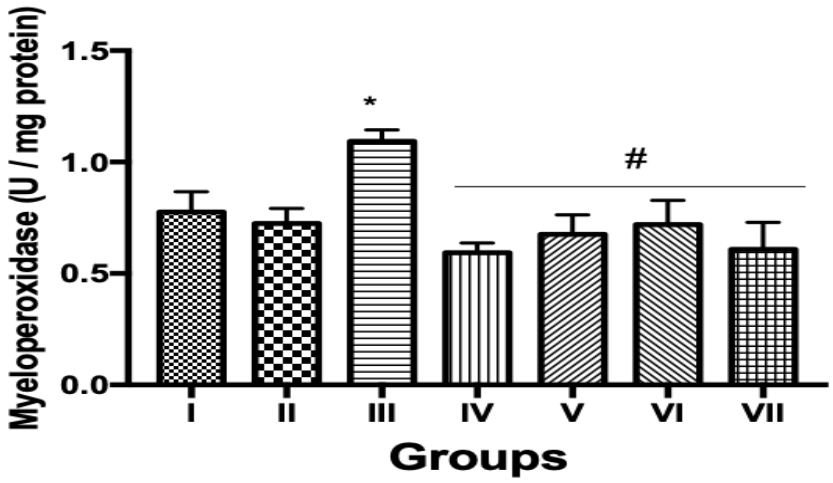

Figure 2.
Myeloperoxidase activity in the gastric tissue of control and experimental groups.
Values are mean±SEM, * indicates values that are significantly different from controls, while # indicates values that are significantly different from group III, the BPA only treatment group. I – Control, II – Vehicle group, III – BPA only, IV – BPA+*G. kola*, V – BPA+kolaviron, VI – *G. kola only*, VII – kolaviron only. MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, GST = glutathione-S-transferase.

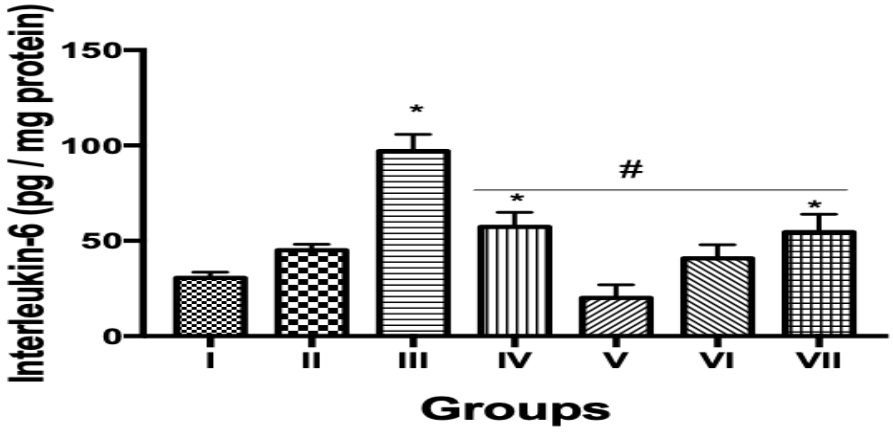

Figure 3.
Gastric interleukin-6 activity in control and experimental groups.
Values are mean±SEM, * indicates values that are significantly different from controls, while # indicates values that are significantly different from group III, the BPA only treatment group. I – Control, II – Vehicle group, III – BPA only, IV – BPA+*G. kola*, V – BPA+kolaviron, VI – *G. kola only*, VII – kolaviron only. MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, GST = glutathione-S-transferase.

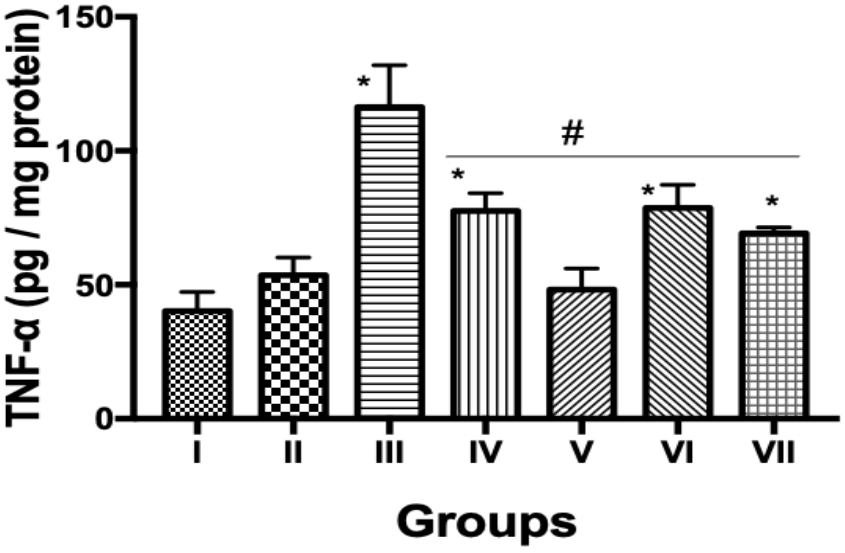

Figure 4.
Tumour necrosis factor – alpha activity in the gastric tissue of control and experimental groups
Values are mean±SEM, * indicates values that are significantly different from controls, while # indicates values that are significantly different from group III, the BPA only treatment group. I – Control, II – Vehicle group, III – BPA only, IV – BPA+*G. kola*, V – BPA+kolaviron, VI – *G. kola only*, VII – kolaviron only. MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, GST = glutathione-S-transferase.

DISCUSSION

Naturally, there is a balance between aggressive forces and protective mechanisms in the stomach, thereby maintaining the integrity of the mucosa (Goel and Sairam, 2003). In cases of an increased aggressor challenge such as exposure to BPA, the defense mechanisms need to be upregulated to avoid pending injury or invasion of the mucosa. Bisphenol A, an "endocrine disrupting" substance found in plastics, has been reported to be absorbed by the gastrointestinal (GI) tract especially the stomach where it has been shown to induce high levels of oxidative stress resulting in a compromise of the gastric structural and functional integrity (Chen *et al.,* 2022). Two important gastroprotective mechanisms involved in maintaining gastric integrity is the protective mucous layer and intrinsic antioxidant defense

system domiciled within the gastrointestinal tract (Goel and Sairam, 2003).

In this study, oral exposure to BPA led to a reduction in the gastric tissue level of mucin and GSH as well as reduced activities of SOD, CAT, and GST suggesting a compromise of gastric integrity and endogenous gastric antioxidant protective mechanisms. Furthermore, and to lend credence to this is the fact that gastric malondialdehyde levels, a marker lipid peroxidation induced by oxidative stress, was elevated in the BPA only treatment group. Myeloperoxidase (MPO), a marker of leucocytes infiltration, which is considered an index of inflammatory and leukocyte-mediated mucosal tissue damage as well as breakdown of the mucosal barrier, was found to be elevated in the gastric tissues of BPA only group, which shows that the integrity of the gastric mucosa in this group may be compromised by mechanisms that can be associated with increased oxidative stress and inflammation. These observations are consistent with finding from other researchers who have reported a gastrotoxic effect of BPA on the gastric mucosa (Ige *et al.,* 2022, Abo-Elsoud *et al.,* 2022).

One of the primary manifestations of increased oxidative stress is inflammation (Sánchez *et al.,* 2015). In this study, the BPA only treated group also exhibited signs of increased gastric inflammation as gastric IL-6 and TNF-α activity were found to be elevated in this treatment group. Bisphenol A has been reported to mimic estrogenic activity resulting in immune dysregulation and thus affect the immune cell signaling pathways and responses. Mucosal injury following exposure to BPA has also been suggested to occur as a result of increased stimulation and production of macrophage TNF-α (Sugimoto *et al.,* 2007). Furthermore, increased TNF-α activates caspase-3 that leads to gastric cell apoptosis (Park *et al.,* 2000). Exposure to BPA has also been linked to an increased expression of gastric IL-6 through mechanisms that are associated with an inflammation mediated amplification of NF-κB production (Zhang *et al*., 2020).

In this study, concomitant treatment of BPA orally exposed animals with *Garcinia kola* or its biflavonoid extract, kolaviron, upregulated the production of gastroprotective biochemicals (SOD, CAT, GSH and GST) when compared with BPA only treated animals suggesting a mitigation of BPA-induced gastric oxidative stress. Furthermore, animals in the BPA+*Garcinia kola*, BPA+kolaviron, *Garcinia kola* only, and kolaviron only treatment groups showed gastric lipid peroxidation values that suggests a potentiation of and increased level of free radical scavenging activity in these treatment groups. The activities of MPO, Il-6 and TNF-α seen in these groups also further suggests a mitigation of BPA induced gastric inflammation. However, mucins which are composed of highly glycosylated proteins that play a tripartite (lubrication, cell signaling, protecting the epithelium) role in the gut, was reduced in all animals exposed to BPA (untreated and treated). This suggest that co-exposure of BPA to either *Garcinia kola* or kolaviron did not attenuate reductions in mucin secretion which have been reported following exposure of the gut to BPA (Gonkowski, 2020). Exposure to either *Garcinia kola* or kolaviron alone, however resulted in increased mucin secretion suggesting a potentiation of gastroprotection in these treatment group.

Isolates of *Garcinia kola* that have been implicated as being responsible for its plethora of its medicinal potentials includes biflavonoids, benzophenones, benzofurans, benzopyran, vitamin E derivatives, xanthones, and phytosterols (Emmanuel *et al.,* 2021). *Garcinia kola* and its major biflavonoid extract, kolaviron, have also been widely touted in various reports for their strong antioxidant and anti-inflammatory potentials (Olatoye and Akindele, 2021). Furthermore, though *Garcinia kola* contains a plethora of medicinal phytochemicals, most of its medical and pharmacological activity could be ascribed to its major biflavonoid, kolaviron (Farombi *et al.,* 2022), which is an active complex of at least 3 compounds consisting of Garcinia Biflavanoid-1 (GB-1), kolaflavanone, and Garcinia Biflavanoid-2 (GB-2). This kolaviron is well known for its potent antioxidant and anti-inflammatory properties that have been explored for therapeutic potentials in several disease models ranging from reproductive toxicity, cardiotoxicity, diabetes mellitus, gastrotoxicity and hepatotoxicity (Erukainure *et al.,* 2021). The potent antioxidant potentials of kolaviron may therefore be responsible for the increased activity of gastric antioxidant biochemicals in the groups co-exposed to BPA and kolaviron when compared with animals co-treated with BPA and *Garcinia kola.*

Taken together, it is likely that the gastroprotection and mitigation against BPA induced gastrotoxicity proffered by both *Garcinia kola* and kolaviron in this study may be due to their ability to potentiate the gastric antioxidant defense mechanism resulting in an upregulation of the production and secretion of SOD, CAT, GSH and GST. The increased SOD produced would have scavenged superoxide ions which would be converted by catalase to water. Increased GSH-GST levels has been reported to protect cells from oxidation caused by BPA via mechanisms such as direct removal of oxidants and increasing the activity of glutathione peroxidase resulting in consumption of glutathione and its conversion into ineffective form of disulfate (Sabour, 2019). The collective actions of the increased antioxidant activity would therefore account for the reduced production of BPA-induced free radicals and thus account for the reductions in gastric inflammatory processes (MPO, Il-6, and TNF-α) seen in the *Garcinia kola* and kolaviron treated BPA exposed groups.

Nitrites have been reported to contribute to host defense mechanism against a number of pathogenic microorganisms in the mouth, stomach and skin (Archer, 2002). It has also been observed to increase gastric mucosal blood flow and mucus thickness (Björne *et al.,* 2004). The reduced values in the BPA only treatment group therefore suggests a likely compromise of gastroprotection via mechanisms that maybe associated with reduced mucosal blood flow and mucus thickness. Nitrites in the groups co-exposed to BPA and either *Garcinia kola* or kolaviron was increased compared to BPA only and comparable to controls suggesting a mitigation of gastric injury in these groups (IV and V), respectively.

In conclusion, this study suggests that *Garcinia kola* and its biflavonoid, kolaviron protects the gastric mucosa against Bisphenol A induced impairment by potentiating gastroprotective biochemicals in male Wistar rats. However, in the duration that this study was conducted, BPA induced reduction of gastric mucin was not attenuated by either *Garcinia kola* or kolaviron

Ethical Approval: For this research related to animals, the Applied and Environmental Physiology Unit, Department of Physiology University of Ibadan approved the experimental procedures and protocol, which has complied with all the guidelines laid down by the Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan and that of the Guide for the Care and Use of Laboratory Animals, 1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA (The Applied and Environmental Unit, Department of Physiology does not issue approval numbers. However, all research studies emanating from this Unit is scrutinised with emphasis on humane care and treatment of animals. Furthermore, the Unit also ensures that all research projects are presented to a larger audience consisting of experts in the field Physiological research. Letters supporting this can be obtained from the Department of Physiology, University of Ibadan if required).

REFERENCES

Abo-Elsoud, R.A.E.A., Ahmed Mohamed Abdelaziz, S., Attia Abd Eldaim, M. (2022). Moringa oleifera alcoholic extract protected stomach from bisphenol A–induced gastric ulcer in rats via its anti-oxidant and inflammatory activities. *Environ Sci Pollut Res* 29, 68830–68841. https://doi.org/10.1007/s11356-022-20543-0

Archer, D. L. (2002). Evidence that ingested nitrate and nitrite are beneficial to health. Journal of Food Protection, 65(5), 872-875.

Björne, H., Petersson, J., Phillipson, M., Weitzberg, E., Holm, L. and Lundberg, J. O. (2004). Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *The Journal of clinical investigation*, 113(1), 106-114.

Chen Zhi, Wang Fen, Wen Di, Mu Ren. Exposure to bisphenol A induced oxidative stress, cell death and impaired epithelial homeostasis in the adult Drosophila melanogaster midgut, *Ecotoxicology and Environmental Safety*, 248, (2022), 114285, https://doi.org/10.1016/j.ecoenv.2022.114285

Corne SJ, Morrissey SM, Woods RJ. Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J Physiol.* 1974 Oct;242(2):116P-117P.

Dogara, A.M., Hamad, S.W., Hama, H.A., Bradosty, S.W., Kayfi, S., Al-Rawi, S.S. and Lema, A.A. (2022). Biological Evaluation of *Garcinia kola* Heckel. Adv Pharmacol Pharm Sci. 2022:3837965. doi: 10.1155/2022/3837965.

Emmanuel, O., Uche, M. E., Dike, E. D., Etumnu, L. R., Ugbogu, O. C., & Ugbogu, E. A. (2021). A review on *Garcinia kola* heckel: traditional uses, phytochemistry, pharmacological activities, and toxicology. Biomarkers, 27(2), 101–117. https://doi.org/10.1080/1354750X.2021.2016974.

Erukainure OL, Salau VF, Chukwuma CI, Islam MS. Kolaviron: A Biflavonoid with Numerous Health Benefits. *Curr Pharm Des*. 2021;27(4):490-504. doi: 10.2174/1381612826666201113094303.

Farombi, E. O., Awogbindin, I. O., Farombi, T. H., Ikeji, C. N., Adebisi, A. A., Adedara, I. A. and Aruoma, O. I. (2022). Possible role of Kolaviron, a *Garcinia kola* bioflavonoid in inflammation associated COVID-19 infection. *American Journal of Biopharmacy and Pharmaceutical Sciences,* 2;3. doi:10.25259/AJBPS_1_2022

Goyal R.K., Sairam K., Anti-ulcer drugs from indigenous sources with emphasis on Musa sapientum, Tamrabhasma, Asparagus racemosus and Zingiber officinale, *Indian J. Pharmacol*. 34 (2002) 100–110.

Gonkowski S. Bisphenol A (BPA)-Induced Changes in the Number of Serotonin-Positive Cells in the Mucosal Layer of Porcine Small Intestine-the Preliminary Studies. *Int J Mol Sci.* 2020 Feb 6;21(3):1079. doi: 10.3390/ijms21031079.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) Glutathione S-Transferases. The First Enzymatic Step in Mercapturic Acid Formation. *Journal of Biological Chemistry*, 249, 7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Ige, A.O, Adebayo, O.O, Adele, B.O, Odetola, A.O, Emediong, I.E, and Adewoye, E.O. (2022) Genistein Mitigates the Gastro-Toxic Effects of Bisphenol A in Male Wistar Rats. *Journal of Biosciences and Medicines*, 10, 60-78. doi: 10.4236/jbm.2022.109006.

Ige, A.O, Adewoye, E.O, Okwundu, N.C, Alade, O.E, Onuobia, P.C (2016) Oral magnesium reduces gastric mucosa susceptibility to injury in experimental diabetes mellitus. *Pathophysiology* 23, 87–93. http://dx.doi.org/10.1016/j.pathophys.2016.04.003

Jollow, D.J., Mitchell, J.R., Zampaglione, N. and Gillete, J.R. (1974) Bromobenzene Induced Liver Necrosis. Protective Role of Glutathione and Evidence for 3,4-Bromo- benzene Oxide as a Hepatotoxic Metabolite. *Pharmacology*, 1, 151-169. https://doi.org/10.1159/000136485

Kayinu, N. A., Adele, B. O., & Ige, A. O. (2024). *Garcinia kola* and Kolaviron Attenuates Bisphenol A-Induced Memory Impairment and Hippocampal Neuroinflammation in Male Wistar Rats. *Journal of Biosciences and Medicines*, 12, 111-130.

Manzoor, M.F., Tariq, T., Fatima, B., Sahar, A., Tariq, F., Munir, S., Khan, S., Nawaz Ranjha, M.M.A., Sameen, A., Zeng, X.A and Ibrahim, S.A. (2022). An insight into bisphenol A, food exposure and its adverse effects on health: A review. *Front. Nutr*. 9:1047827. doi: 10.3389/fnut.2022.1047827.

Misra, H.P. and Fridovich, I. (1972) The Role of Superoxide Anion in the Autoxida- tion of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry*, 247, 3170-3175. https://doi.org/10.1016/S0021-9258(19)45228-9

Muzeza, C., Ngole-Jeme, V. and Msagati, T.A.M. (2023). The Mechanisms of Plastic Food-Packaging Monomers' Migration into Food Matrix and the Implications on Human Health. *Foods.* 2023; 12(18):3364. https://doi.org/10.3390/foods12183364.

National Research Council (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington DC.

Obuzor, G.U. and Onyedikachi, U.B. (2023). Chemical Leaching into Food and the Environment Poses Health Hazards. In: Nwaichi, E.O. (eds) Modernity in Health and Disease Diagnosis: The Account from STEM Women. Sustainable Development Goals Series. *Springer*, Cham. https://doi.org/10.1007/978-3-031-34963-8_14.

Ogwu, M.C., Ogwu, H.I., Osawaru, M.E., Izah, S.C. (2024). *Garcinia kola* Heckel. (Clusiaceae): An Overview of the Cultural, Medicinal, and Dietary Significance for Sustainability. In: Izah, S.C., Ogwu, M.C., Akram, M. (eds) Herbal Medicine Phytochemistry. Reference Series in Phytochemistry. *Springer*, Cham. https://doi.org/10.1007/978-3-031-21973-3_74-1.

Olaleye S.B., Adaramoye O.A., Erigbali P.P., Adeniyi O.S.(2007) Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats, *World J. Gastroenterol*. 13 (38) 5121–5126.

Olaleye, S. B., Farombi, E. O., Adewoye, E. A., Owoyele, B. V., Onasanwo, S. A., & Elegbe, R. A. (2000). Analgesic and anti-inflammatory effects of kolaviron (a *Garcinia kola* seed extract). *African journal of biomedical research*, 3(3), 171-174.

Olatoye, F. J. and Akindele, A. J. (2021). Ninety-day oral toxicological profiling of Kolaviron (an extract of Garcinia kola) in male and female rats. Drug and Chemical Toxicology, 46(1), 1–14. https://doi.org/10.1080/01480545.2021.1997543.

Oyagbemi, A.A., Omobowale, T.O., Adedapo, A.A. and Yakubu, M.A. (2016). Kolaviron, Biflavonoid Complex from the Seed of *Garcinia kola* Attenuated Angiotensin II- and Lypopolysaccharide-induced Vascular Smooth Muscle Cell Proliferation and Nitric Oxide Production. *Pharmacognosy Res.* Mar;8(Suppl 1): S50-5. doi: 10.4103/0974-8490.178647. PMID: 27114693; PMCID: PMC4821108.

Papastergiadis, A., Mubiru, E., Van Langenhove, H. and De Meulenaer, B. (2012). Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. *Journal of agricultural and food chemistry*, 60(38), 9589-9594.

Park I, Park M, Choe T, Jang J, Hong S, Lee SJIJOO. TNF-alpha induces apoptosis mediated by AEBSF-sensitive serine protease (s) that may involve upstream caspase-3/CPP32 protease activation in a human gastric cancer cell line. *Int J Oncol*. 2000;16(6):1243–1251.

Sabour AN. The effect of bisphenol A on some antioxidants in white male rats. *Sci J Med Res*. 2019;3(10):83–86.

Sánchez, A., Calpena, A. C. and Clares, B. (2015). Evaluating the oxidative stress in inflammation: role of melatonin. International journal of molecular sciences. 16(8), 16981-17004.

Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972 Jun;47(2):389-94. doi: 10.1016/0003-2697(72)90132-7. PMID: 4556490.

Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, Sugimura H, Hishida AJJOG, Hepatology Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *J Gastroenterol Hepatol*. 2007;22(1):51–59. doi: 10.1111/j.1440-1746.2006.04442.x.

Szymańska, K., Makowska, K., Całka, J. and Gonkowski, S. (2020). The Endocrine Disruptor Bisphenol A (BPA) Affects the Enteric Neurons Immunoreactive to Neuregulin 1 (NRG1) in the Enteric Nervous System of the Porcine Large Intestine. *Int J Mol Sci*. Nov 19;21(22):8743. doi: 10.3390/ijms21228743.

Tauchen, J., Frankova, A. and Manourova, A. (2023). Garcinia kola: a critical review on chemistry and pharmacology of an important West African medicinal plant. *Phytochem Rev* 22, 1305–1351 (2023). https://doi.org/10.1007/s11101-023-09869-w.

Vandenberg, L.N., Russ, H., Michele, M., Nicolas O. and Wade V. W. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology*. Volume 24, Issue 2: Pages 139 177, ISSN 0890-6238. https://doi.org/10.1016/j.reprotox.2007.07.010.

Xia Y, Zweier JL. Measurement of myeloperoxidase in leukocyte-containing tissues. Anal Biochem. 1997 Feb 1;245(1):93-6. doi: 10.1006/abio.1996.9940.

Yoon, K., Kwack, S. J., Kim, H. S., & Lee, B. M. (2014). Estrogenic Endocrine-Disrupting Chemicals: Molecular Mechanisms of Actions on Putative Human Diseases. *Journal of Toxicology and Environmental Health*, Part B, 17(3), 127–174. https://doi.org/10.1080/10937404.2014.882194.

Zhang S, Li J, Fan J, Wu XJJOB, Toxicology M. Bisphenol A triggers the malignancy of acute myeloid leukemia cells via regulation of IL-4 and IL-6. *J Biochem Mol Toxicol*. 2020;34(1):e22412. doi: 10.1002/jbt.22412.

Zhang, C., Wang, A., Sun, X., Li, X., Zhao, X., Li, S., & Ma, A. (2013). Protective effects of Lycium barbarum polysaccharides on testis spermatogenic injury induced by bisphenol A in mice. *Evidence-based complementary and alternative medicine*, 2013:690808. doi: 10.1155/2013/690808.