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ORIGINAL ARTICLE

Bisphenol A toxicity induced hepatotoxicity and altered biochemical, histopathology, and immunohistochemical parameters: the metal chelating and antioxidant roles of naringin

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Abstract

Bisphenol A (BPA) is an environmental pollutant used in the polymer industry to produce plastics. BPA has been reported to cause deleterious effects in both humans and animals. Naringin is one of the flavonoids with antioxidant and metal chelating properties. This study was carried out to assess the potential ameliorative effect of naringin on BPA-induced hepatotoxicity in cockerel chicks. Thirty-one-day-old cockerel chicks were used for this study and randomly divided into 6 groups of five chicks per group as follows: Group 1 (control), Group 2 (BPA 100 ppm), Group 3 (BPA + 100 mg/kg naringin), Groups 4 (BPA + 200 mg/kg naringin), Group 5 (100 mg/kg naringin), and Group 6 (200 mg/kg naringin), respectively. The administration of BPA was via drinking water and naringin through oral gavage. BPA intoxication caused significant ($p < 0.05$) increases in ALT, ALP, AST, TC, TAG, and LDL, but decrease total protein and HDL-cholesterol when compared with the control. Also, there were significant increases in hepatic H_2O_2 generation and MDA contents with concomitant decrease in reduced glutathione content, glutathione S-transferase, and superoxide dismutase activity in BPA intoxicated chicks. Histology revealed a moderate to diffuse sinusoidal congestion, with a severe periportal cellular infiltration in BPA intoxicated chicks. Immunohistochemistry revealed higher expression of hepatic caspase 3 and TNF-α in chicks exposed to BPA alone relative to the control and naringin treated chicks (100 mg/kg and 200 mg/kg). Findings from this study show that naringin administration restored hepatoxicity, improved antioxidant status, and lowered exaggerated values of total cholesterol, oxidative stress indices, inflammation, and ameliorated ultrastructure anarchy. Combining all, the incorporation of naringin into poultry feeds could serve as a metal chelator and toxicant binder with beneficial effects in the reduction of toxicities associated with environmental pollutants such as bisphenol A.

Keywords Antioxidant · Bisphenol A · Cockerel chicks · Hepatotoxicity · Metal binding · Naringin

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Introduction

Over the years, there has been an increase in the exposure of living organisms to endocrine-disrupting chemicals (EDCs) that are available in the environment. The persistency of these environmental pollutants in the environment has become a source of great concern to human and animal health (Kelly et al. 2020; Kasonga et al. 2021). These EDCs are known to interfere with the function of the endocrine system, hence, leading to fatal health challenges in animals or its offspring (EC 1999). Bisphenol-A (BPA) is a typical example of EDC. BPA is known to mimic estrogenic activity**,** and this has attracted several research interests owing to its negative impacts on human and animal health (Rochester 2013). Staples et al. (2018) reported an increase in the global production of BPA from 5 to 8 million tons (MT) between 2010 and 2016. An estimate of 10.2 MT of BPA production by the year 2022 has also been predicted (Xing et al. 2022). BPA is one of the organic, synthetic compound pollutants in the environment (Rahman et al. 2017). The structure of BPA is similar to oestradiol and diethylstilbestrol. Hence, it has the tendency of stimulating cellular response via estrogen receptor binding (Rubin 2011). As reported by Mohapatra et al. (2010), sources of BPA include manufacturing industry effluents, landfill leaching, wastewater treatment plants, sludge discharge, and dumpsite wastewater. Industrial utilization of BPA has been employed in pipe material production, plastic materials, polyesters, and polyacrylate production (Tomza-Marciniak et al. 2018). The environmental exposure of humans and animals to BPA is ubiquitous, and majorly occurs through oral, respiratory, and dermal routes (Rahman et al. 2017). However, dietary ingestion is the main exposure route to living organisms. Birds are also exposed to BPA via plastic materials such as overhead water tanks, drinkers, and feeding trays (Escande et al. 2006). Previous study on animal models reported that BPA can cause reproductive toxicity through the induction of oxidative stress (Kumar et al. 2019). For instance, BPA toxicity has been reported to cause sperm cell depletion, shorter sperm transit time, and reduction in mitochondrial activity (Matuszczak et al. 2019). A study conducted by Abdel-Gwaad et al. (2020) revealed a significant reduction in the activity of liver catalase activity following BPA exposure.

Flavonoids belong to a class of plant secondary metabolites. In plants, they are the origin of bioactive compounds (Ghasemzadeh and Jaafar 2013). Flavonoids have varying structures in the form of glycones or glycosides in fruits and vegetables (Li et al. 2017). They have good health benefits based on their anti-cancer, anti-inflammatory, antioxidant, and anti-mutagenic effects. Naringin is a natural flavonoid. Different studies have established that naringin contains antioxidant, anti-apoptotic, anti-osteoporotic, anti-inflammatory, anti-carcinogenic, and anti-ulcer properties (Wang et al. 2013; Chen et al. 2016). Naringin also plays a significant function in reducing triglycerides, cholesterol, together with improvement in immune function and antioxidant status. Therefore, naringin, as a powerful antioxidant, has great capacity to scavenge free radicals and prevent lipid peroxidation (Changxing et al. 2018). Naringin has been reported to inhibit radiation-induced reduction of the antioxidant defense system (Chen et al. 2020). Goliomytis et al. (2019) reported that naringin supplementation in poultry feeds could favorably elongate the shell-life of eggs. Goliomytis et al. (2019) also reported the ability of naringin to improve yolk color without any side effects on egg trait quality and egg production. The dietary inclusion of naringin at 100 mg/kg daily was found to lower plasma fatty acid levels, and improved liver mitochondrial dysfunction, and glucose intolerance (Alam et al. 2013). Furthermore, naringin was reported to lower blood sugar level in rat and prevent the development of hyperglycemia (Jung and Kim 2014; Parmar et al. 2012) and reduce LDL-cholesterol, total cholesterol, and triglyceride levels in chicken (da Silva et al. 2001).

The consumption of leaked BPA into the feeds and water of poultry animals is detrimental to the health of the animals. Various means of reducing the toxic effect of BPA on different organs through the usage of various flavonoid-based supplements have been employed such as orange-peel powder in a rat model (Abdel-Gwaad et al. 2020) and naringin in male rats (Elsawy et al. 2021). There are limited studies on the effect of BPA toxicity in birds, and how naringin could be used to reverse its deleterious effect. This study was designed to determine the ameliorative effect of naringin and its mechanism of action on BPA toxicity in the liver of cockerel chicks. Gao et al. (2021) reported the induction of non-alcoholic fatty liver disease in laying hens exposed to BPA toxicity. In another finding, Li et al. (2020) documented that BPA toxicity enhanced abdominal fat deposition in aged hens via induction of oxidative stress and free radical generation. Öznurlu et al. (2021), Atay et al. (2020), and Mentor et al. (2020) also reported structural developmental abnormalities such as birth defects in chicken embryos exposed to BPA. Chen et al. (2022a, b), Eldefrawy et al. (2021) and Sharin et al. (2021) from their research findings, reported in vitro and in vivo hepatotoxicity induced by BPA toxicity. Toxicity of BPA to chicken trachea and induction of autophagy in chicken kidney have been documented (Yin et al. 2023). BPA toxicity has been reported to induce renal autophagy, and aggravate renal apoptosis and necroptosis in chicken especially in selenium-deficient chickens via oxidative stress and PI3K/AKT pathway (Chen et al. 2022a). The novelty of the present study from that of Mahdavinia et al. (2021) is that the mechanism of BPA-induced hepatotoxicity

via oxidative stress, inflammation, and apoptosis was elucidated in cockerel chicks. Therefore, this study aimed at elucidating the beneficial effects of naringin supplementation in poultry against environmental pollutants such as BPA.

Materials and methods

Chemicals

Naringin and bisphenol A were purchased from Akscientific, USA, while albumin, hydrogen peroxide (H_2O_2), phosphate buffer, sodium hydroxide, copper sulphate, potassium tartarate, trichloroacetic acid (TCA), tris-potassium chloride, ammonium ferrous sulphate, sorbitol, sulfuric acid, carbonate buffer, adrenaline, Ellman's reagent (DTNB) reduced glutathione (GST), thiobarbituric acid (TBA), 2-dichloro-4-nitrobensene (CDNB), sodium azide, and O-dianiside dihydrochloride were purchased from Sigma Chemical Co., USA. Biotinylated secondary antibody (2-step plus Poly HRP antirabbit/mouse IgG detection system (E-IR-R217) with DAB solution, caspase 3 monoclonal antibody (E-AB-22213), and TNF-α polyclonal antibody (E-AB-63550) were purchased from Elabscience Biotechnology, China.

Experimental animals

In this study, thirty-one-day-old cockerel chicks purchased from CHI FARMS Limited Ibadan, Nigeria, were utilized. The chicks were housed at the experimental rearing facility of Avian diseases unit, Department of Veterinary Medicine, University of Ibadan. Brooding of the chicks was for 2 weeks by supply of additional heat and then further reared for 4 weeks. The chicks were fed age-appropriate poultry feed from Top Feeds® limited, Ibadan, and clean drinking water ad libitum was given liberally. All the cockerels were kept in open-sided tropical rearing spacious cages with all necessary conditions.

Ethical approval to conduct the study.

Ethical approval to conduct the study was approved and the approval UI-ACUREC/048–0521/21 was assigned.

Experimental design

Cockerels with uniform weight were randomly allotted into 6 groups (5 chicks per group). Bisphenol A at 100 ppm was administered continuously in drinking water for a period of 6 weeks, while the treated groups were administered different dosages of naringin through oral gavage for a period of 2 weeks. Thirty-one-day-old cockerel chicks used for this study were randomly divided into 6 groups of five chicks per group as follows: Group 1 served as the control group and received only clean tap water, Group 2 BPA 100 ppm, Group 3 BPA + 100 mg/kg naringin, Groups 4 BPA + 200 mg/kg naringin, Group 5 100 mg/kg naringin, and Group 6 200 mg/kg naringin. The administration of BPA was via drinking water, while naringin was administered through oral gavage (1 g/10 mL of distilled water). The dosage of BPA was chosen based on the previous study of Gharibi et al. (2013), while naringin was administered according to the study of Alam et al. (2013) and Li et al. (2022).

Sample collection and preparation of hepatic post-mitochondrial fractions

Twenty-four hours post administration of the last treatment, chickens were euthanized by quick cervical dislocation. The whole liver was harvested by rapid dissection on ice, rinsed with distilled water, blotted with filter paper, and weighed on electronic digital scale. Subsequently, about 2 g each of the liver samples were collected, sliced, and homogenized in 0.1 M aqueous potassium phosphate buffer at pH 7.4. Liver post mitochondrial fractions (PMFs) were obtained after centrifuging at 10,000 g for 10 min with a cold centrifuge at − 4 °C. The supernatant was collected for biochemical assay.

Biochemical assays

Total protein concentration in the supernatant was determined using the Biuret method as described by Gornal et al. (1949). The method described by Wolff (1994) was used for the determination of hydrogen peroxide (H_2O_2) generation. The method of Varshney and Kale (1990) was employed in the determination of the lipid peroxidation (malondialdehyde (MDA) concentration). The hepatic reduced glutathione (GSH) concentration determination was quantified with the method described by Ellman (1959). The method of Misra and Fridovich (1972) with slight modification by Oyagbemi et al. (2015) was followed for the superoxide dismutase (SOD) activity. The methods described by Buetler et al. (1963) and Habig et al. (1974) were followed for the determination of glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities, respectively. The liver function tests such as total protein, albumin, total bilirubin, conjugated bilirubin, aspartate transferase (AST), alanine transferase (ALT), and alanine phosphatase (ALP) were determined using the commercial colorimetric kits as described by Manterys et al. (2016) and Adeyemo et al. (2018).

Histopathology

Liver tissues were fixed in 10% formalin, embedded in paraffin wax, and sections of 5–6 mm in thickness and thereafter stained with hematoxylin and eosin (H&E) as previously described by Drury and Wallington (1976). Finally, the stained sections were examined with light microscopic.

Immunohistochemistry

In this study, the immunohistochemistry procedure for the determination of hepatic caspase 3 and tumor necrosis factor-alpha (TNF-α) as described by Oyagbemi et al. (2019) was adopted.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare the means of different groups. Test of significance between two experimental groups was estimated with Student's *t*-test. *P*-value < 0.05 was considered statistically significant. The GraphPad Prism version 5.0 software was deployed.

Results

Liver function tests

From the present study, Fig. 1 shows the results obtained for the liver function tests. The values of total protein, albumin, and total bilirubin of chicks intoxicated with BPA (100 ppm) were significantly ($p < 0.05$) lower than that of the control and chicks treated with naringin (Fig. 1 a, b, c). However, there was significant improvement in the values of total protein, albumin, and total bilirubin of chicks treated with naringin (100 and 200 mg/kg) and those that received only naringin (100 and 200 mg/kg). From our results, we observed a significant ($p < 0.05$) increase in the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase in birds intoxicated with BPA in comparison to the control (Fig. 1 d, e, f). Treatment with naringin (100 and 200 mg/kg) significantly reduced the heightened values of AST, ALT, and ALP, respectively, in BPA intoxicated chicks. This is indicative of hepatoprotective action of naringin.

Lipid profiles

The total cholesterol (TC), triacylglyceride (TAG), and low-density lipoprotein cholesterol (LDL-c) values in BPA (100 ppm) exposed birds were significantly ($p < 0.05$) higher when compared with the value of TC, TAG, and LDL in the control group (Fig. 2). The values of TC, TAG, and LDL-c in BPA (100 mg/kg) + naringin (100 mg/kg) and BPA (100 ppm) + naringin (200 mg/kg) treatment groups were significantly ($p < 0.05$) lower than the BPA (100 ppm) untreated chicks. Again, the values of TC, TAG, and LDL-c levels of chicks treated with only naringin (100 mg/kg and 200 mg/kg) were similar in values when compared to that of the control group (Fig. 2a, b, d). The value of high-density lipoprotein cholesterol (HDL-c) in the BPA (100 ppm) group was significantly ($p < 0.05$) lower when compared with the HDL-c of the control group (Fig. 2c). Also, there was an increase in the values of HDL-c of BPA (100 ppm) + naringin (100 mg/kg) treated group, though not significantly different when

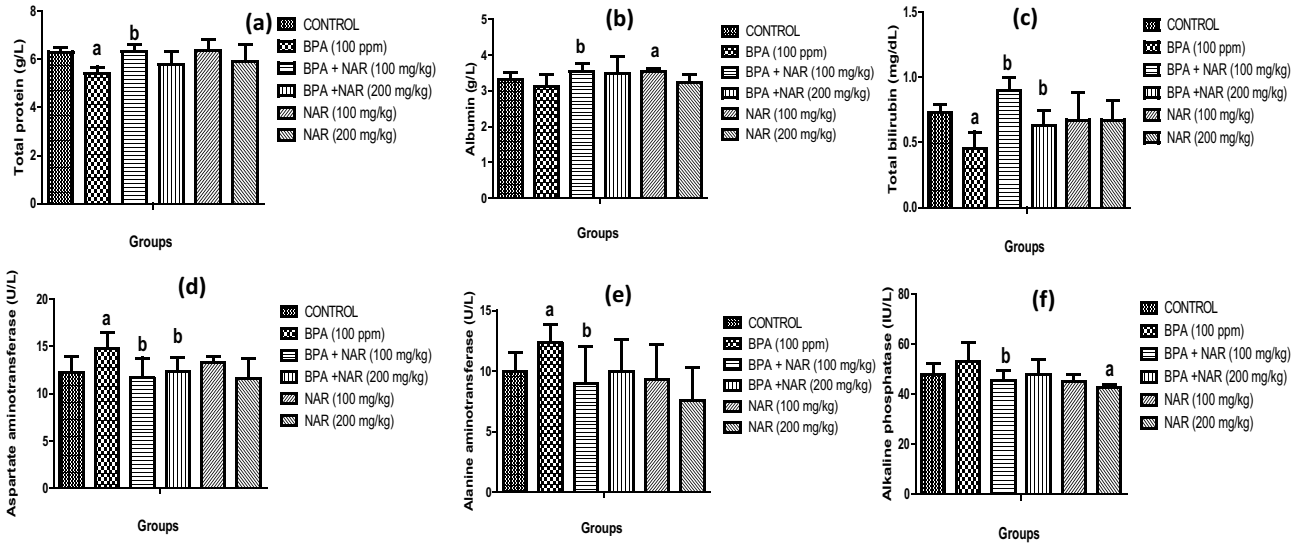

Fig. 1 Liver function tests showing **a** total protein, **b** albumin, **c** total bilirubin, **d** aspartate aminotransferase, **e** alanine aminotransferase, and **f** alkaline phosphatase for various groups. Superscript (a) indicates significant difference at $p < 0.05$ when compared with the control group (Group A). Superscript (b) indicates significant difference when compared with the bisphenol A group only (Group B). The results are shown in mean ± SD ($n = 6$). Group A: control, Group B: BPA (100 ppm), Group C: BPA and naringin (100 mg/kg), group D: BPA and naringin (200 mg/kg), Group E: naringin (100 mg/kg), and Group F: naringin (200 mg/kg)

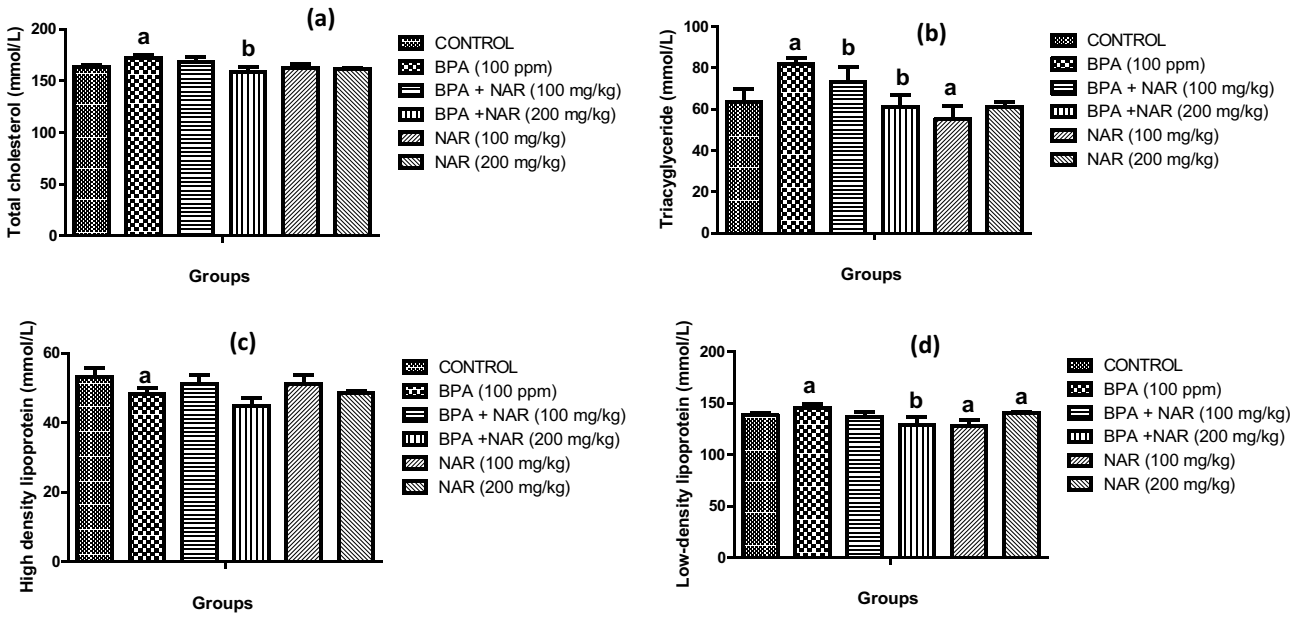

Fig. 2 Lipid profile showing **a** cholesterol, **b** triacyglyceride, **c** high-density lipoprotein, and **d** low-density lipoprotein for various groups. Superscript (a) indicates significant difference at $p < 0.05$ when compared with the control group (Group A). Superscript (b) indicates significant difference when compared with the bisphenol A group only (Group B). The results are shown in mean ± SD ($n = 6$). Group A: control, Group B: BPA (100 ppm), Group C: BPA and naringin (100 mg/kg), Group D: BPA and naringin (200 mg/kg), Group E: naringin (100 mg/kg), and Group F: naringin (200 mg/kg)

compared to BPA untreated chicks. The values of HDL-c of chicks administered with only naringin (100 mg/kg and 200 mg/kg) were like that of the control group, respectively.

Markers of oxidative stress and antioxidant defense system in the liver

The values of hydrogen peroxide (H_2O_2) generation and contents of lipid peroxidation product (MDA) in the liver of the BPA intoxicated chicks were significantly ($p < 0.05$) higher when compared with the control group (Fig. 3a, b). Data from Fig. 3 also show significant ($p < 0.05$) reduction in the level of H_2O_2 generation and MDA contents in the liver of BPA-treated chicks with naringin (100 mg/kg and 200 mg/kg) and naringin only (100 mg/kg and 200 mg/kg) in comparison to BPA-untreated chicks. The observed significant ($p < 0.05$) reduction in the contents of MDA was dose-dependent in naringin treated chicks (Fig. 3b), thus,

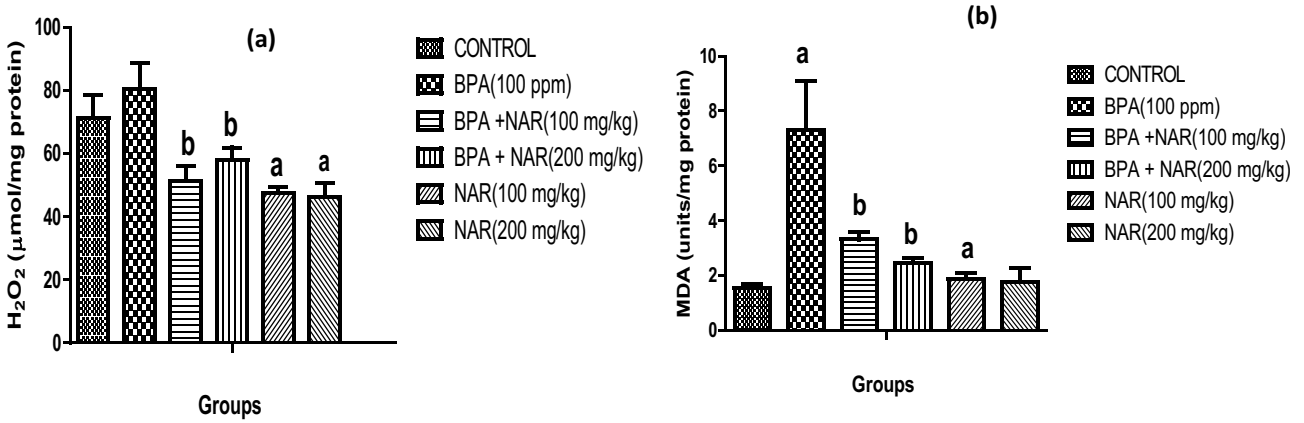

Fig. 3 Markers of oxidative stress in liver **a** H2O2 and **b** MDA. Superscript (a) indicates significant difference at $p < 0.05$ when compared with the control group (Group A). Superscript (b) indicates significant difference when compared with the bisphenol A group only (Group B). The results are shown in mean ± SD ($n = 6$). Group A: control, Group B: BPA (100 ppm), Group C: BPA and naringin (100 mg/kg), Group D: BPA and naringin (200 mg/kg), Group E: naringin (100 mg/kg), and Group F: naringin (200 mg/kg)

indicating the antioxidative action of naringin. Again, our result showed a significant ($p < 0.05$) decrease in the contents of GSH and activity of GST and SOD in the BPA-exposed chicks when compared with the control group (Fig. 4a, b, c), while BPA toxicity enhanced the activity of GPx greater than that of the unexposed chicks (control) as indicated in Fig. 4d. The noticeable increase in the activity of hepatic GPx might be due to consumption of GSH due to BPA toxicity. However, treated of intoxicated chicks with naringin significantly improved the content of GSH and the activities of GST, SOD, and GPx (Fig. 4a, b, d).

Histopathology

Histology revealed a moderate to diffuse sinusoidal congestion, with severity in the histopathology of hepatic tissues of chicks administered only BPA (Fig. 5). We observed that treatment of BPA-exposed chicks with naringin ameliorated the ultra-structure anarchy and hepatic tissue pathology as recorded in Fig. 5.

Immunohistochemistry

The immunolocalization of hepatic caspase 3 and tumor necrosis factor (TNF-α) revealed higher expression in BPA exposed group relative to the control and groups treated with naringin (Figs. 6 and 7). The reduction in the expression of TNF-α as well as caspase 3 is indicative of amelioration of hepatic inflammation, apoptosis, and hepato-protective effects of naringin against BPA toxicity. The hepato-protective effects of naringin were demonstrated as reduction in the expression of TNF-α and caspase 3 in the chicks treated with naringin and those administered naringin only.

Discussion

Different health-related deleterious effects are associated with BPA exposure in living organisms, with oxidative stress as a major player in the pathogenesis of BPA toxicity (Singh et al. 2016). BPA induces oxidative stress by generating reactive oxygen species (ROS) through generation of free radicals, and thereby causing an imbalance between ROS and antioxidant defenses in favor of ROS production (Kobroob et al. 2018; Amjad et al. 2020). The hepatotoxic effect of BPA was established in this study with statistically significant increase in the activities of ALP, AST, and ALT, and a concomitant decrease in total protein and albumin in BPA-exposed chicks. This is in line with the study carried out by Oguazu et al. (2015) and Abdel-Gwaad et al. (2020) as they reported an exaggerated increase in the activities of ALT, AST, and AST as biomarkers of hepatotoxicity induced by BPA toxicity. Naringin is an example of a flavonoid that has been reported to possess several biological and pharmacological effects such as anti-inflammatory,

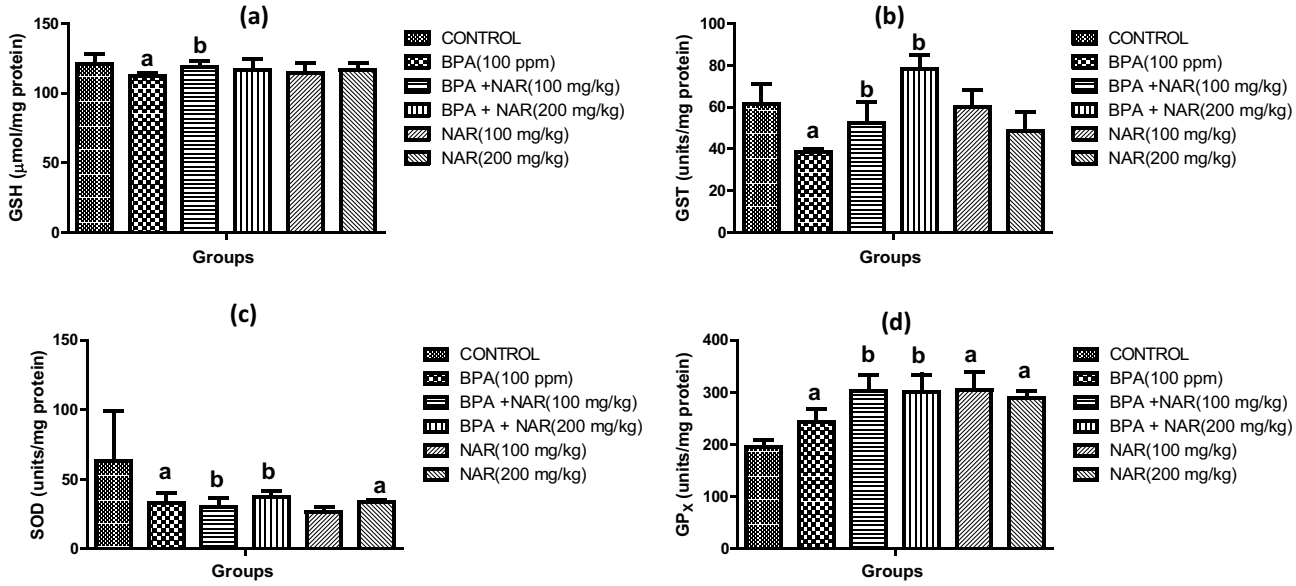

Fig. 4 Liver antioxidant enzyme profile following BPA exposure and naringin application for **a** GSH, **b** GPx, **c** GST, and **d** SOD. Superscript (a) indicates significant difference at $p < 0.05$ when compared with the control group (Group A). Superscript (b) indicates significant difference when compared with the bisphenol A group only (Group B). The results are shown in mean ± SD ($n = 6$). Group A: control, Group B: BPA (100 ppm), Group C: BPA and naringin (100 mg/kg), Group D: BPA and naringin (200 mg/kg), Group E: naringin (100 mg/kg), and Group F: naringin (200 mg/kg)

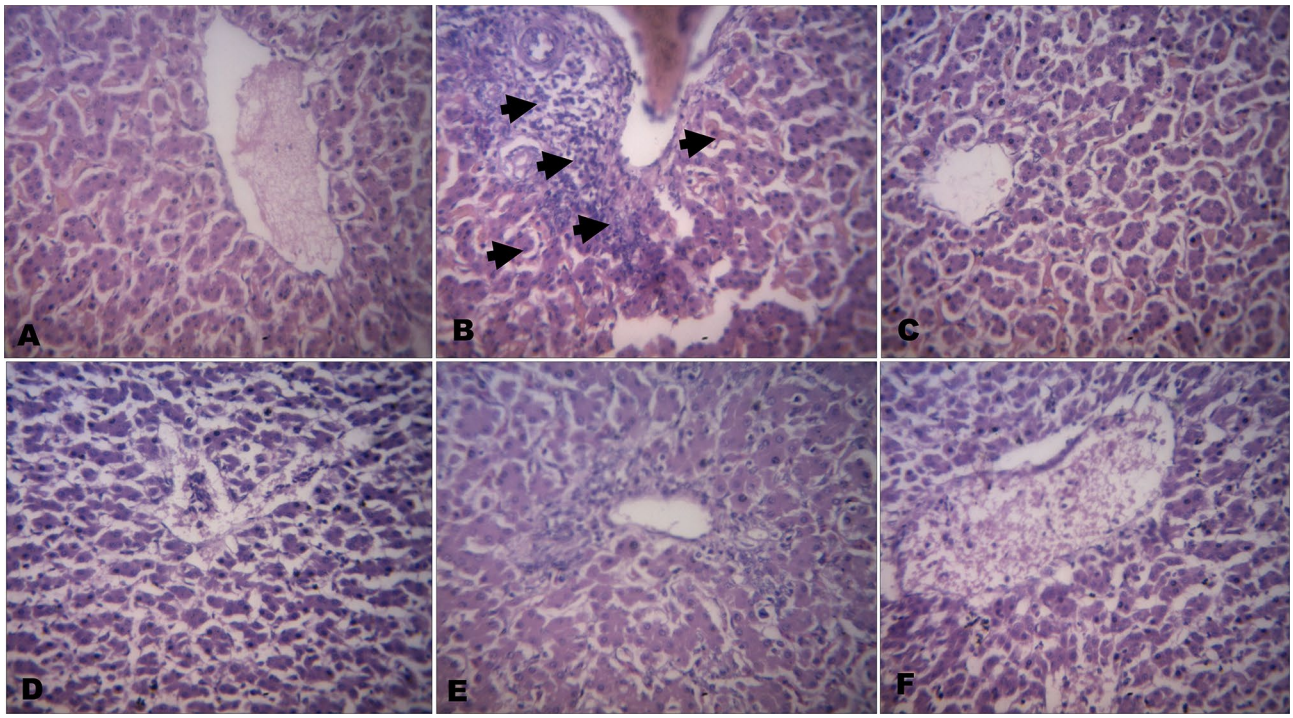

Fig. 5 The histology of the kidney. Group A (Control) shows no visible lesions; Group B (bisphenol A; 100 mg/kg) shows moderate diffuse sinusoidal congestion, with a severe periportal cellular infiltration (arrowheads); Group C (bisphenol A + naringin 100 mg/kg) shows no visible lesions; Group D (bisphenol A + naringin 200 mg/kg) shows no visible lesions; Group E (naringin 100 mg/kg) shows no visible lesions; and Group F (naringin 2000 mg/kg) shows no visible lesions. Slides stained with high definition hematoxylin and eosin (H&E) at × 100 magnification

hypolipidemic, antioxidant, antiviral, antimicrobial, antiapoptotic, hepatoprotective, and immunomodulatory effects (Changxing et al. 2018).

Hepatoprotective activities were observed in the naringin-treated groups as there were significant reductions in ALT, AST, and ALP with a corresponding increase in total

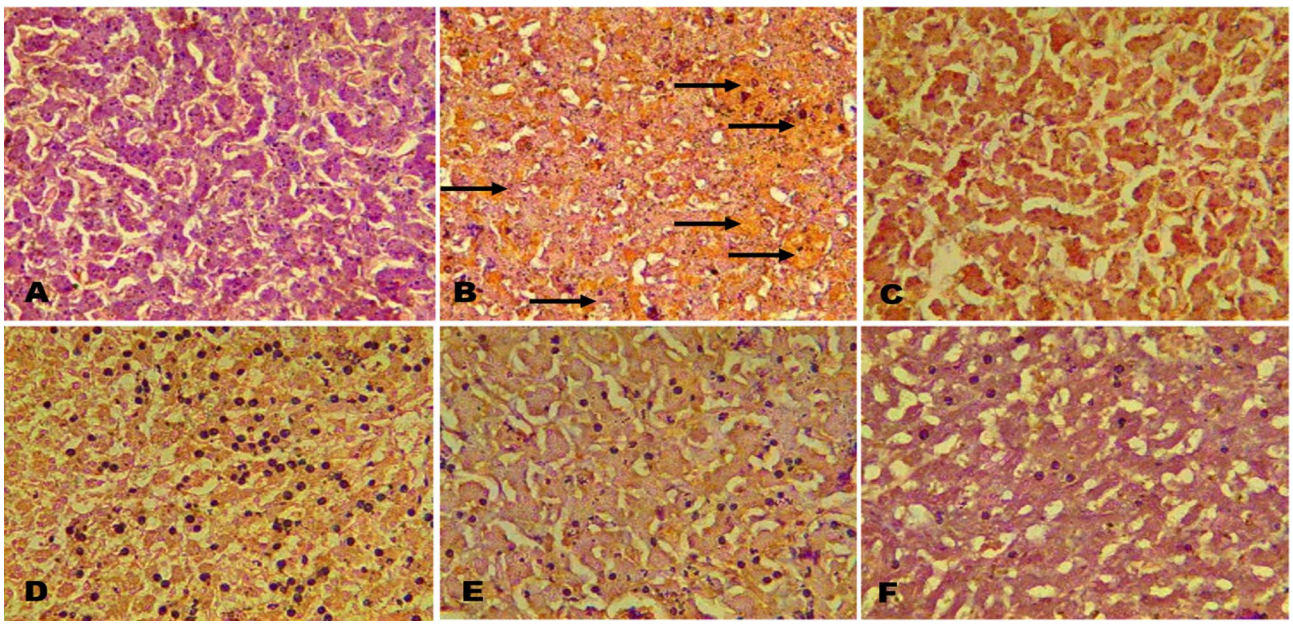

Fig. 6 The immunohistochemistry of hepatic caspase 3. **A** control; **B** bisphenol A; 100 ppm; **C** bisphenol A + naringin 100 mg/kg; **D** bisphenol A + naringin 200 mg/kg; **E** naringin 100 mg/kg; **F** naringin 2000 mg/kg). Slides stained with high-definition hematoxylin. (magnification × 100)

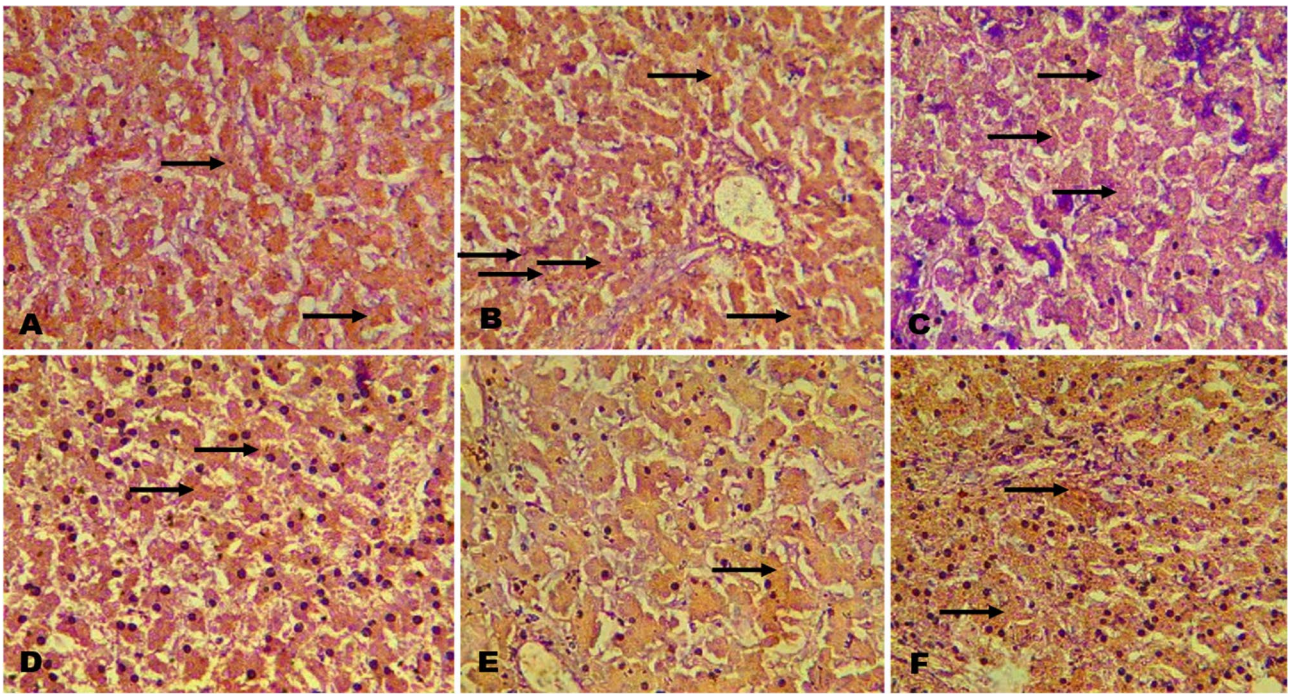

Fig. 7 The immunohistochemistry of hepatic tumor necrosis factor alpha (TNF-α). **A** control; **B** bisphenol A; 100 ppm; **C** bisphenol A + naringin 100 mg/kg; **D** bisphenol A + naringin 200 mg/kg; **E** naringin 100 mg/kg; **F** naringin 2000 mg/kg. Slides stained with high-definition hematoxylin (magnification × 100)

protein. This finding corroborate the earlier study of Imam et al. (2016) who reported that co-administration of naringin with aluminum chloride intoxication ameliorated aluminum-induced liver damage and oxidative stress. Furthermore, the levels of TC, TAG, and LDL-c were significantly ($p < 0.05$) higher in BPA intoxicated chicks with a concurrent decrease in HDL-c as observed in the present study. This finding agrees with the report of Eweda et al. (2020) who observed that BPA induced dyslipidemic state and increased triglycerides (steatosis) and cholesterol accumulation in the liver tissue of rat. Marmugi et al. (2012) also reported that the exposure of rat to BPA resulted in hypertriglyceridemia, hypercholesterolemia, and alterations of fatty acids composition in the liver. More so, there was upregulation of genes which are linked with de novo lipogenesis and cholesterol synthesis in the liver. Kumar et al. (2019) reported that treatment with naringin gave a noteworthy amelioration against the developments of metabolic syndrome which is characterized with hyperlipidemia. The anti-hyperlipidemic effect of naringin was established in this study as naringin reversed the toxicity of BPA by decreasing LDL-c, TC, TAG, and increasing HDL-c in chicks treated naringin.

The scavenging and neutralization of free radicals such as superoxide radicals, hydrogen peroxides (H_2O_2), and hydroperoxides are performed by antioxidants, thereby preventing oxidation reactions. During oxidative stress, generation of free radicals increases in the body (Amjad et al. 2020). This study showed an increase in H_2O_2 contents in the liver of BPA-exposed chicks. This is in concordance with the study of da Silva et al. (2018) who reported an exaggerated increase H_2O_2 generation in thyrocytes of female Wistar rats intoxicated with BPA. More so, Kabuto et al. (2003) observed that BPA caused overproduction of H_2O_2 with resultant oxidative stress. In our study, naringin reduced the level of H_2O_2 in the BPA-treated chick and naringin-only treated groups. The antioxidant and free radical scavenging power of naringin was earlier reported by Rashmi et al. (2018) who showed the H_2O_2 radical scavenging potential of naringin in streptozotocin-induced liver damage. Also, Miles and Calder (2021) reported that naringin supplementation increased liver and kidney expression of anti-inflammatory transcription factors.

In this study, the increase in the level of MDA caused lipid peroxidation as BPA-induced oxidative stress as earlier reported by Kobroob et al. (2018) and Eweda et al. (2020). Lipid peroxidation of biological membranes is known to cause loss of membrane fluidity, increases membrane permeability, alters receptor function, and changes in membrane potential. It can be observed in this study that the MDA level was exaggerated in the liver of BPA intoxicated chickens. However, as recorded in birds co-treated with naringin, there was observable reduction in the MDA level, as previously reported by Rajadurai and Stanely (2006) and Alam et al. (2014). This therefore demonstrates the anti-oxidative and

free radical scavenging action of naringin by preventing lipid peroxidation precipitated by BPA toxicity. Hence, supplementation of poultry feeds with lysine as a precursor of naringin could offer significant health benefits via improvement of in vivo antioxidant capacity.

Glutathione (GSH) is one of the non-enzymatic antioxidants that is depleted during oxidative stress. In this study, a decreased level of GSH was observed in the liver of BPA-exposed chicks, which depicts ongoing oxidative stress caused by BPA toxicity. Kobroob et al. (2018) reported a decrease in GSH in rats exposed to BPA. However, birds co-treated with naringin showed improvements in the concentration of GSH. This supports the study of Kumar et al. (2019) who stated that administration of naringin to diabetic rats raised the concentration of GSH. The GPx, SOD, and GST are known to protect tissues from damage caused by oxidative stress by detoxification of several substrates generated from cellular oxidative processes (Sravani et al. 2016). We observed increase in the hepatic GPx activity in BPA-exposed chicks, and this could be as a result of the adaptive response. We proposed that this might result from nuclear translocation of nuclear erythroid-related factor 2 (Nrf2). Nrf2 is usually low when it is not activated by stress factors. In the presence of ROS such as superoxide, hydroxyl, and peroxyl radicals, cysteine residues (the main sulfhydryl group) become oxidized on the Keap1 segment. This changes the conformity of Keap1 segment and prevents it from attaching to Nrf2. The Nrf2 released within the cytosol enters the nucleus and heterodimerize with small Maf proteins, and further binds to regulatory gene recognized as antioxidant response elements (ARE). Substances that can initiate activity of ARE include environmental pollutants, H_2O_2, and nitric oxide. The Nrf2-Maf-ARE complex then initiates subsequent specific antioxidant and detoxification genes such as that of GST, GPx, GSH, and SOD, thereby increasing antioxidant activities and inactivating ROS (Baird and Dinkova-Kostova 2011; Oyagbemi et al. 2017). The antioxidant effect of naringin was established as naringin restored the levels of GSH, GST, GP_X, and SOD in the hepatic tissue of BPA-treated chicks.

TNF-α is an example of a pro-inflammatory cytokine that plays a significant role in the host's defense against injury. It was stated that BPA toxicity exacerbated the proinflammatory cytokine expression along with interleukins such as IL-1β, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) as previously reported (Wang et al. 2019). In the present study, we recorded higher expression of TNF-α in the liver of the BPA-exposed chicks when compared to the control and naringin-treated chicks. Naringin has been reported to possess a hepatoprotective effect by regulating inflammatory cytokines and increasing antioxidant enzymes (Caglayan et al. 2018). This study establishes that naringin reduces the expression of TNF-α in birds co-administered with naringin and BPA. Caspase-3 is a biomarker of cell death. In this study, we observed increase in expression of caspase-3 in the liver of BPA intoxicated chicks. This shows that hepatic apoptosis might have resulted from inflammation and oxidative stress following BPA toxicity (Abdel-Rahman et al. 2018; Liu et al. 2022a, b). Previous study has reported that BPA-induced non-alcoholic fatty liver disease, apoptosis, and necroptosis in bursa of Fabricius in chicken through regulating oxidative stress and PI3K/AKT pathway (Gao et al. 2021; Liu et al. 2021; Gharibi et al. 2013). The anti-apoptotic effect of naringin was exhibited in the BPA group co-treated with naringin and naringin treated groups by cleaving caspase-3 and inhibiting the genes and proteins involved in apoptotic pathways such as P53 and P $16^{INK\ 49}$ (Yuan and Yang 2022).

Conclusion

Based on the findings in this study, it can be inferred and concluded that naringin abated the toxicity of bisphenol A-induced liver dysfunction. Naringin exhibited its hepatoprotective property by revitalizing cells that were damaged due to BPA-induced oxidative stress. The use of naringin supplementation in poultry feeds could offer potential health benefits and also serves as a natural and safe feed supplement in poultry nutrition.

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Author contribution The authors, Leah Oluwaseyanu Esuola, Oluwaseun Esan, Adamu Shafiu Maikifi, Ademola Adetokunbo Oyagbemi, and Temidayo Olutayo Omobowale, designed the experiment. Leah Oluwaseyanu Esuola, Oluwaseun Esan, and Adamu Shafiu Maikifi performed the immunohistochemistry and biochemical assays. The blood pressure and electrocardiogram were performed by Leah Oluwaseyanu Esuola, Oluwaseun Esan, and Temidayo Olutayo Omobowale. Moses Olusola Adetona, Ademola Adetokunbo Oyagbemi, Temidayo Olutayo Omobowale, Oluwafemi Oguntibeju, Evaristus Nwulia, and Momoh Audu Yakubu supervised, proof-read, and approved the submission.

Availability of data and materials Data will be made available on request.

Compliance with ethical standards

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Ethics approval The study was conducted following guidelines approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan (approval number: UI-ACUREC/048–0521/21).

Informed consent Informed consent was obtained from all individual participants included in the study.

Consent for publication For this type of study consent for publication is not required.

Consent to participate Not applicable.

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