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Genistein Mitigates the Gastro-Toxic Effects of Bisphenol A in Male Wistar Rats

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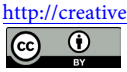

Abstract

Exposure to Bisphenol A (BPA) worldwide is on the increase. Its toxicities in various tissues expressing estrogen receptors have been reported. However, limited information exists on its effects on the gastric tissue. This study therefore investigated the gastric effects of BPA exposure and the likely ameliorative potential of genistein, a phytoestrogen antioxidant, known to interact with estrogen receptors. Eighty-four male Wistar rats were randomly divided into 6 groups (n = 14) and orally treated for 28 days. Group I-IV received distilled water (0.2 ml), corn oil (3 ml/kg), BPA (50 mg/kg) and genistein (50 mg/kg) respectively. Group V was pre-treated daily with BPA one hour prior to genistein administration while Group VI was pre-treated daily with genistein one hour prior to BPA treatment. Thereafter, blood was collected into heparinized bottles for hematological analysis. Gastric juice was collected for pH and electrolytes determination. Gastric tissue was excised and analyzed for superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde, nitric oxide (NO), mucin, parietal and mucous cell counts, and histology. The BPA only treatment group exhibited significant increases ($p < 0.05$) in white blood cell count, neutrophil-lymphocyte ratio, mucin concentration, NO, and malondialdehyde while gastric juice pH, bicarbonate, SOD, and GSH levels were reduced compared to control. The gastric mucosa also showed pathologies consistent with inflammation. Genistein pre-and post-treatment in BPA exposed rats significantly ($p < 0.05$) ameliorated these effects of BPA. However, some signs of gastric inflammation were still evident in the mucosal samples. Bisphenol A induces gastro-toxicity by increasing gastric acidity, reducing gastric juice bicarbonate level and disrupting pro-oxidant/antioxidant balance. Genistein pre- and post-treatment ameliorated these gastro-toxic effects of Bisphenol A via gastroprotective, antioxidant and anti-inflammatory mechanisms.

Keywords

Bisphenol A, Genistein, Stomach, Gastro-Protection, Gastric Impairment

1. Introduction

Gastric health is dependent on the balance between aggressive factors such as hydrochloric acid, pepsin, oxidative markers and the protection provided by several gastric mucosal defense mechanisms which include pre-epithelial, epithelial and sub-epithelial mechanisms and anti-oxidant systems [1] [2]. These gastric mucosal defense mechanisms are the first line of protection against ingested food material, chemicals and environmental contaminants [3].

Industrialization worldwide has led to the development of a massive number of chemicals used in the plastic industry for manufacturing containers for diverse food products [4]. This increased production of plastics has been observed to constitute an environmental pollution problem. More importantly, the continuous intentional and unintentional consumption of plastic particles released mainly from plastic containing packaging that are in direct contact with food and beverages is now an emerging human health risk [4].

Bisphenol A (BPA) is a man-made organic chemical widely used in the production of polycarbonate plastics and epoxy resins which are incorporated in numerous consumer products like food and water container bottles, resin lining of food and beverage cans, dental sealants and toys [5]. Exposure of humans to BPA has been largely attributed to intake of BPA-contaminated food products [6]. Bisphenol A has been observed to leach into food products and thus contaminate them when consumables stored or placed in BPA containing plastics are subjected to heat (≥70˚C) or acidity [7]. Human exposure to BPA has been confirmed by its presence in plasma and urine [8] and this exposure has been associated with several disease conditions including neural impairment and repro-toxicity [9], hypertension and cardiovascular diseases [10], diabetes mellitus and obesity [11], and cancer [12]. Bisphenol A is often categorized as an endocrine disrupting agent as it disrupts hormonal systems by interfering with the synthesis, secretion, transport, binding and or elimination of natural hormones from the body [13]. It has been reported to induce toxicity in the body by interacting with estrogen receptors in different tissues [14], disturbing pro-oxidant/anti-oxidant balance [15] and inducing DNA damage [16] amongst other mechanisms. Gastric cells especially gastric parietal and neuronal cells have been reported to express both alpha (α) and beta (β) estrogen receptors (ERs) [17] and hence are likely to react to BPA. The toxicity of BPA in various tissues and organs of the body has been reported. However, there is paucity of information concerning its effects on the stomach.

Genistein is an isoflavone compound derived from soy products and is known to possess estrogenic, anticancer, antioxidant, antineoplastic and antiangiogenic

activities [18] [19]. Due to its heterocyclic phenol rings, it is capable of binding both ERs (α and β) and sex-hormone binding globulins [20]. Studies have also demonstrated that genistein exerts gastro-protective effect via its ability to interact with gastric estrogen receptors [21] [22]. This study is therefore designed to investigate the effects of BPA on gastric mucosal integrity in the presence or absence of estrogenic receptor agonist, genistein.

2. Materials and Methods

2.1. Animals and Groupings

Eighty-four (84) male Wistar rats (140 - 160 g) were housed in well aerated cages, maintained on standard rat pellets allowed access to water *ad libitum*, exposed to natural atmospheric conditions, room temperature (25˚C - 27˚C) and alternating day and night cycles. Animals were acclimatized to laboratory conditions for 14 days prior to any experimental procedures. All experimental procedures were carried out according to the guidelines of the Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan, Nigeria and that of the Guide for the Care and Use of Laboratory Animals [23], published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA. Thereafter animals were randomly divided into six groups of 14 rats each as follows: Group I (control) was treated with distilled water (0.2 ml), animals in group II (vehicle group) received corn oil at 3 ml/kg [24] while animals in Group III and IV received Bisphenol A (50 mg/kg) [25] and genistein (50 mg/kg) [21] respectively. Animals in group V were pre-treated daily with Bisphenol A (50 mg/kg) one hour prior to genistein (50 mg/kg) administration while Group VI animals were pre-treated daily with genistein (50 mg/kg) one hour prior to Bisphenol A (50 mg/kg) treatment. Bisphenol A and Genistein were constituted separately in corn oil. All treatments were administered orally for 28 days in each group, respectively.

2.2. Blood Collection and Haematological Analysis

Blood was collected from five rats per group through cardiac puncture under mild sodium thiopental anaesthesia (50 mg/kg *i.p.*) into heparinized bottles. The collected heparinized blood samples were analyzed for red blood cell count and total white blood cell count using a heamocytometer. Differential white blood cell (neutrophils and lymphocytes) count was evaluated using the Wright's stain [26], packed cell volume was evaluated as described by Billett, [27] while heamoglobin concentration was assessed using Sahli's method.

2.3. Evaluation of Gastric Mucin Concentration

After blood collection, the animals were opened up by an abdomino-pelvic incision to excise the stomach and the gastric barrier mucous was estimated as described by Corne *et al.* [28]. Briefly, the excised stomachs from each group were soaked for 2 hours in 0.1% Alcian blue dissolved in buffer solution containing

0.1M sucrose and 0.05M Sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10 ml aliquots of 0.5M $MgCl_2$ for 2 hours. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase was measured at 605 nm using a spectrophotometer.

2.4. Evaluation of Gastric Secretion Acidity and Electrolytes

Post-treatment, animals (n = 5) were subjected to surgery under light ether anaesthesia according to Brodie and Knapp [29]. Briefly, under light ketamine anaesthesia (40 mg/kg) the abdomen of each animal was opened through a mid-line epigastric incision, and the stomach was exposed. The pyloric end was identified and a fine thread was tied round the pylorus, care was taken to avoid inclusion of adjacent blood vessels. The wound was then closed with catgut and the animal returned to its cage where it subsequently regained consciousness. After 4 hours the animal was again anaesthetized, opened up and stomach was removed after clamping the pylorus and the lower end of the oesophagus. 4-hour gastric secretion was washed out with 5 ml distilled water into a graduated test tube and centrifuged at 3000 rpm for 10 min at room temperature [30]. The clear supernatant collected was analyzed for gastric electrolytes (Na^+, K^+, HCO^{3-}, Cl^-) using commercially available electrolyte assay kits and total gastric acidity using a pH meter (Hanna Instruments, USA).

2.5. Evaluation of Gastric Tissue Antioxidants

The excised stomachs were weighed and then homogenized on ice with ice-cold 0.1 M phosphate buffer (1:4 w/v, pH 7.4). The homogenates obtained was centrifuged at 2500 rpm for 10 min at 4˚C and the resulting supernatants were frozen at −4˚C until use. Aliquots of the supernatants were thereafter analysed for superoxide dismutase (SOD) [31], reduced glutathione (GSH) [32], malondialdehyde (MDA) [33] and nitric oxide (NO) [34], respectively.

2.6. Histological Studies

Gastric tissues from the remaining 4 animals in each group were also excised, fixed in 10% formalin and evaluated for mucous cell count using Periodic Acid Schiff (PAS) reaction technique while parietal cell count, gastric structure and architecture was evaluated using the Haematoxylin and Eosin staining technique [3].

2.7. Statistical Analysis

Data are presented as mean ± standard error of mean (SEM) and statistical analysis was carried out using Graph Pad Prism 7.0 (GraphPad Software Inc., USA). Statistical significance was assessed using one-way analysis of variance (ANOVA) and pairwise comparisons were conducted using Newman Keul's

post-hoc test at $p < 0.05$.

3. Results

3.1. Haematological Parameters in Control and Experimental Groups

The values for total red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (HGB) were not significantly different between control and all other experimental groups (Table 1). However, total white blood cell (WBC) (mm^{-3}) in vehicle (corn oil) (3288.30 ± 87.50), Bisphenol A (BPA) only (3250.26 ± 170.78), BPA + GEN (3825.26 ± 329.46) and GEN + BPA (3712.50 ± 394.43) treatment groups were increased compared to control (2600.10 ± 133.05). Compared to BPA only treatment values, WBC values in the BPA + GEN and GEN + BPA treatment groups were 17.69% and 14.22% increased, respectively. Differential neutrophil and lymphocyte counts were reduced in the BPA + GEN and GEN + BPA treatment groups compared to control, vehicle (corn oil) and BPA only treatment groups respectively. The neutrophil-lymphocyte ratio (NLR) was also increased in the BPA + GEN (0.60 ± 0.06) and GEN + BPA (0.64 ± 0.04) treatment groups compared to control (0.42 ± 0.02), vehicle (corn oil) (0.41 ± 0.02), and BPA only (0.45 ± 0.04) treatment groups, respectively (Table 1).

3.2. Gastric Mucin Concentration, Antioxidants Status and Nitric Oxide Levels in Control and Experimental Groups

Gastric mucin concentration (mg/ml) was significantly increased ($p < 0.05$) in the BPA only treatment group (0.21 ± 0.01) compared to control (0.14 ± 0.01), vehicle (0.15 ± 0.03) treated, GEN only (0.15 ± 0.02), BPA + GEN (0.13 ± 0.01) and GEN + BPA (0.14 ± 0.03) treatment groups, respectively (Figure 1). The values for superoxide dismutase (SOD) were significantly reduced ($p < 0.05$) in the BPA only treatment group compared to control and vehicle groups respectively. However, the BPA + GEN and GEN + BPA treatment groups exhibited a

Table 1. Effects of Bisphenol A (BPA) and Genistein (GEN) on haematological parameters.

	CONTROL	CORN OIL	BPA	GEN	BPA + GEN	GEN + BPA
RBC ($\times 10^6$ mm^{-3})	6.84 ± 0.22	7.42 ± 0.26	6.87 ± 0.22	7.01 ± 0.24	6.85 ± 0.19	6.57 ± 0.37
PCV (%)	40.80 ± 1.39	43.80 ± 1.56	41.01 ± 1.38	41.40 ± 1.60	40.03 ± 1.48	39.04 ± 1.64
Haemoglobin (g/dl)	13.62 ± 0.39	14.64 ± 0.60	13.64 ± 0.53	13.68 ± 0.52	13.5 ± 0.58	12.94 ± 0.59
Total WBC (mm^{-3})	2600.10 ± 133.05	3288.30 ± 87.50*	3250.26 ± 170.78*	2537.40 ± 190.80	3825.26 ± 329.46*	3712.50 ± 394.43*
Neutrophils (%)	28.20 ± 1.02	28.01 ± 1.14	29.80 ± 1.66	28.40 ± 1.81	36.20 ± 2.48^{a*†}	37.20 ± 1.39^{a*†}
Lymphocytes (%)	68.20 ± 1.36	68.40 ± 1.36	67.01 ± 1.73	67.40 ± 2.09	61.05 ± 2.30^{a*†}	58.20 ± 1.36^{a*†}
NLR	0.42 ± 0.02	0.41 ± 0.02	0.45 ± 0.04	0.43 ± 0.04	0.60 ± 0.06^{a*†}	0.64 ± 0.04^{a*†}

Values are Mean ± SEM. *indicates values that are significant different from control ($p < 0.05$). †indicates values that are significant different from vehicle (corn oil) treatment group. ^{a}indicates values that are significant different from Bisphenol A only treatment group.

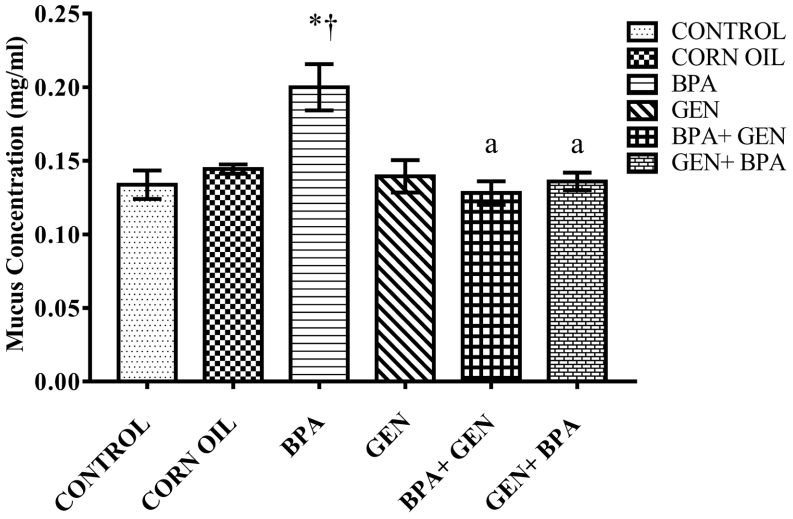

Figure 1. Effects of Bisphenol A (BPA) and Genistein (GEN) on mucus concentration (mg/ml) in the stomach tissues of rats. Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. [a] indicates values that are significant different from Bisphenol A only treatment group.

46.42% and 41.60% increase ($p < 0.05$) in SOD values compared to BPA treatment group. The SOD values obtained in the BPA + GEN and GEN + BPA treatment groups were also comparable to values obtained in the control, vehicle and GEN only treatment groups, respectively (**Figure 2**). Reduced glutathione (GSH) (µg/ml) values declined ($p < 0.05$) in the BPA treatment compared to control and vehicle group respectively. Values for GSH obtained in the BPA + GEN and GEN + BPA were also significantly reduced ($p < 0.05$) compared to controls. However, animals in the genistein only treatment group exhibited a significant increase ($p < 0.05$) in GSH levels compared to BPA only treatment group (**Figure 3**). Malondialdehyde (MDA) (U/µg protein), a marker of lipid peroxidation, was significantly increased ($p < 0.05$) in the BPA only treatment group compared to all other treatment groups respectively, except the GEN + BPA treatment group. Values observed in the GEN, BPA + GEN and GEN + BPA showed MDA levels that were 10.54%, 30.15% and 8.67% reduced compared to BPA only treatment group (**Figure 4**). Nitric oxide (NO) (µmol/g) observed in the BPA only treatment group exhibited a 19.44% and 44.02% increase compared to control and vehicle treatment groups, respectively. Compared to values obtained in the BPA only group, NO values in the GEN, BPA + GEN and GEN + BPA were 50.36%, 29.34% and 9.02% reduced, respectively (**Figure 5**).

3.3. Electrolyte Composition and Acidity of Gastric Secretion in Control and Experimental Groups

Sodium ion concentration (mmol/L) observed in the gastric secretions collected from control and experimental were not significantly different across the groups. However, potassium ion concentrations (mmol/L) were significantly reduced (p

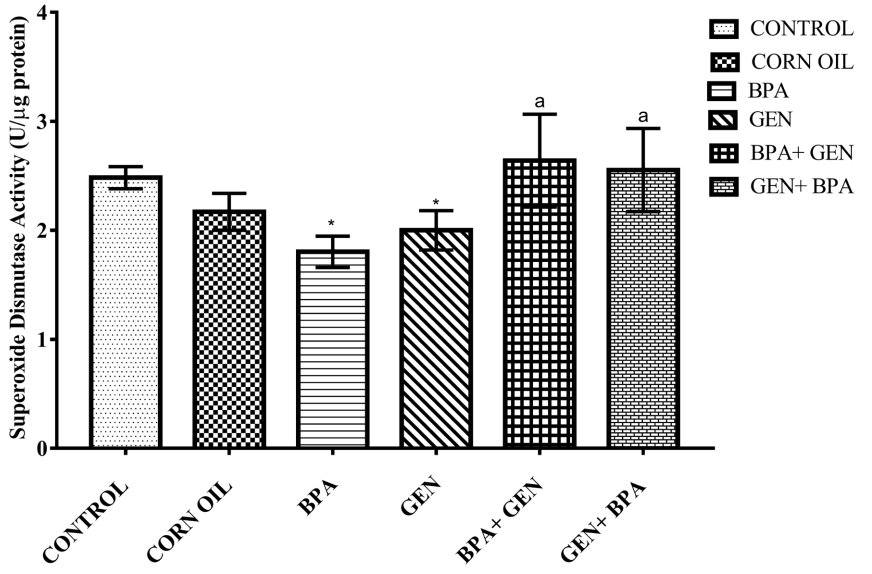

Figure 2. Effects of Bisphenol A (BPA) and Genistein (GEN) on superoxide dismutase activity (U/µg protein) in the stomach tissues of rats. Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). [a] indicates values that are significant different from Bisphenol A only treatment group.

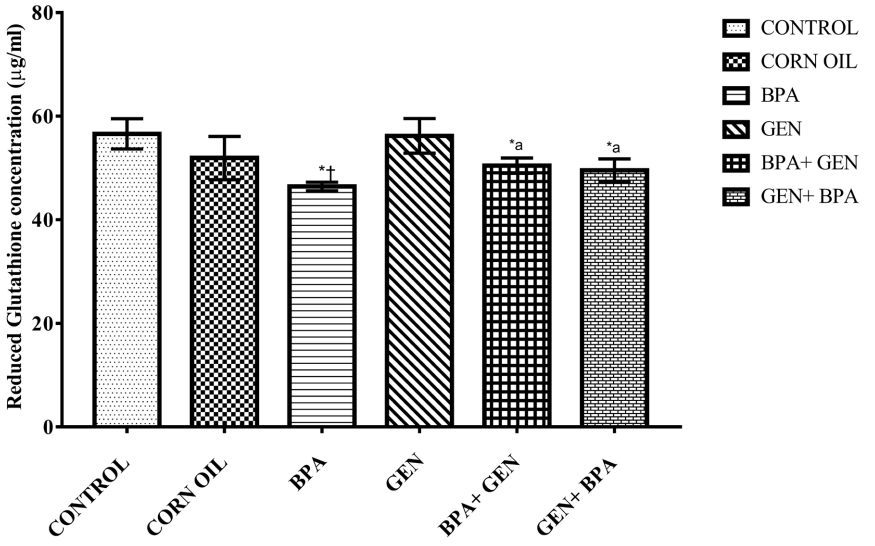

Figure 3. Effects of Bisphenol A (BPA) and Genistein (GEN) on reduced glutathione concentration (µg/ml) in the stomach tissues of rats. * Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). [a] indicates values that are significant different from Bisphenol A only treatment group.

< 0.05) in the vehicle and GEN + BPA treatment groups compared to control. Sodium: Potassium ratio (Na:K) was also not significantly different across all groups (Table 2). Bicarbonate levels (mmol/L) reduced in the BPA only treatment group compared to control and vehicle only treatment groups, respectively. Bicarbonate values obtained in the GEN (57.82 ± 8.15), BPA + GEN (59.10 ± 8.48) and GEN + BPA (35.56 ± 8.11) were significantly increased ($p < 0.05$)

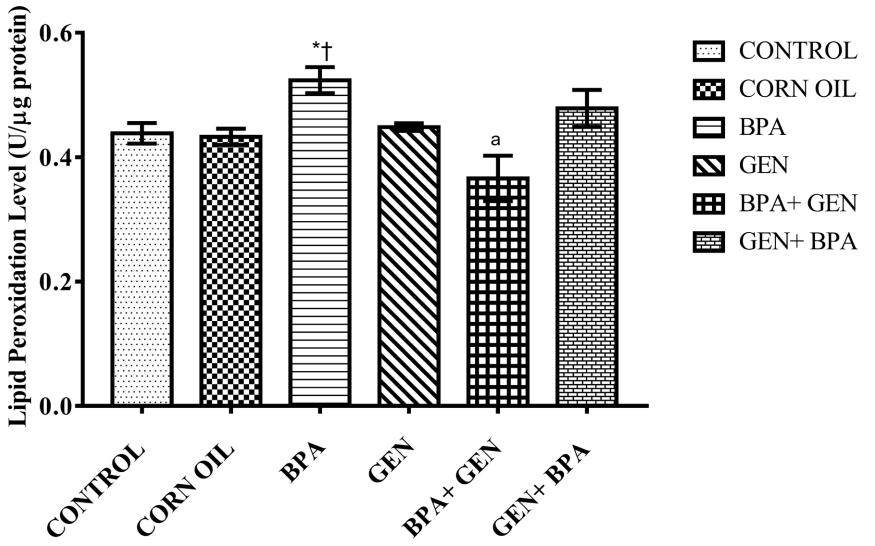

Figure 4. Effects of Bisphenol A (BPA) and Genistein (GEN) on lipid peroxidation level (U/µg protein) in the stomach tissues of rats. Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. [a] indicates values that are significant different from Bisphenol A only treatment group.

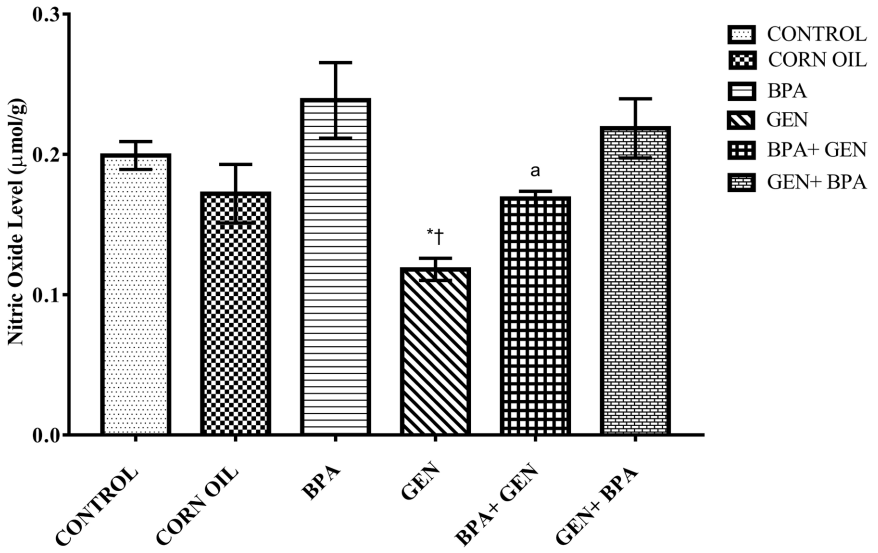

Figure 5. Effects of Bisphenol A (BPA) and Genistein (GEN) on nitric oxide level (µmol/g) in the stomach tissues of rats. Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. [a] indicates values that are significant different from Bisphenol A only treatment group.

compared to BPA only (16.22 ± 0.83), respectively (Table 2). Compared to control values, chloride ion was reduced in all experimental groups with significant reductions ($p < 0.05$) observed in the BPA only, GEN only, BPA + GEN and GEN + BPA treatment groups respectively (Table 2). The pH value of gastric secretion in the BPA only group was significantly reduced ($p < 0.05$) compared

Table 2. Effects of Bisphenol A (BPA) and Genistein (GEN) on gastric juice electrolytes.

Treatment groups	Sodium (mmol/L)	Potassium (mmol/L)	Na:K	Bicarbonate (mmol/L)	Chloride (mmol/L)
CON	38.06 ± 2.52	6.00 ± 1.11	7.27 ± 1.43	40.38 ± 6.16	16.76 ± 3.57
CORN	32.54 ± 0.93	3.52 ± 0.22*	9.40 ± 0.70	57.22 ± 2.93	12.15 ± 1.55
BPA	40.40 ± 4.942	4.29 ± 0.77	10.40 ± 1.70	16.22 ± 0.83*†	10.16 ± 1.40*
GEN	36.48 ± 0.90	4.34 ± 0.70	9.14 ± 1.19	57.82 ± 8.15 [a]	8.87 ± 0.98*
BPA + GEN	36.90 ± 0.68	4.18 ± 0.63	9.61 ± 1.36	59.10 ± 8.48[a]	12.83 ± 1.77
GEN + BPA	36.46 ± 2.81	3.77 ± 0.34*	9.72 ± 0.28	35.56 ± 8.11[a]	9.10 ± 0.75*

Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. [a] indicates values that are significant different from Bisphenol A only treatment group.

to control and vehicle (corn oil) treated group. The pH of the gastric secretion from the GEN only, BPA + GEN and GEN + BPA exhibited 89.35%, 86.99% and 27.87% increases compared to BPA only treatment group (Figure 6).

3.4. Parietal and Mucous Cell Counts in Gastric Sample of Control and Experimental Groups

Parietal cell count (cells/field) in all treatment were comparable except for values observed in the vehicle (corn oil) and GEN + BPA treatment groups which were significantly reduced ($p < 0.05$) compared to control. Compared to the control group, mucous cell count (cell/field) was reduced in all experimental groups with significant reductions ($p < 0.05$) noted in the GEN only and BPA + GEN treatment groups respectively (Table 3).

3.5. Structural Evaluation of Gastric Samples in Control and Experimental Groups

The gastric mucosa of the control group (group I) had normal architecture with preserved mucosa epithelial cell layer (a), the mucosa layer showed no infiltration of the gastric glands and lamina propria. The submucosal layers appear normal and are not infiltrated by inflammatory cells (d), the circular muscle layer (c) also appears normal (Figure 7(I)). Animals in the vehicle (corn oil) treatment group (group II) exhibited gastric samples with normal architecture that had preserved mucosa epithelial cell layer (a). The mucosa layer of samples in this group showed no infiltration of the gastric glands and lamina propria. The submucosal layers seen were also not infiltrated by inflammatory cells. The circular muscle layer appeared normal, however, mild vascular congestion (d) was observed (Figure 7(II)). The BPA only treatment group (group III) exhibited gastric samples with poor architecture, the mucosa epithelial cells layer shows moderately denudated layer. Infiltration of the gastric glands and lamina propria (a) was observed in the mucosa layer and submucosal layers (b) respectively (Figure 7(III)). The genistein only treatment group (group IV) showed

Table 3. Effects of Bisphenol A (BPA) and Genistein (GEN) on parietal and mucous cell counts (cells/field) in the stomach tissues of rats.

Treatment groups	Parietal Cell Count (cells/field)	Mucous Cell Count (cells/field)
CON	338.03 ± 17.47	529.30 ± 19.41
CORN	279.70 ± 20.79*	459.01 ± 25.87
BPA	338.30 ± 34.45	489.03 ± 30.02
GEN	381.03 ± 13.11	259.70 ± 76.21^{a*†}
BPA + GEN	344.30 ± 26.77	422.30 ± 34.05*
GEN + BPA	250.30 ± 28.79*	493.05 ± 58.77

Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. ^{a} indicates values that are significant different from Bisphenol A only treatment group.

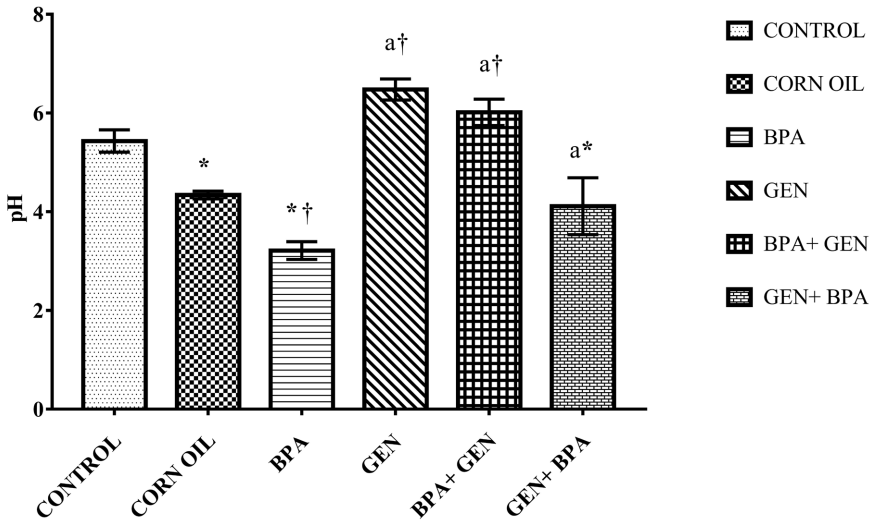

Figure 6. Effects of Bisphenol A (BPA) and Genistein (GEN) on gastric juice pH in the stomach tissues of rats. Values are Mean ± SEM. * indicates values that are significant different from control ($p < 0.05$). † indicates values that are significant different from vehicle (corn oil) treatment group. ^{a} indicates values that are significant different from Bisphenol A only treatment group.

gastric samples with normal architecture, the mucosa epithelial cells layer was well preserved (a), the mucosa layer showed no infiltration of the gastric glands and lamina propria. The submucosal layers appear normal despite the presence of mild infiltration by inflammatory cells (b). The circular muscle layers also appeared normal (c) (**Figure 7(IV)**). Gastric samples from group V (BPA + GEN) exhibited moderate architecture with mucosa epithelial cell that were also moderately preserved. The mucosa layer in this group showed mild infiltration of the gastric glands and lamina propria (a) and vascular congestion (b) in the submucosal layer (**Figure 7(V)**). Sample from group VI (GEN + BPA) exhibited poor gastric architecture, and there are focal areas of ulcer on the gastric mucosa epithelial cells

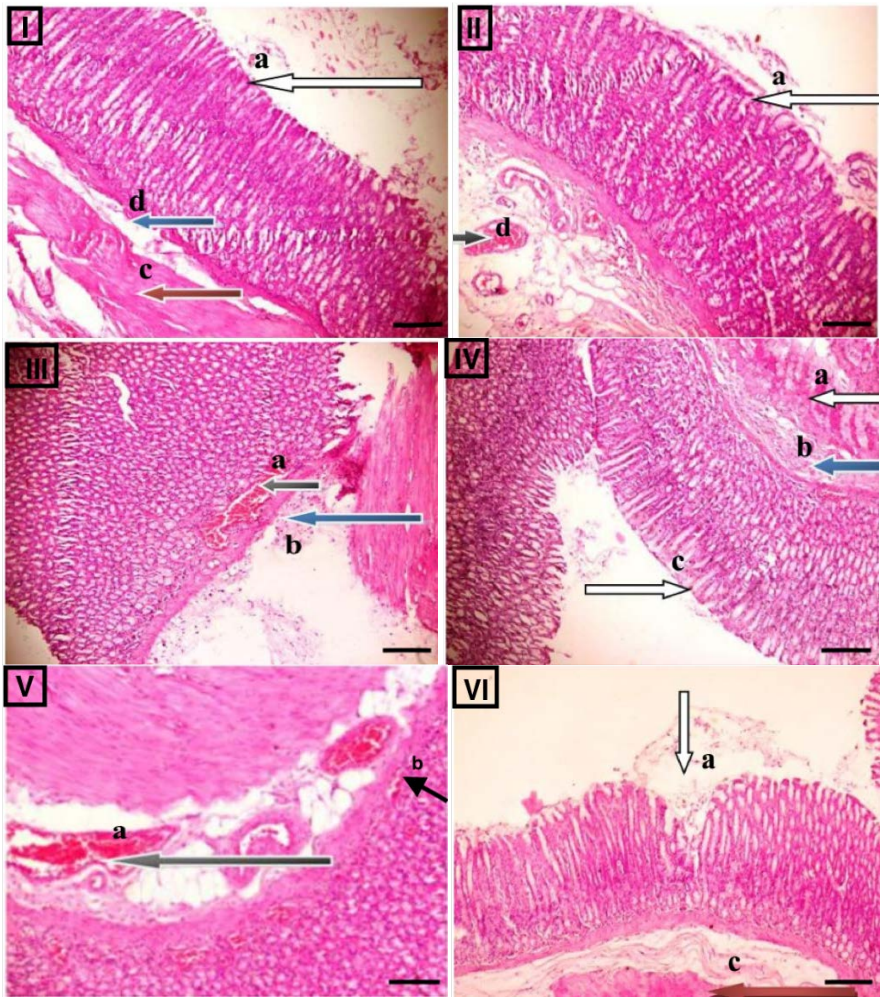

Figure 7. (I-VI) Photomicrograph of stomach samples in control and experimental groups (×400, Scale bar 50 μm). Section shows controls with normal architecture with preserved mucosa epithelial cell layer (a), the mucosa layer showed no infiltration of the gastric glands and lamina propria. The submucosal layers appear normal and are not infiltrated by inflammatory cells (d), the circular muscle layer (c) also appears normal (I). Vehicle (corn oil) treated also exhibited normal architecture that had preserved mucosa epithelial cell layer (a) The mucosa and submucosa layers showed no infiltration by inflammatory cells. The circular muscle layer is normal, however, mild vascular congestion (d) is observed (II). The BPA only treatment group showed samples with poor architecture, the mucosa epithelial cells layer shows moderately denudated layer. Infiltration of the gastric glands and lamina propria (a) was observed in the mucosa layer and submucosal layers (b) respectively (III). Genistein only treatment group (group IV) exhibited samples with normal architecture, well preserved mucosa epithelial cells layer (a), the mucosa layer showed no infiltration. The submucosal layer was normal despite the presence of mild infiltration by inflammatory cells (b). The circular muscle layers also appeared normal (c) (IV). Sections from the BPA + GEN group exhibited moderate architecture with mucosa epithelial cell that were also moderately preserved. The mucosa layer showed mild infiltration of the gastric glands and lamina propria (a) while vascular congestion (b) was seen in the submucosal layers (V). Sections from the (GEN + BPA) exhibited poor gastric architecture, and focal areas of ulcer on the gastric mucosa epithelial cells layer (a). The mucosa layer shows no infiltration of the gastric glands and lamina propria, the circular muscle layer (c) also appeared normal (VI).

layer (a). The mucosa layer shows no infiltration of the gastric glands and lamina propria and the circular muscle layer appeared normal (c) (**Figure 7(VI)**).

4. Discussion

The increase in the number of chemicals produced following increased industrialization will continue to be of concern to human health as continuous and unintentional consumption of these chemicals has been suggested to be the root cause of many present-day disease conditions. Bisphenol A, an endocrine disruptor, is one of such chemicals that humans may be unintentionally exposed to, being a constituent of plastic and epoxy resin-made food containers. In assessing the toxicological effects of a substance, the hematological profile following exposure to the substance remains the frontline indicator for identifying toxicity. According to guidelines issued by the US environmental protection agency (EPA), the proposed values for the no-observed-adverse-effect-level (NOAEL) and the lowest-observed-adverse-effect-level (LOAEL) for BPA exposure has been set at 5 mg/kg body weight and 50 mg/kg body weight respectively [35]. This study utilized the LOAEL dose in which studies on the effects of BPA on red cell indices have reported no alterations at doses of ≤50 mg/kg [36] However, treatments with BPA at higher doses have been associated with marked reductions in red cell indices. This study, which had experimental animals being treated with BPA at 50 mg/kg, is therefore consistent with other reports as red cell indices (RBC count, PCV and haemoglobin) were not significantly different compared to controls (**Table 1**). However, white cell indices (total WBC, differential neutrophil and lymphocyte counts) were elevated in the BPA exposed groups suggesting an activated immune response which is consistent with previous reports that have also noted this observation albeit with no change in red cell indices [36]. While systemic WBC level is considered potential predictive marker for pathogenesis [37], the Neutrophil-Lymphocyte Ratio (NLR) has been reported to be an indicator of inflammatory status of a subject [38]. Hence it is not unlikely that the increased WBC counts, neutrophil, NLR, and reduced lymphocytes observed in the BPA pretreated GEN (group V) and BPA post-treated GEN (group VI) animals may be due to activation of inflammatory processes that has been reported to occur following exposure to BPA. Observations from the results on haematological perturbation following genistein treatment either post- or pre-BPA treatment did not attenuate and restore WBC count and NLR to control levels (**Table 1**).

The integrity of the gastric mucosa has been reported to be dependent on a variety of factors which include mucus-alkaline secretion and the activity of gastric antioxidant enzymes such as SOD, GPx that protect the stomach against exogenous and endogenous irritants [39]. The mucus-bicarbonate barrier is regarded as the first line of defence in the protection of the gastric mucosa [1]. The mucus cells of the gastric epithelium produce a viscoelastic gel, mucus, and small amounts of bicarbonate to form a pre-epithelial continuous mucus-bicarbonate

barrier against gastric erosion and invasion. The mucus produced also reduces the shear stresses on the gastric epithelium and contributes to barrier function through various mechanisms, which include binding to bacteria thus preventing epithelial colonization and retarding diffusion of agents that can damage the epithelial surface e.g. acid secretion [3]. Furthermore, damage from the highly acidic conditions in the gastric lumen to the epithelium is prevented by bicarbonate ion, produced by mucus cells and in gastric juice, which serves to neutralize the acid and thus present a neutral pH along the epithelial membrane [40]. This study shows that exposure to oral BPA can lead to a decrease in gastric juice pH (increased acidity) (**Figure 6**) and decreased bicarbonate secretion (**Table 2**) resulting in an imbalance between gastro-aggressive and gastro-protective factors and thus predispose the gastric epithelium to erosion and inflammation. This may account for the erosion of the gastric epithelium, infiltration of gastric glands and inflammation observed in the gastric samples obtained from the BPA only treated group (**Figure 7(III)**). The increased mucus (evaluated as mucin) observed in the BPA only group (**Figure 1**) may likely be a result of direct activation of gastric mucus cells to produce more mucus to counteract the observed BPA induced increase in gastric juice acidity (**Figure 6**). This hypersecretion of mucus may have upset the balance between mucous cell exhaustion and replacement, thus accounting for the slight reduction in mucus cell counts observed in the BPA only treatment group (**Table 3**). Parietal cell count in the BPA only treatment group was however unchanged compared to control (**Table 3**) suggesting that BPA may lower gastric juice pH through non-genomic pathways *i.e.* in a manner that does not disrupt parietal cell mass.

There are several studies that have indicated that oral exposure to BPA inhibits intestinal motility and mucosal mucin secretion [41], induces apoptosis and inhibition of intestinal mucosa cell proliferation, impairs the intestinal barrier, increases intestinal permeability [42], and affects the neurochemical coding of neuronal cells and nerves in the ENS [43]. Its effects on the stomach are however, sparse, scarce and not fully elucidated [44]. It has however been stated that since there is a constant interplay between signals that control motility and secretory activities between the stomach and intestines, it is likely that the effects of BPA on the intestines may replicate itself in the stomach [45]. More so that the effects of BPA on the intestines are mediated by binding to estrogen receptors (α and β) [45] which have also been identified in the gastric epithelium. It is therefore not unlikely that the gastro-aggressive effects observed in the BPA only treatment may be ascribed to gastric estrogen receptor agonist activities exerted by BPA. Treatment with genistein alone or genistein either after BPA exposure or before BPA exposure in this study, appeared to suppress gastro-aggressive factors and potentiate gastroprotective factors as increased gastric juice pH (reduced acidity) in these treatment groups (**Figure 6**) was accompanied by increased gastric juice bicarbonate secretion (**Table 2**) and no change in mucus secretion (**Figure 1**). Furthermore, gastric samples from these treatment groups

(BPA + GEN and GEN + BPA) exhibited characteristics that appear to be less severe than that seen in the BPA treatment group (**Figure 7(V)**, **Figure 7(VI)**), suggesting a likely protective effect being exhibited by genistein on BPA-induced gastric impairment. It is however interesting that parietal cell count was reduced in the vehicle and GEN + BPA treatment groups while mucous cells were observed to be reduced in the GEN and BPA + GEN treatment groups respectively (**Table 3**). This however did not seem to impair the gastroprotection observed in these treatment groups. Genistein, a phytoestrogen and tyrosine-specific protein kinase inhibitor [46] that binds to both estrogen receptors, has been shown to possess anti-neoplastic, anti-inflammatory, anti-oxidant, and anti-atherogenic properties [47]. In rodents with H. pylori-induced gastropathy, it has also been reported to exert gastroprotective effects via a reduction of pro-inflammatory mediators, nuclear receptor NF-κB expression and gastric mucosal apoptosis [47]. The similarity between the structure of genistein and estrogen, especially estradiol, suggests that it binds to estrogen receptors in estrogen responsive tissues. However, studies have shown it maybe a weak estrogen agonist [48] and thus may also present with estrogen antagonistic effects [49]. Gastroprotection ascribed to genistein has also been attributed to its ability to reduce gastric acid secretion and suppress TNF-alpha and cytokine-induced neutrophil chemoattractant (CINC)-1, which are inflammatory cytokines related to gastric ulceration [21]. Given these aforementioned effects of genistein, the gastroprotective mechanism against BPA induced gastro-toxicity observed in this study may therefore be attributed to the interaction of genistein with gastric estrogen receptors.

Gastric antioxidants are an essential component of the gastrointestinal defense system. Their ability to scavenge free radicals is known to play an important role in the prevention of gastric lesions [50]. Bisphenol A on the other hand, is known to induce free radical generation [51] and thus result in a leftward shift in the prooxidant/antioxidant equilibrium [52] and increased inflammation [53]. This ability of BPA to increase oxidative stress and result in inflammation was also observed in the BPA only treatment group which exhibited an increase in gastric lipid peroxidation (**Figure 4**), depleted antioxidants (**Figure 2** and **Figure 3**) and increased nitric oxide (NO) levels. Treatment with genistein, a well reported antioxidant and anti-inflammation agent, either post- or pre-BPA exposure ameliorated lipid peroxidation and stimulated an increase in antioxidant, especially SOD, production. This antioxidant activity of genistein may also be a sequential potentiation of the endogenous antioxidant system starting with SOD, a first line antioxidant enzyme [54]. Interestingly, NO levels were only reduced in the group treated with genistein after BPA treatment (BPA + GEN), which is consistent with the report of Jalili *et al.* [55], and not in the genistein pre-treated BPA group (GEN + BPA) suggesting the likely persistence of some level of inflammation in the gastric tissue in this group.

Electrolyte balance at gastric level is closely related with gastric acid secretion,

gastric acidity and electrolyte composiotion of gastric juice. Hence depletion of electrolytes in the extracellular fluid will likely affect the intracellular fluid concentration of the parietal and non-parietal cells that both contribute to the electrolyte composition of gastric juice [56]. While the exact effects of BPA exposure on the composition of gastric juice appear at present to be unknown, this study shows a reduction in bicarbonate (as discuss above previously) and chloride ion concentrations respectively, with no alterations in either sodium and potassium ion concentrations or the ratio of sodium to potassium (Na: K) in the BPA only treatment group. Treatment with genistein alone, and either genistein prior to or after BPA exposure resulted in increased bicarbonate content of gastric juice which would be expected to potentiate gastroprotective mechanisms (previously discussed). Potassium ion levels were also reduced in all treatment groups which could have been caused by the treatment vehicle, corn oil, whose group (group 2) also exhibited significant reductions in potassium ion compared to control. Treatment with genistein alone or genistein post- or pre-BPA exposure did not also reverse the observed reductions in gastric juice chloride ion concentration likely caused by BPA.

In conclusion, oral exposure to bisphenol A, an endocrine disrupter that acts via estrogen receptors, may increase the susceptibility of the gastric mucosa to erosions by increasing gastro-aggressive factors such as decreased gastric juice pH while decreasing its bicarbonate composition, depleting gastric antioxidant defense mechanisms and stimulation of inflammatory mechanism within the gastric epithelium. Treatment with genistein, a phytoestrogen, either pre- or post-exposure may ameliorate bisphenol A induced gastric pathologies by interacting with gastric estrogen receptors resulting in a stimulation of gastroprotective, antioxidant and anti-inflammatory mechanisms.

Conflicts of Interest

The authors declare that there are no conflicts of interest with respect this study.

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