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Garcinia Kola and Kolaviron Attenuates Bisphenol A-Induced Memory Impairment and Hippocampal Neuroinflammation in Male Wistar Rats

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Abstract

Bisphenol A (BPA), a toxicant which can leach into food from plastic containers, is reported to induce neurotoxicity among others via oxidative mechanisms. However, antioxidant compounds have been suggested to mitigate BPA-induced toxicities. *Garcinia kola* (GK) and its bioactive compound, kolaviron, are well-established natural antioxidants, which can exert protective effects against BPA-induced toxicities. This study was designed to investigate the likely mitigating effect of GK and kolaviron on BPA-induced memory impairment and hippocampal neuroinflammation in male Wistar rats. Thirty-five rats were equally grouped and treated as follows: I and II received distilled water and corn oil, respectively at 0.2 mL, while III - VII received BPA (50 mg/kg), BPA + GK (200 mg/kg), BPA + kolaviron (200 mg/kg), GK and kolaviron, respectively for 28 days *p.o*. Thereafter, behavioral studies were done using the Novel Object Recognition and Y maze tests. Subsequently under anaesthesia, the hippocampus in each animal was dissected out, homogenized and analysed for malondialdehyde, superoxide dismutase, catalase, reduced glutathione, glutathione transferase, nitrites, interleukin-6, tumour necrosis factor-*α*, acetylcholinesterase, glutamate acid decarboxylase, and arginase activity. Data were analyzed by ANOVA and Tukey Post-hoc test at $p < 0.05$. Animals in group III significantly ($p < 0.05$) exhibited memory impairment which was accompanied by increased oxidative stress, neuroinflammation and altered hippocampal neurochemicals. Treatment with GK and Kolaviron (groups IV and V) significantly mitigated the aberrations observed in the BPA only exposure group. This study suggests that *Garcinia kola* and Kolaviron mitigate bisphenol A-induced memory impairment and neuroinflamma-

tion via antioxidant potentiation and neurotransmitter balance.

Keywords

Bisphenol A, Memory Impairment, Neuroinflammation, Neuroprotection, Garcinia Kola, Kolaviron, Antioxidant

1. Introduction

Bisphenol A (BPA) is a chemical compound largely used in the production of epoxy resins, polycarbonate plastics and polyvinyl chloride plastics widely dispersed in consumer products and healthcare consumables [1] [2]. Products containing BPA are widely used as kitchenware and other household items such as plastic bottles, food containers, toys, wire insulation and food/beverage cans, thus, increasing human contact with BPA [3]. Studies have shown that BPA monomers can leach out from these products, when subjected to washing processes, heating or contact with acidic or basic pH, and hence enter the human body through occupational and food contact [4]. Compared with other sources of exposure, diet is known to make a significant contribution due to the leaching of BPA into food materials from packing or storage containers [3].

Globally, the BPA burden is on the increase as it is known to mediate toxicities even at low doses [2] [5]. On exposure, BPA binds to both nuclear and membrane estrogen receptors without excluding thyroid, glucocorticoid and PPAR receptors to exert its effects on hormone-dependent, immune, developmental activities and behavior in both experimental animals and humans [6] [7] [8]. Other toxic effects are epigenetic modification as well as neurobehavioral disruption [9] [10]. The neuropsychological disorders and neurobehavioral disturbances linked to BPA are a result of its reported ability to pass through the blood-brain barrier [11] and cause damage to protein and lipid structures via free radicals mediated mechanisms [12]. This could also be associated with the presence of estrogenic receptors which BPA could interact with [13]. Given the increasing exposure of humans to plastics and the pleiotropic effects of BPA due to the vast expression of estrogenic receptors in the human body, there is an urgent need for treatments to abate the increase in the occurrence of BPA-related neurotoxicity.

Garcinia kola is a tree with all its parts used in the folkloric treatment of different diseases and disorders [14]. Most especially, *Garcinia kola* seed and one of its bi-flavonoid extracts, kolaviron, have been reported in various studies to possess diverse biological activities ranging from antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiviral and antiulcer properties [15] [16]. However, the neuroprotective effect of *Garcinia kola* seed and kolaviron is scarcely reported. As an antioxidant, they could help prevent oxidative mechanisms involved in BPA-induced neurobehavioural impairments and mitigate BPA-associated neu-

rotoxicity. This study was therefore designed to investigate the effects of *Garcinia kola* and kolaviron on Bisphenol A-induced behavioural impairment and hippocampus neurotoxicity.

2. Materials and Methods

2.1. Plant Preparation and Extraction of Kolaviron

Garcinia kola seeds were procured from a local vendor in Kaduna state, Nigeria and authenticated at the University of Ibadan Herbarium where a voucher specimen already existed. The seeds were peeled, sliced, air-dried, powdered and stored in a sterile plastic container until needed. Fresh solutions of *G. kola* suspended in distilled water were prepared daily and administered at a dose of 200 mg/kg [17].

Kolaviron was extracted using the methods of Olaleye *et al*. [18]. Briefly, dried powdered *G. 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 kolaviron-rich crude extract. The crude acetone extract was concentrated to about 100mL with a rotary evaporator at 40˚C, diluted to twice its volume with distilled water and then partitioned with ethyl acetate (to extract nonpolar flavonoids). The ethyl acetate fraction was concentrated to get a yellow-brown powder known as kolaviron which was administered at a dose of 200 mg/kg [19].

2.2. Animal Grouping and Experimental Protocol

Thirty-five male adult Wistar rats (165 - 180 g) acclimatized to laboratory conditions were grouped into seven (7) (n = 5) and treated orally as follows: Group I - control (distilled water, 0.2 mL/day), Group II- vehicle (corn oil, 0.2 mL/day), III - VII were treated with BPA only, BPA + *G. kola*, BPA + Kolaviron, *G. kola* only, and kolaviron only, respectively. Bisphenol A was administered at 50 mg/kg (the lowest-observed-adverse-effect-level for BPA [20]) while *G. kola* and kolaviron were given at 200 mg/kg (therapeutic doses according to Akpantah *et al*. [17] and Faronmbi *et al*. [19]) respectively for 28 days. Bisphenol A was administered alone to evaluate neurotoxicity, while BPA co-administration with either *G. kola* or kolaviron was carried out to evaluate the possible therapeutic potentials of the plant and its extract in mitigating BPA induced neurotoxicity. *Garcinia kola* and kolaviron were also administered separately to evaluate potential benefits or otherwise in the absence of BPA induced toxicity. All experimental protocols were approved by the Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC/063-0723/12) and also followed the Guide for the Care and Use of Laboratory Animals, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA [21].

2.3. Neurobehavioural Studies

Twenty-eight days post-treatment, animals were assessed for likely neurobehavioral impairments using the novel object recognition test (NORT) [22] and Y maze [23] test. Briefly, NORT consists of three phases: habituation, familiarization (trial), and test phase.

The habituation phase aims to accustom the animals to the open-field arena. The arena was cleaned with 70% ethanol before placing each animal in the arena. In the habituation phase, each animal was allowed to freely explore the open-field arena in the absence of objects for a period of 5 minutes. After 5 minutes each animal was returned to their cage for a period of 4 hours before the commencement of the trial phase.

The trial or familiarization phase started 4 hours after the habituation phase. Each animal was exposed to two identical objects placed in the open-field arena. In the trial phase, animals were placed in the middle of two adjacent identical objects (A and B) at a distance of 8 cm from the walls and 34 cm from each other in the open-field chamber for 5 min. the open-field arena was cleaned with 70% ethanol before the reintroduction of each animal into the arena to eliminate animal clues either from urine or feces.

The test phase commenced 24 hours after the trial phase, in the test phase the novel object was introduced to replace one of the two similar objects. During the test phase, object B was replaced with object C, which was novel to the animals and different from either object A or B, each animal was then left to explore objects A and C for a period of 5 min. The apparatus was cleaned after each test with 70% ethanol to eliminate animal clues. The test phase was for each animal was recorded with the aid of a phone camera which was set up using a tripod stand. The time spent (in seconds) exploring each of the objects (familiar and novel objects) was recorded in the test phase with the aid of a stopwatch.

The discrimination index (DI) evaluates the discrimination between the novel and familiar objects, by using the difference in time of exploration of the novel object and familiar object, followed by dividing this value by the total amount of time (sum) of exploration of both the novel and familiar objects represented by the formula:

$$\mathrm{DI} = \frac{\mathrm{T_N} - \mathrm{T_F}}{\mathrm{T_N} + \mathrm{T_F}}$$

where $\mathrm{T_N}$ is the time (seconds) spent exploring the novel object, and $\mathrm{T_F}$ is the time spent exploring the familiar object.

The result from the discriminative index can vary between +1 and −1, where a positive score indicates increased time spent with the novel object, while a negative score indicates more time spent with the familiar object, and a score of zero indicates neutral preference.

The recognition index (RI) is used as an index of retention which is the time spent exploring the novel object relative to the total time spent observing both objects. It is determined by the time of exploration of the novel object divided by

the total amount of time exploring both objects represented in the formal below:

$$RI = \frac{T_N}{T_N + T_F}$$

where T_N and T_F are also as above.

The result from the recognition index is interpreted as the index of retention where an animal is able to recognize an unfamiliar object (novel) and a familiar object. High recognition index score indicates high retention while low scores indicate low retention when compared between groups or animals.

In the Y-maze test, each animal was placed in the Y-shaped maze with three labeled arms, A, B and C and allowed to explore all three arms freely for 5 min. The number and sequence of arm entries were recorded. An entry was scored when the four paws of the animals were completely in the arms of the Y-maze. The percentage alternation, which is a measure of memory function, was calculated by dividing the total number of alternations by the total number of arm entries, subtracted by two, and then multiplied by 100. Animals were introduced into the maze at arm "A".

2.4. Sample Collection, Preparation and Biochemical Assays

Animals were thereafter anaesthetized by an intraperitoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg) [24], euthanized, and the brain was excised and rinsed in ice cold phosphate buffered saline. The brain excision process was done with the aid of surgical scissors, iris scissors and forceps to first separate the skin from the skull and then to separate the skull from the brain, the brain was then separated into left and right hemispheres, the cortex of each hemisphere was separated from the mid line to expose the hippocampus, a C shaped gray structure, with surrounding structures such as the corpus callosum and cortex which were carefully removed. Both left and right hippocampus was excised from the two hemispheres over ice to maintain a low temperature which was aimed to reduce enzyme activity and to preserve the hippocampus tissue. The tissue was then homogenized in ice cold 0.1M sodium phosphate buffer (pH 7.4) to preserve the tissue by preventing proteins denaturing. The homogenate obtained was centrifuged for 10minutes at 10,000 rpm at 4˚C. The clear supernatant obtained was kept in aliquots at −4˚C, and later analyzed for the following biochemicals:

2.5. Malondialdehyde Estimation

Malondialdehyde was measured as an index of lipid peroxidation using the assay of thiobarbituric reacting substances (TBARs) by the method of Nagababu *et al*., [25]. Briefly, 100 µL of supernatant was diluted ten times in 0.15 M Tris-KCl buffer, and deproteinized with 500 µL trichloroacetic acid (30%). The mixture was centrifuged in a bench top centrifuge at 4000 rpm for 10 min at room temperature. 200 µL of the supernatant was then removed into Eppendorf tubes, and 200 µL of thiobarbituric acid (1%) was added. The mixture was heated at 80˚C

for 1 hour. The tubes were cooled by placing on ice and 200 µL of the mixture was removed into microtitre plate and absorbance measured at 532 nm. The result was calculated using an index of absorption for MDA (molar extinction coefficient 1.56×10^5/M/cm). The concentration of TBARS in the tissues was expressed as ηmol MDA/ mg protein.

2.6. Determination of Superoxide Dismutase (Sod)

The level of SOD activity was determined by the method of Misra and Fridovich [26]. Superoxide dismutase activity is determined based on its ability to inhibit the autoxidation of adrenaline in sodium carbonate buffer (pH 10.7). Briefly, 50 µL of 2X diluted supernatant was added into a microtitre plate containing 150 µL of carbonate buffer. The reaction was started by the addition of 30 µL of freshly prepared 0.3mM adrenaline to the mixture. Blanks were prepared using 50 µL of distilled water. The increase in absorbance at 495 nm was monitored every 60 seconds for 300 seconds in the LT-4500 microplate reader (Labtech, UK). The SOD activity was expressed as U/mg protein.

2.7. Catalase Enzyme Assay

Catalase activity in the supernatants of the hippocampus was determined using the colorimetric assay based on the yellow complex with molybdate and H_2O_2, which was described by Goth *et al.* [27]. Briefly, 50 µL of 2X diluted supernatant was added into a microtitre plate, and then 50 µL of a reaction mixture containing 65 mmol/mL of H_2O_2 in sodium-potassium phosphate buffer (60 mM, pH 7.4) was added. The enzymatic reaction was incubated for 3 min and stopped with 100 µL of ammonium molybdate (64.8 mM) in sulfuric acid. The absorbance at 405 nm was measured in a LT-4500 microplate reader (Labtech, UK). The catalase enzyme activity unit was expressed as U/ mg protein.

2.8. Reduced Glutathione Level

Reduced glutathione (GSH) as a non-enzymic antioxidant maker was measured in the supernatant of the hippocampus supernatants according to Jollow *et al.* [28]. Briefly, 100 µL of supernatant was diluted ten times in 0.15 M Tris-KCl buffer, and deproteinized with 500 µL trichloroacetic acid (30%). The mixture was centrifuged in a bench top centrifuge at 4000 rpm for 10 min at room temperature. 100 µL of the deproteinized supernatant was mixed 100 µL of 5^1, 5^1-Dithios-nitrobenzoic acid (DTNB, 0.0006 M) in a microplate plate. The absorbance was read within 5 min at 405 nm in a LT-4500 microplate reader (Labtech, UK). The glutathione concentration was extrapolated from standard curve of glutathione (0 - 200 µM) and expressed as a µM GSH/ mg protein.

2.9. Determination of Glutathione-S-Transferase Activity

The glutathione-s-transferase enzyme activity was determined by the method of Habig *et al.* [29]. A reaction mixture of 1-chloro-2,4-dinitrobenzene, glutathione

and the hippocampal tissue supernatant was prepared and mixed thoroughly in a test tube. The reaction mixture was incubated at 37˚C for a period of 30 minutes for a catalytic reaction by the enzyme. Trichloroacetic acid was added to terminate the reaction and thoroughly mixed to ensure inactivation of the enzyme to terminate the reaction. The absorbance of the solution was read at 340nm with increase in absorbance correlated to enzyme activity with units expressed as units per milligram of protein (U/mg protein).

2.10. Estimation of Nitrites

Nitrite is a major intravascular store for nitric oxide. Hippocampal Nitrite Levels were measured using Griess method [30]. Briefly, the reaction between Nitrite and Griess Reagent which consisted of sulfanilamide and N-(1-napthyl)-ethylenediamine, produced a violet color solution. The solution was then measured for standards at varying concentrations (to determine an absorbance curve) and samples for the determination of maximum absorbance wavelength. Photometric absorbance at 538nm accurately determines nitrite concentration. Nitrite levels were extrapolated from the curve and expressed as μmoles/mg protein.

2.11. Estimation of Interluekin-6 and Tumor Necrosis Factor-Alpha

The levels of Il-6 and TNF-α in the supernatant were determined using the Biolegend Enzyme linked immunosorbent assay (ELISA) kit, (USA) specific to the cytokines of interest. All the measurements were done at room temperature in accordance with manufacturer's instructions and read at 450 nm using a microplate reader. The concentration of Il-6 and TNF-α from the supernatant were extrapolated from the standard curves of IL-6 and TNF-α standards included in the assay kits and expressed as pg/mL.

2.12. Estimation of Acetyl-Cholinesterase Activity

The procedure described by Ellman *et al.* [31] was used to estimate acetylcholinesterase (AChE) activity in the supernatant of the brain tissues. Briefly, 50 μL aliquots of hippocampal supernatant was diluted 50 μL of phosphate buffer (0.1 M, pH 7.4) followed by addition of 50 μL of DTNB (0.0001 M) in a 96-well plate. The initial absorbance was first measured after 5 min of incubation with DTNB. Thereafter, 50 μL of acetylthiocholine iodide (0.028 M) was added to the mixture for 3 min and the absorbance was again measured at 405 nm in a microplate reader (LT4500, UK). The rate of acetyl-cholinesterase activity (μmol/min/mg tissue) was calculated as described below:

$$R = 5.74 \times 10^{-4} \times A/Co$$

where:

R = Rate in moles of substrate hydrolyzed/min/g tissue,

A = Change in absorbance/min,

Co = Original concentration of the tissue.

2.13. Determination of Glutamic Acid Decarboxylase

The procedure described by Yu *et al.* [32] was used to estimate glutamic acid decarboxylase (GAD) activity in the supernatant of the hippocampal tissues. Briefly, 50 µL aliquots of 2X diluted hippocampal tissue supernatant was incubated with reaction mixture containing 20 mM sodium acetate, 70 µM bromocresol, 10 mM pyridoxal-5-phosphate (PLP) and 2 µL glutamate (from a 1M stock in acetate buffer). The increase in absorbance at 630 nm for 5 min was read in a microplate reader (LT4500, UK). The unit of enzyme was expressed as µmoles min^{-1} mg $protein^{-1}$.

2.14. Determination of Arginase Enzyme Activity

Arginase is a manganese-containing enzyme that catalyzes the conversion of arginine to urea and ornithine. Based on this principle, a colorimetric assay in which ornithine gives a color product with ninhydrin was used. One unit of arginase activity is defined as the amount of Mn^{2+} activated enzyme that produces 1 µmol of ornithine/min at 37˚C [33]. The arginase enzyme activity unit was expressed as U/mg protein.

2.15. Statistical Analysis

Data obtained were expressed as mean ± SEM. Statistical significance was established at $p < 0.05$ using one way ANOVA followed by Tukey multiply comparisons.

3. Results

3.1. Body Weight and Relative Brain Weight in Control and Experimental Groups

Groups III (BPA only), IV (BPA + *G. kola*), V (BPA + kolaviron), VI (G. *kola* only) and VII (kolaviron only) exhibited a 24.31% ± 5.29%, 10.67% ± 1.80%, 19.16% ± 3.13%, 8.01% ± 2.90%, and 9.43% ± 2.69% increase in body weight when compared with their individual day 0 values (Table 1).

Table 1. Effect of Garcinia kola and kolaviron on body weight changes in control and experimental groups.

Groups	Body weight (g)					Percent weight change (%)
	Day 0	Day 7	Day 14	Day 21	Day 28	
I	177.29 ± 9.96	175.57 ± 9.23	177.71 ± 9.50	176.71 ± 9.06	190.71 ± 9.44	7.89 ± 1.98
II	177.00 ± 9.87	178.29 ± 8.88	186.00 ± 9.46	183.86 ± 9.16	201.29 ± 8.52	14.38 ± 3.30*
III	178.29 ± 9.44	183.29 ± 7.85	194.43 ± 7.95	198.86 ± 6.84	219.57 ± 7.66	24.31 ± 5.29*
IV	175.43 ± 4.59	179.00 ± 5.00	180.14 ± 6.62	182.00 ± 7.73	194.43 ± 7.54	10.67 ± 1.80[a]
V	183.33 ± 6.56	189.00 ± 6.68	195.50 ± 4.72	196.33 ± 4.32	217.33 ± 3.35	19.16 ± 3.13*
VI	173.86 ± 8.05	176.43 ± 7.59	180.14 ± 7.32	178.14 ± 6.42	187.29 ± 8.22	8.01 ± 2.90[a]
VII	176.29 ± 6.11	180.57 ± 5.93	182.14 ± 6.46	181.29 ± 6.88	192.71 ± 7.20	9.43 ± 2.69[a]

Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

Relative brain weight (%) significantly reduced in group III (0.75 ± 0.02) compared with groups I (0.86 ± 0.04) and II (0.82 ± 0.01). *Garcinia kola* however significantly increased relative brain weight (0.82% ± 0.03%) compared with BPA only (0.75% ± 0.02%) but no significant change was observed in group V when compared with group III (Figure 1).

3.2. Cognitive Function in Control and Experimental Groups

Discrimination and recognition indices were significantly reduced in BPA only compared with control. However, treatment with *Garcinia kola* and kolaviron significantly increased these indices compared with BPA only (Figure 2 and Figure 3).

Spontaneous alternation (a measure of spatio-temporal memory) reduced significantly in BPA only (64.62 ± 1.42) compared with control (81.79 ± 3.62). This was significantly increased by *Garcinia kola* and kolaviron in groups IV (87.33 ± 1.63) and V (71.34 ± 1.84), respectively when compared with group II (Figure 4).

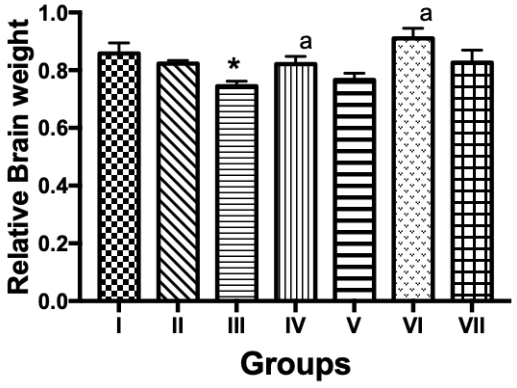

Figure 1. Relative brain weight in control and experimental groups. Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

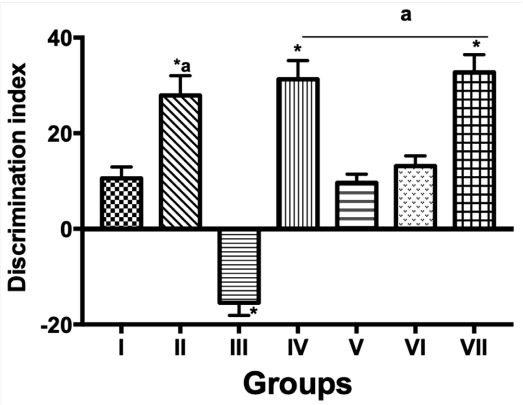

Figure 2. Novel object recognition test (discrimination index) in control and experimental groups. Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

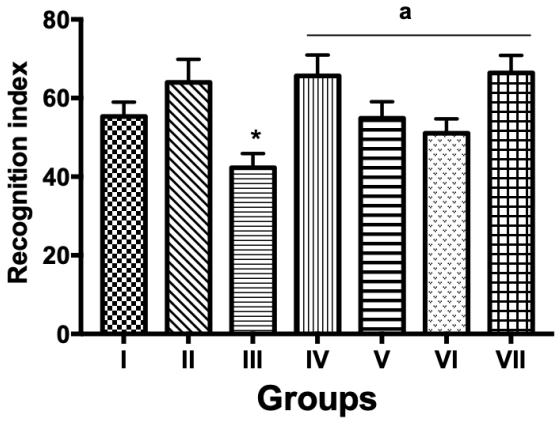

Figure 3. Novel object recognition test (recognition index) in control and experimental groups. Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

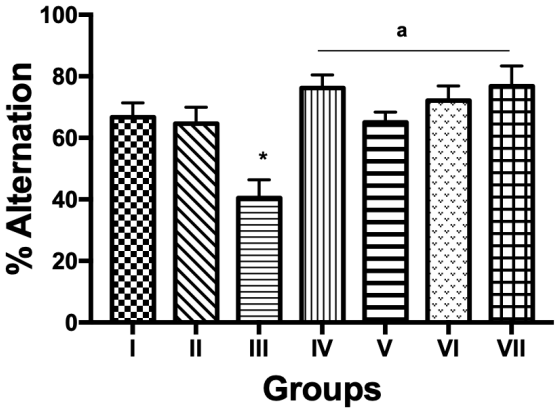

Figure 4. Y-maze test in control and experimental groups. Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

3.3. Oxidative and Nitrosative Stress in Control and Experimental Groups

Hippocampal MDA (3.76 ± 0.18 vs 2.23 ± 0.16) and nitrites (3.38 ± 0.34 vs 1.47 ± 0.07) levels increased significantly while SOD activity (0.69 ± 0.02 vs 0.87 ± 0.05) and GSH level (5.26 ± 0.39 vs 7.62 ± 0.45) reduced significantly in group III compared with control. *Garcinia kola* and kolaviron significantly reversed BPA effect on MDA, nitrites level, SOD and GSH activity (Table 2).

3.4. Inflammatory Markers in Control and Experimental Groups

Tumour necrosis factor-∝ (TNF-∝) and interlukin-6 (Il-6) levels in group III were significantly increased ($p < 0.05$) compared control (group I). Compared to group III, TNF-∝ values in groups IV and V, were reduced by 36.78% and 41.29% (Figure 5) while Il-6 levels in groups IV, V were also reduced by 29.85% and 25.58%, respectively (Figure 6).

Table 2. Oxidative stress indices in the hippocampus of control and experimental groups.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmoles/mg protein)	GST (U/mg protein)	Nitrites (µmoles/mg protein)	MDA (ηmoles/mg protein)
I	0.87 ± 0.04	15.88 ± 0.84	7.62 ± 0.45	0.115 ± 0.006	1.47 ± 0.07	2.23 ± 0.16
II	0.99 ± 0.06	15.08 ± 0.51	7.53 ± 0.26	0.157 ± 0.014	1.57 ± 0.08	2.28 ± 0.07
III	0.69 ± 0.02*	12.60 ± 0.46	5.26 ± 0.39*	0.116 ± 0.025	3.38 ± 0.34*	3.76 ± 0.18*
IV	0.83 ± 0.05[a]	14.15 ± 1.13	7.76 ± 0.86[a]	0.180 ± 0.009*[a]	1.64 ± 0.07[a]	2.08 ± 0.16[a]
V	1.58 ± 0.14*[a]	15.33 ± 0.26	7.54 ± 0.34[a]	0.135 ± 0.006*	1.74 ± 0.15[a]	2.47 ± 0.10[a]
VI	1.20 ± 0.04*[a]	13.25 ± 0.52	6.52 ± 0.46	0.187 ± 0.015*[a]	1.41 ± 0.13[a]	1.90 ± 0.17[a]
VII	1.09 ± 0.03*[a]	17.13 ± 0.85	10.16 ± 0.45*[a]	0.152 ± 0.009*[a]	1.91 ± 0.21[a]	2.07 ± 0.17[a]

Values are mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

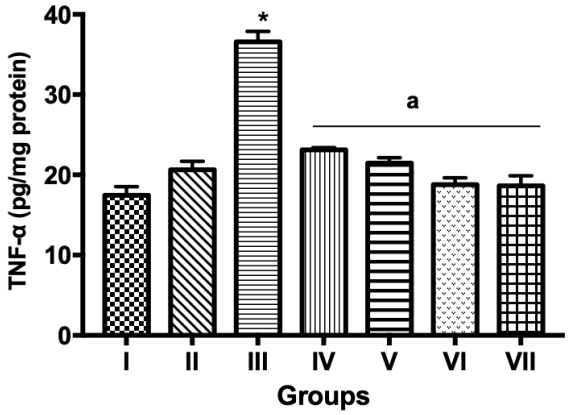

Figure 5. Tumour necrosis factor-alpha (TNF-∝) levels in the hippocampus of control and experimental groups. Values are Mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

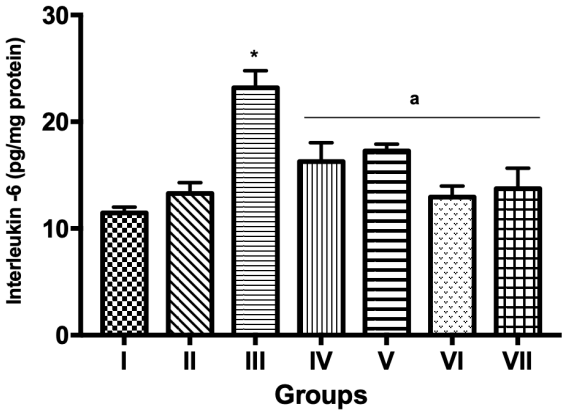

Figure 6. Interleukin 6 levels in the hippocampus of control and experimental groups. Values are Mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

3.5. Neurotransmitter Activity in Control and Experimental Groups

Acetylcholinesterase activity (μmoles/min/mg protein) reduced significantly in BPA groups treated with *Garcinia kola* (0.37 ± 0.03) and kolaviron (0.36 ± 0.01), respectively when compared with BPA only group (0.72 ± 0.07). Glutamic acid decarboxylase (GAD) activity (U/mg protein) did not differ significantly in all the experimental groups. Arginase activity increased significantly in groups IV and V relative to group III (Table 3).

4. Discussion and Conclusion

The brain is an organ with complex functions ranging from control of learning, memory, speech and language, and control of bodily organs. Control of learning, memory and behavior is associated with the hippocampus [34], and any damage to this organ results in the impairment of memory and cognition. The hippocampus also possesses estrogenic receptors [13] which would therefore make it susceptible to the toxic effects of BPA.

In this study, memory and cognitive function were impaired in the BPA only treatment group as the discrimination index and recognition index declined in this group compared with controls. This is consistent with the reports of Zhou *et al.* [35] who also reported a marked decrease in cognitive performance following exposure of experimental animals to BPA. Furthermore, BPA exposure has been reported to cause a decline in spatial learning and memory abilities as a result of mechanisms associated with reduced expression of glutamate receptors (NR2 and GluR1) in male rat hippocampus and primary visual cortex [36]. Studies have shown that cognitive dysfunction is often associated with significant changes in the levels and activity of several neurotransmitters, with the cholinergic system being mostly affected [37]. Exposure to BPA has been reported to induce choline toxicity resulting in decreased acetylcholine transferase activity [38] and increased acetylcholinesterase activity which leads to decreased availability of acetylcholine as a neurotransmitter for cognitive activity [39]. This study also observed increased acetylcholinesterase activity in the BPA only group, suggesting

Table 3. Neurotransmitter enzyme activity in the hippocampus of control and experimental groups.

Groups	Acetylcholinesterase (μmoles/min/mg protein)	GAD (U/mg protein)	Arginase (U/mg protein)
I	0.42 ± 0.01	11.21 ± 0.48	0.86 ± 0.07
II	0.40 ± 0.03	11.90 ± 0.74	0.86 ± 0.11
III	0.72 ± 0.07*	10.14 ± 0.17	1.12 ± 0.10*
IV	0.37 ± 0.03[a]	11.17 ± 1.16	0.87 ± 0.15[a]
V	0.36 ± 0.01[a]	11.62 ± 1.04	0.85 ± 0.09[a]
VI	0.35 ± 0.02[a]	10.38 ± 0.10	0.91 ± 0.22
VII	0.33 ± 0.04[a]	10.52 ± 0.94	0.76 ± 0.04[a]

Values are Mean ± SEM. * indicates values that are significantly different from controls. [a] indicates values that are significantly different from the BPA only treatment group.

the presence of cognitive and memory impairment in this treatment group. Furthermore, glutamic acid decarboxylase (GAD), an intracellular enzyme whose physiologic function is the decarboxylation of glutamate to gamma-aminobutyric acid (GABA) [40], was reduced in the BPA only treatment group, suggesting the likely presence of glutamate accumulation resulting in excitotoxicity and hence, cognitive and memory impairment [41]. Furthermore, relative brain weight was reduced in the BPA only treated animals suggesting impaired mental function [42] and hence the likely presence of memory and cognitive impairment in this group. Arginase is an enzyme that utilizes arginine as a substrate to generate ornithine and urea. It competes with nitric oxide synthase (NOS) for utilization of arginine [43]. When increased in neuronal samples, it often suggests a reduction in the supply of L-arginine required for the production of nitric oxide by nitric oxide synthase [44], resulting in increased formation of reactive oxygen species and neuroinflammation mediators [45]. This study also demonstrates increased hippocampal neuroinflammation likely via oxidative stress related mechanisms as arginase levels and activity were significantly increased in the BPA only treatment group. The presence of increased hippocampal oxidative and nitrite stress in the BPA only treatment group was confirmed as samples in the group also exhibited increased lipid peroxidation and depleted levels of intracellular antioxidants compared to controls. This increased hippocampal oxidative and nitrite stress was accompanied by increased hippocampal production of pro-inflammatory cytokines and hence neurotoxicity which was typified by elevated TNF-∝ and IL-6 [46].

Taken together the observations in the BPA (50 mg/kg) treatment group suggests BPA induces memory and cognitive impairment via impaired neurotransmitter enzymatic activity (decreased acetylcholine transferase and glutamic acid decarboxylase activity), increased oxidative and nitrite stress (increased lipid peroxidation and depletion of intracellular antioxidants) and neuroinflammation (elevated TNF-∝ and IL6) within the hippocampus. In addition to this, weight gain was also evident in the BPA only treatment group which may be ascribed to mechanisms previously described by Naomi *et al.* [47].

Oxidative damage and neuro-inflammation, have been described as the most probable reason for brain dysfunction as it, *i.e.*, the brain has been reported to be an organ with inadequate antioxidant defences to meet up with its high rate of oxygen free radical production [48]. In addition to this, the brain has also been reported to contain large amounts of phospholipids which are composed of polyunsaturated fatty acid side chains with a tendency for peroxidation by oxygen free radicals [49]. It therefore is likely that antioxidants, especially those of natural origin, may exert significant therapeutic effects against diseases, disorders, and toxicities (such as BPA induced neuronal toxicity) that have increased oxidative stress as being central to their aetiology.

This hypothesis was tested in this study using two well-established antioxidants, *Garcinia kola* seeds [15] and one of its most active bioflavonoids, kolaviron [50]. The results obtained in this study showed that memory and cognitive

impairments as well as reduced relative brain weight caused by BPA were prevented when the animals were co-treated with either *G. kola* or kolaviron. This protective effect may be attributed to the reported ability of *G. kola* [51] and kolaviron [52] to inhibit acetylcholinesterase activity (as observed in this study) and hence increase the availability of acetylcholine as a neurotransmitter for cognitive activity. Furthermore, glutamic acid decarboxylase (GAD) activity in the groups treated with BPA and either *G. kola* or kolaviron were comparable to controls suggesting an availability of this enzyme (which decreased in the BPA only treatment group) to decarboxylate glutamate to gamma-aminobutyric acid (GABA) [40], and hence prevent excitotoxicity as well as cognitive and memory impairment. The BPA-induced increase in arginase activity was also prevented in animals that were treated with either *G. kola* or kolaviron combined with BPA, suggesting an increase in the availability of arginine and hence neuronal nitric oxide synthase use of arginine for the production of nitric oxide, which has been observed to function as a retrograde neurotransmitter in synapses, mediate neural blood flow and facilitate intracellular signalling in order to maintain neuronal metabolic health and normal dendritic spine growth [53].

In addition to this, increased hippocampal lipid peroxidation and accumulation of reactive nitrite species which was observed in the BPA only treatment group was reduced following co-exposure to both BPA and either *G. kola* or kolaviron. This may likely be a result of the potentiation of the intracellular antioxidant defence mechanism which was also observed in the co-exposure groups. The production of hippocampal neuroinflammation mediators (TNF-∝ and Il-6) was also reduced in the animals treated with BPA and either *G. kola* or kolaviron possibly as a result of increased availability of arginine for nitric oxide production resulting neuronal health and well-being, increased potentiation of antioxidant activity and hence reduced stress.

Garcinia kola especially its seeds has been widely recognized to possess antioxidant properties, that is, they have been reported to detect and prevent oxidative propagation, stabilize any radical generated and thus reduce oxidative damage [15]. The effects of *G. kola* have been attributed to several different classes of compounds such as biflavonoids, benzophenones, benzofurans, benzopyran, vitamin E derivatives, xanthones, and phytosterols, which have been isolated from *G. kola*, many of which appear to only be found only in it. Some of these include garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, garcipyran and kolaviron [16]. These compounds have been well reported and recognized for their antioxidant, anti-inflammatory and neuroprotective activities, which may therefore account for the observation in the animals exposed to BPA and treated with *G. kola*. Amongst all the constituents of *G. kola*, the most extensively studied is kolaviron, to such an extent that it is often perceived by many studies as being its active principle [54]. It (kolaviron) has on its own been reported to exert diverse biological effects including antioxidant, anti-inflammatory and antigenotoxic effects [55] [56]. It is therefore not unlikely that the protection against BPA-induced weight gain, memory and cognitive impairments, and hip-

pocampal oxidative stress, depleted antioxidants and neuroinflammation by *G. kola* and kolaviron may be attributed to its reported antioxidant and (especially) anti-inflammatory potentials.

Garcinia kola seed on consumption possesses a bitter astringent taste and has been reported to occupy a pivotal position in African hospitality and ethnomedicine [57]. In Africa, it is also widely consumed recreationally. This study also evaluated the effects of *Garcinia kola* and kolaviron, on memory function and neuroinflammation mediators in the absence of BPA-induced toxicity. Observations seen in this study suggest that *G. kola* and kolaviron enhanced the activity of hippocampal detoxifying (GST) and antioxidant enzymes (especially kolaviron), and kept the activity of neurotransmitters and neuroinflammatory mediators at values comparable with controls suggesting the absence of hippocampal neuroinflammation. Body and relative brain weights were also kept relatively constant suggesting the benefits of *G. kola* and kolaviron in weight management [58] and prevention of learning impairment. Memory function appeared unimpeded following *G. kola* treatment and potentiated with kolaviron treatment which may perhaps be via mechanisms associated with its increased antioxidant potentiating activities (**Table 2**).

In conclusion, this study adds to the growing body of knowledge indicating the adverse effects that oral exposure to Bisphenol-A exerts on the brain which include impaired memory and cognitive functions as well as inducing increased neuroinflammation. This study also shows that *Garcinia kola* and kolaviron in Wistar rats mitigate bisphenol A induced memory and cognitive impairment, and neuroinflammation through mechanisms that can be associated with potentiation of antioxidant activities, suppression of lipid peroxidation and nitrite stress, increased activity of detoxifying enzymes, and inhibition of acetylcholinesterase and arginase activity in the hippocampus.

Conflict of Interest Statement

The authors declare no conflicts of interest regarding the publication of this paper.

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