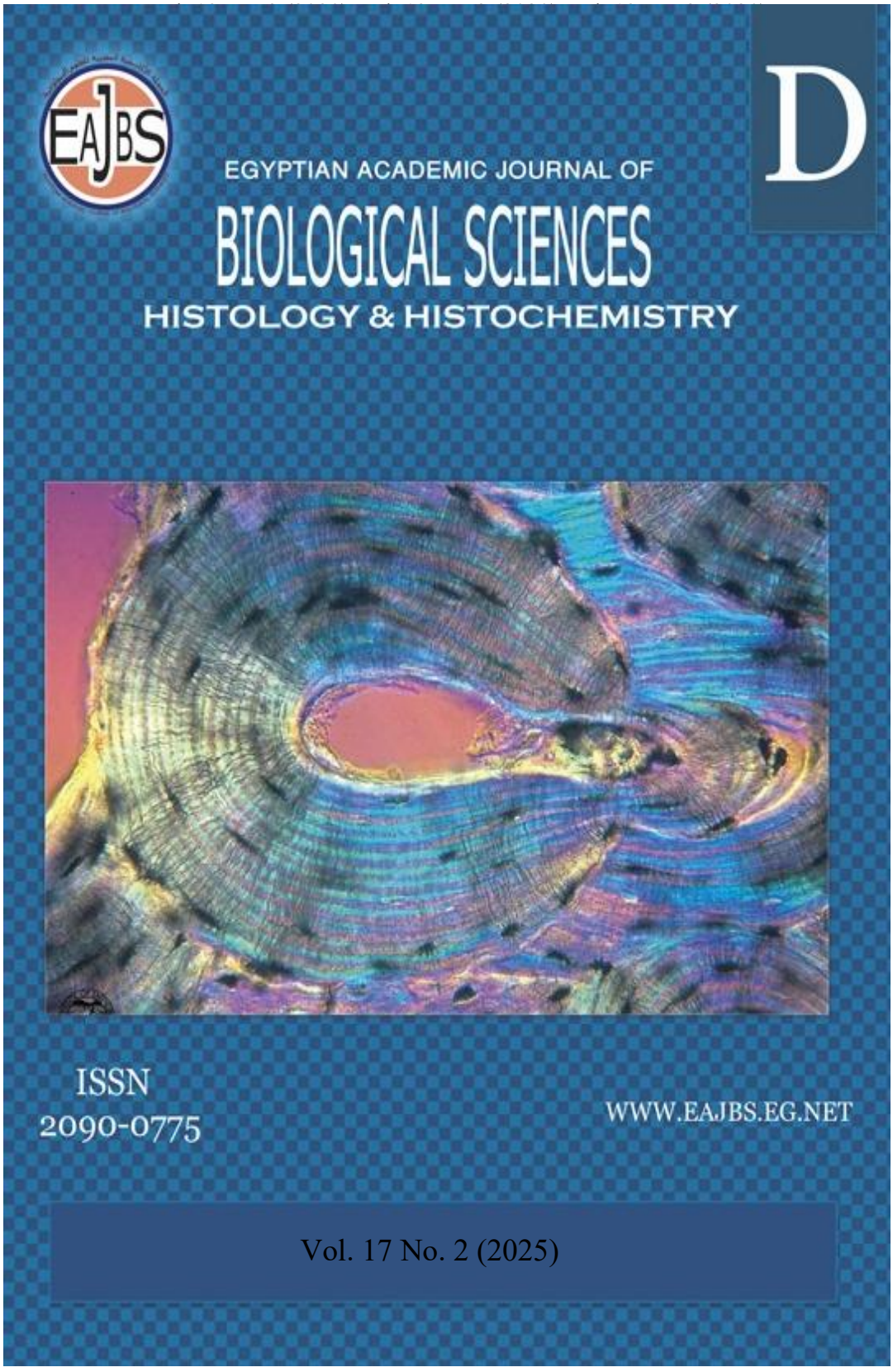

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Possible Protective Role of Selenium on Adverse Effect of Bisphenol A on the Adrenal Cortex in Adult Male Albino Rats: A Histological, Immunohistochemical, and Biochemical Study

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ABSTRACT

Background: Bisphenol A (BPA) is a common endocrine disruptor that induces oxidative stress and apoptosis in endocrine organs. The adrenal cortex, essential for stress hormone regulation, may be particularly vulnerable. Selenium, a potent antioxidant, has been proposed as a protective agent. **Methods:** Twenty-one adult male albino rats were randomized into three groups (n = 7 each): control, BPA (10 mg/kg/day), and BPA + sodium selenite (0.5 mg/kg/day). Adrenal tissues were evaluated histologically, immunohistochemically (caspase-3), and biochemically (Malondialdehyde, Glutathione Peroxidase, Adrenocorticotropic Hormone corticosterone). Morphometric analyses quantified cortical thickness, collagen deposition, and apoptotic index. **Results:** BPA exposure disrupted adrenocortical architecture, with cytoplasmic vacuolations, capsular fibrosis, and significant reduction in zone thickness (ZG: 68.14 ± 1.91 µm; ZF: 256.54 ± 12.51 µm; ZR: 100.61 ± 6.52 µm, $p < 0.001$). Caspase-3 expression and collagen deposition increased markedly (23.41 ± 2.54%). Biochemically, BPA elevated MDA (167.23 ± 3.84) and ACTH (385.03 ± 6.44), while reducing GPx (59.97 ± 4.55) and corticosterone (51.07 ± 1.38). Selenium co-treatment significantly reversed these effects: cortical thickness improved, caspase-3 and fibrosis were reduced (3.02 ± 0.63%), MDA decreased (61.07 ± 3.02), and GPx activity increased (112.11 ± 3.37). Hormone levels normalized toward control values. **Conclusion:** Se Supplementation significantly attenuates BPA-induced oxidative stress, fibrosis, and apoptosis in the adrenal cortex, thereby restoring both structural and functional integrity. These findings support selenium's potential as a protective agent against endocrine disruptor–induced adrenal injury, although further studies on dosing, duration, and clinical translation are warranted.

INTRODUCTION

One of the most common environmental contaminants and a famous endocrine disruptor is bisphenol A (BPA), which is used in polycarbonate plastics such as water and baby bottles. It is also present in polymers used in dental materials and food packaging (Chapin *et al.,* 2008).

BPA is a potential endocrine-disrupting chemical (EDC), because it can strongly interfere with the function of endocrine glands and can affect many organs in the body.

The effects of its toxicity have been discussed in several experimental and clinical studies (Sartain and Hunt, 2016). Current knowledge regarding the impact of BPA on the histological, morphometric, and immune-histochemical characteristics of the adult adrenal cortex remains scarce (Medwid, 2017). The cellular toxicity of BPA is primarily linked to oxidative stress, driven by excessive production of harmful free radicals. Reactive oxygen species (ROS) play a pivotal role in regulating normal cellular functions; however, when their levels rise, they can damage DNA, RNA, and proteins, ultimately leading to cellular dysfunction and apoptosis (Huang *et al.,* 2018; Rahman *et al.,* 2019).

The oxidative stress of BPA results in functional disruption and cell apoptosis with caspase 3 acting as a key player (Li *et al.,* 2009).

BPA intake can also induce structural changes in the adrenal cortex, such as loss of normal arrangement of cells, increased vacuolization of cytoplasm, nuclear pyknosis, thickening of the capsule, and reduction of the thickness of all three layers (Bushra and Hassanin, 2023).

Selenium (Se) has shown antioxidant properties against oxidative stress. It inhibits the generation of free radicals to alleviate damage to cells (Ozturk and Ozdemir, 2023).

Sodium selenite is a synthetic form of selenium that is used to promote growth and prevent selenium deficiency diseases. Selenium compounds such as sodium selenite are generally absorbed from the gastrointestinal tract (Fordyce, 2012). Selenium has a wide antioxidant effect on different body organs (Dominiak *et al.,* 2016).

Although Se has several advantages, there is limited literature investigating its effects on BPA-induced adrenal damage. The aim of this study is to fill in this gap by looking into whether Se can reduce the damage produced by BPA in the adrenal gland by modulating the levels of oxidative stress. Specifically, we examined the histopathological, immunohistochemical, and molecular changes in adrenal tissue. By elucidating the BPA's cytoprotective mechanisms, this research could pave the way for targeted therapies against BPA poisoning and other oxidative stress-related disorders

MATERIALS AND METHODS

1. Chemicals:

A- Bisphenol A (BPA): In the form of powder dissolved in distilled (EL-Gomhoria Company, Egypt). Each milliliter contained 10 mg/kg (Olukole *et al.,* 2019).

B- Sodium selenite (SS): In the form of powder dissolved in distilled water. Each milliliter contained 0.5 mg/kg (Boyacioglu *et al.,* 2021).

2. Animals and Ethical Approval:

This experiment was carried out following the ethical guidelines of the Laboratory Animal Committee at Kasr El-Ainy and with prior approval from the Institutional Review Board (Approval No.: CU-III-F-2-23). A total of 21 adult male Sprague-Dawley albino rats, weighing between 180 and 220 g, were used. The animals were supplied by the Animal House, Faculty of Medicine, Cairo University, and were allowed a two-week acclimatization period before the initiation of the study. They were maintained in cages under controlled laboratory and environmental conditions, with free access to standard rat chow, pellets, and water.

3.Experimental Design and Grouping:

Twenty one rats were randomly allocated into three groups: (a) control group, (b) BPA group that received 10 mg/kg/day BPA *via* gastric gavage for 4 weeks (Olukole et al., 2019), and (c) Se group that received sodium selenite 0.5 mg /kg /day orally in the form of suspension *via* gastric gavage (Ahmed Zaki *et al.,* 2021; Boyacioglu *et al.,* 2021) for 4 weeks.

4.Humane Endpoints Monitoring and Specimen Collection:

At the end of the 4-week experiment, blood samples were collected from the rat tail vein after applying local anesthetic cream for serological studies. Then the rats were sacrificed by intraperitoneal injection of phenobarbitone sodium (50 mg/kg) after application of local anesthetic cream on abdominal skin (Khafaga *et al.,* 2019). Then the abdomen was opened through a ventral midline incision, periadrenal fat was removed, and the adrenal glands of both sides were rapidly dissected out. The right adrenal gland was fixed in 10% formol saline solution for the histological and immunohistochemical studies, while the left gland was preserved in phosphate buffered solution for biochemical studies.

5. Serological Study:

Serum Levels of ACTH and Corticosterone:

Plasma ACTH levels were measured using the chemiluminescence technique with an IMMULATE automated analyzer (DPC, Los Angeles, CA, USA), and results were reported as pg ACTH/ml of plasma. Serum corticosterone concentrations were assessed with a commercial ELISA kit (Immunodiagnostic System Ltd, Boldon, UK), and values were expressed as ng CORT/ml of serum (Xi *et al.,* 2011).

6. Biochemical Study:

A- Tissue Level of Malondialdehyde (MDA):

MDA, a marker of lipid peroxidation, was measured as an indicator of oxidative stress in tissues. The assay kits were obtained from Bio Diagnostics (Egypt). The procedure involved mixing 0.5 ml of tissue homogenate with 2.5 ml of 20% trichloroacetic acid and 1 ml of 0.6% thiobarbituric acid (TBA). The mixture was then heated in a boiling water bath for 30 minutes and rapidly cooled. Subsequently, 4 ml of n-butanol was used to extract the resulting chromogen. The absorbance of the organic layer was read at 530 nm using a spectrophotometer, with butanol as the blank. MDA concentration was calculated based on a standard curve (Onaolapo *et al.,* 2017)

B- Determination of Adrenal Glutathione Peroxidase (GPx) Activity:

Enzymatic activity in the tissue homogenate was assessed by evaluating the inhibition of nitroblue tetrazolium reduction by superoxide anions generated through the xanthine/xanthine oxidase system. One unit of GPx activity was defined as the enzyme quantity required to produce 50% inhibition in 1 ml of reaction mixture per gram of tissue protein, with results expressed as U/g of tissue (Ahmed *et al.,* 2021).

7. Histological (Light microscopic) Study:

Tissue samples from all groups were fixed in 10% formol saline solution, followed by dehydration in graded alcohol, clearing in xylene, and paraffin embedding. Five µm sections were cut and stained with hematoxylin and eosin for routine histological evaluation (Avwioro, 2010) and with Masson's trichrome to demonstrate collagen (Ross and Pawlina, 2011).

8. Immunohistochemical Staining: Caspase-3: (Sadek *et al.,* 2021):

Deparaffinized tissue sections were rehydrated in distilled water and incubated with 3% H2O2. To minimize nonspecific binding, the sections were blocked with 1.5% goat serum in PBS and then incubated with the primary antibody for 45 minutes at room temperature. Immunoreactivity was assessed using a rabbit monoclonal anti-caspase-3 antibody (#ab184787, 1:1000, Abcam, Cairo, Egypt).

9. Quantitative Morphometric Study:

A. Measurement of the Thickness of Adrenocortical Zones:

The mean thickness of the three classical zones of the adrenal cortex was measured in 7 serial H&E-stained sections in all groups **(**Fig. 1**)**.

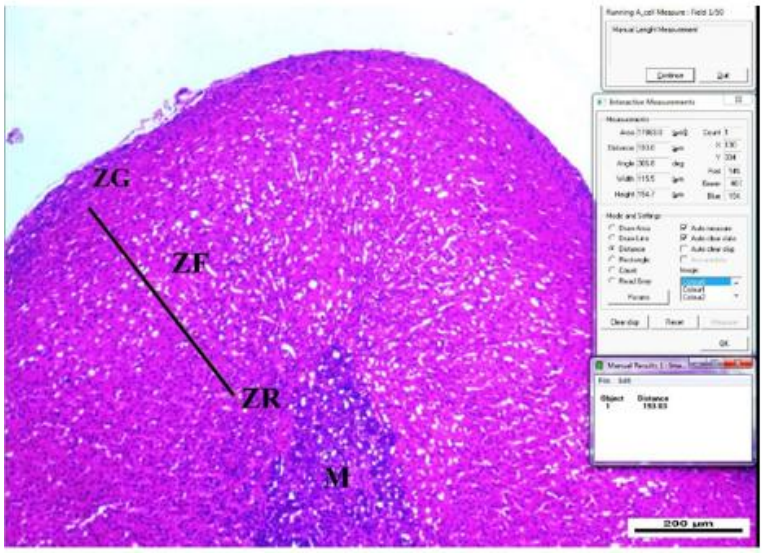

Fig (1): Representative image from the analyzer monitor showing measurements of the adrenal cortex zones' thickness in a section of the adrenal gland from an adult male rat. **(Hx &E x100)**

B. Measurement of the Area Percentage of Collagen Fibers in Masson's Trichrome Stained Sections:

This was observed at the X40 objective lens in 7 non-overlapping fields for each adrenal cortex in all groups. Measurements were generated by a binary image for the blue color in the stroma (Fig. 2).

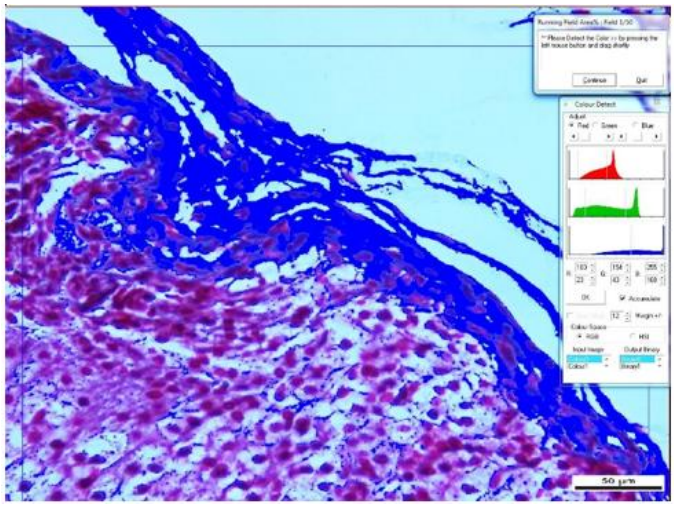

Fig. (2): Representative display from the image analyzer monitor showing measurement of the area percentage of collagen fibers in a Masson's trichrome stained section of the adrenal gland from an adult male rat. Collagen fibers appear in blue and are delineated within the standard measuring frame. **(Masson's trichrome x400).**

C. Measurement of the area percentage of immuno-expression in caspase-3-stained sections:

This was done in 7 fields per adrenal cortex by analyzing the percentage of tissue labeling in each field and the absorbance of caspase-3 immuno-positive cells (Fig. 3).

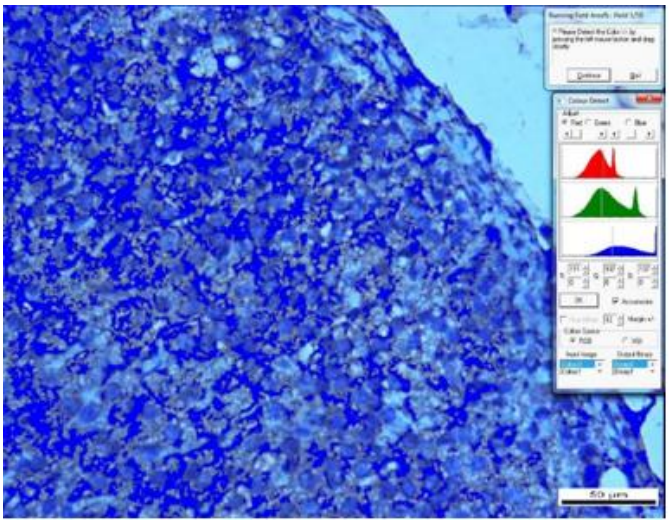

Fig (3): Representative display from the image analyzer monitor showing the measurement of the area percentage of caspase-3 immunoreactivity in a section of the adrenal gland from an adult male rat. Caspase-3–positive areas are masked in blue for quantification in the immunostained sections. **(caspase-3 x400)**

Statistical Analysis:

Data were tabulated, analysed using SPSS, version 26 and represented as means and standard deviations. Group comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. All analyses were two-tailed. Significance was recorded at a p-value ≤ 0.05.

RESULTS

1.Adrenal Cortex Structure Revealed by H&E Staining:

The BPA group showed the highest severity of inflammation and maximum decrease in the mean of the thickness of ZG, ZF, and ZR, which was 68.14 ± 1.91, 256.54 ± 12.51, and 100.61 ± 6.52 µm. These findings confirm that BPA induces significant adrenal cortex injury. The BPA + Se group exhibited a considerable reduction in adrenal cortex damage severity compared to the BPA group. In the Se group, inflammation decreased, and the thin capsules were restored. These reductions imply that Se administration reduces BPA-induced adrenal cortex (Figs 4,5).

2.Masson's trichome results:

As proved by the Mean ± SD of area % of collagen fibers In the BPA-treated group was 23.41 ± 2.54, $p < 0.001$, showed marked evidence of thickening of the capsules with lamellar separation of the capsular collagen bundles while use of Se lead to restoration of the thin capsules with fine thin collagen fibers arranged between the cells of the ZG and ZF, as evidenced by the mean of area percent was 3.02 ± 0.63. (Figs 6,7)

3. Immunohistochemical Assessment of Caspase-3:

As proved by Mean ± SD of Caspase 3 area% in the BPA-treated group (23.41 ±2.54, $p < 0.001$), there was a marked increase in caspase-3 immunoreactivity, with widespread brown cytoplasmic staining evident in alveolar and bronchial epithelial cells indicating increased apoptotic activity triggered by BPA-induced oxidative stress. Co-treatment with Se significantly attenuated this expression to 3.02 ±0.63 with a visibly reduced staining intensity and distribution, suggesting a protective, anti-apoptotic effect of selenium. (Figs. 6,8). In both Se -only and control groups, caspase-3 expression remained low and comparable (3.02 ±0.63 % vs. 2.10 ±0.28%), insuring the non-toxic profile of Se under basal physiological conditions (Figs. 6,8).

4.Oxidative Stress Markers:

Exposure to BPA led to a marked increase in MDA level with Mean ± SD of tissue level of 167.23 ±

3.84 with downregulation in the GPx level with Mean ± SD of tissue level of 59.97 ± 4.55, serum ACTH and Corticosterone serum levels in BPA group showed a Mean ± SD of 385.03 ± 6.44, 51.07 ± 1.38 respectively indicating significant oxidative stress and impaired antioxidant defense. Treatment with Se significantly decreased MDA level with Mean ± SD of tissue level of 61.07 ± 3.02 with a visible elevation of GPx level with Mean ± SD of tissue level of 112.11 ± 3.37.

5- Serum Levels of ACTH and Corticosterone:

Treatment of Se significantly decreased the serum level of ACTH (216.47 ± 4.91) and increased serum level of corticosterone (12.31 ± 1.01) compared to the control group (Figs. 9,10).

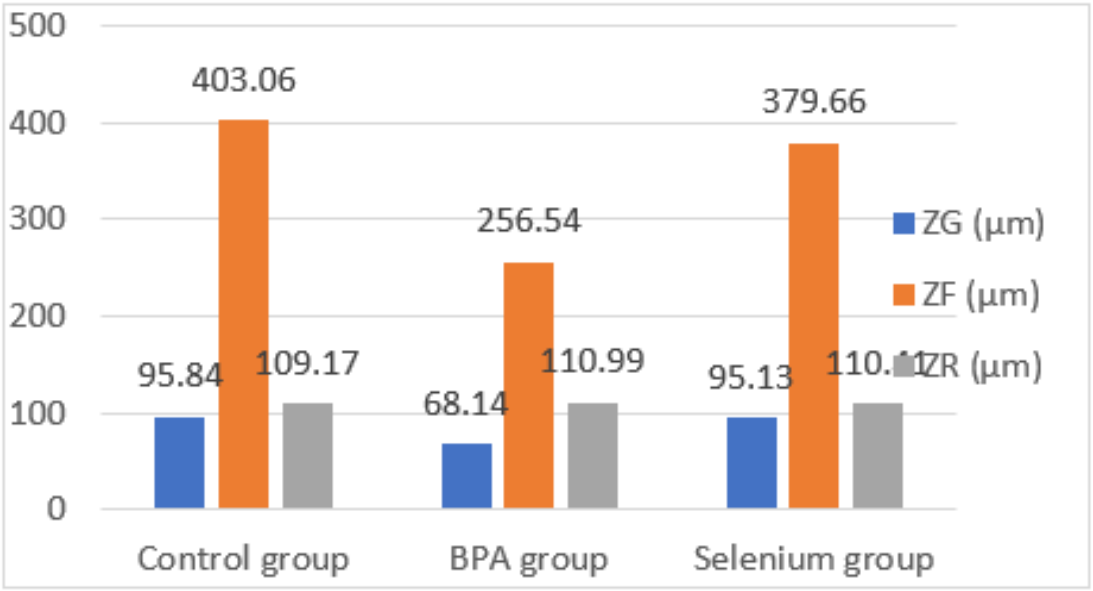

Fig. 4 Bar chart shows mean thickness of ZG, ZF and ZR in all groups.

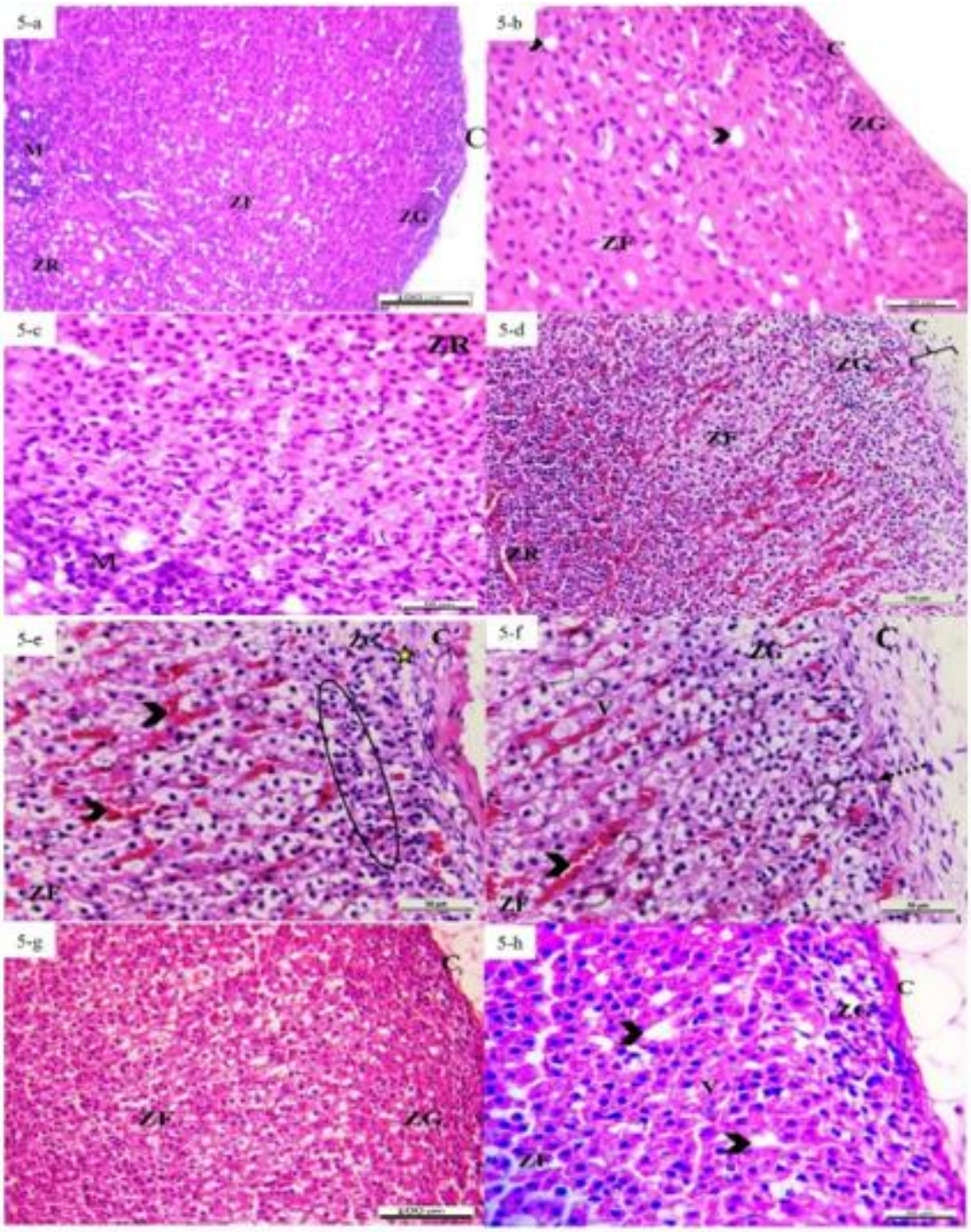

Fig. 5 The morphology of the adrenal cortex in various groups. Fig. 5- a-c shows normal adrenal gland structure in the control group: the capsule (C), The medulla (M) is also observed, with the regular polyhedral cells of ZG and ZF arranged as regular columns of oval cells separated by blood sinusoids (arrowheads). Fig. 5-d-f, the BPA-affected group shows disruption of part of the covering capsule (C) and the three cortical layers, thickening of the covering capsule (C) and its separation from ZG (yellow star); marked disorganization of ZG cells; and congested blood sinusoids between the ZF cells (arrowheads). Notice: A narrow zone of closely packed cells of zona intermedia is seen between the ZG and ZF (marked area), marked congestion (arrowhead), and vacuolation (V) in ZG and ZF layers. Fig 5- g, h Selenium group shows a thin fibrous connective tissue capsule (C) and three layers of adrenal cortex with restoration of the normal arrangement of the cells of ZG and adjacent ZF with minimal vacuolations (V). The intercellular blood sinusoids show no congestion (arrowheads) (ZG: zona glomerulosa, ZF: zona fasciculata, and ZR: zona reticularis) . The scale bar measures 100 m in (Fig. 5-a, g), 50 m in (Fig. b-f, h), and H&E.

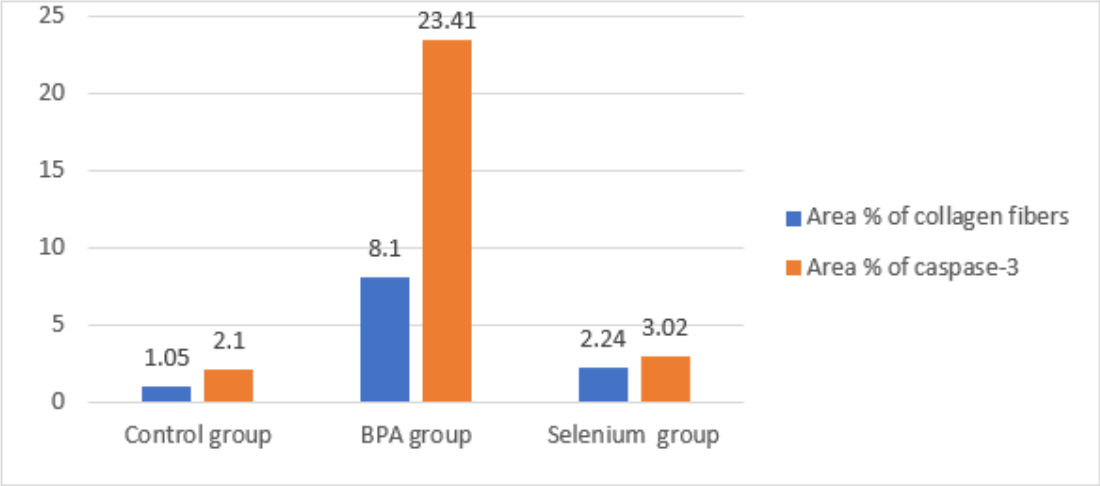

Fig. 6 presents a bar chart that displays the mean percentage area of collagen fibers stained with Masson's trichrome and the levels of caspase-3 across all groups.

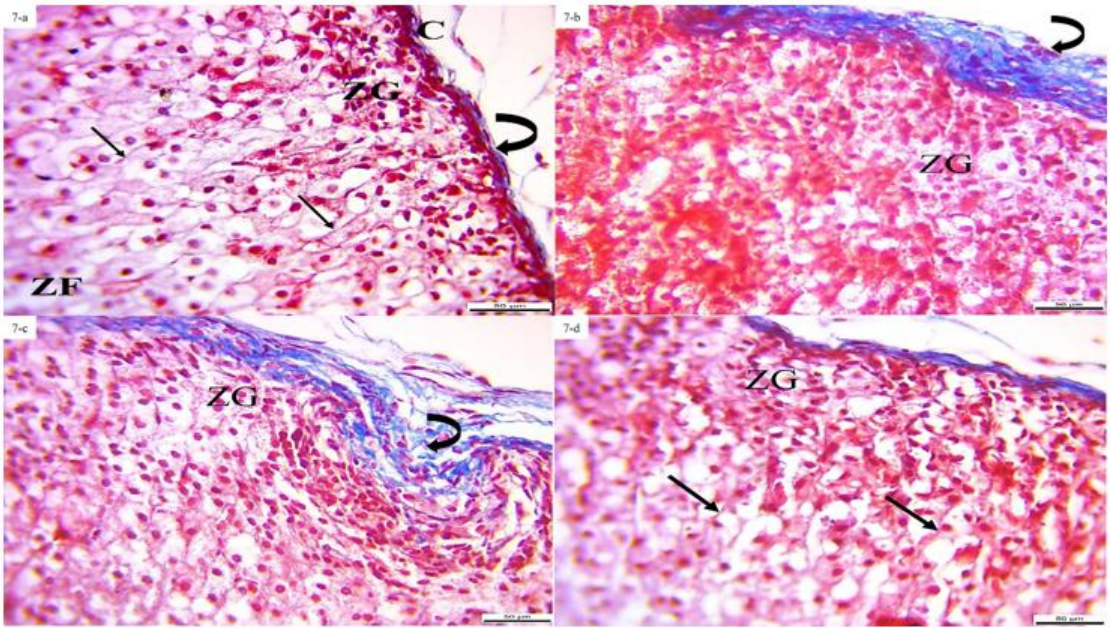

Fig. 7 The collagen fiber distribution in the adrenal cortex in various groups: Fig 7a—The control group shows the capsule (C) with a fine network of collagen fibers (curved arrow). The capsule (C) exhibits a regular arrangement of parallel collagen fibers that intervene between the columns of ZF cells, as indicated by the arrows. Fig. 7b, c BPA group shows marked thickening and lamellar separation of collagen fibers of the capsule (curved arrow). Fig 7 d- Selenium group shows a thin, regularly arranged capsule with fine parallel collagen fibers intervening between columns of zona glomerulosa (ZG) cells (arrows). The scale bar measures 50 m, Masson's trichrome.

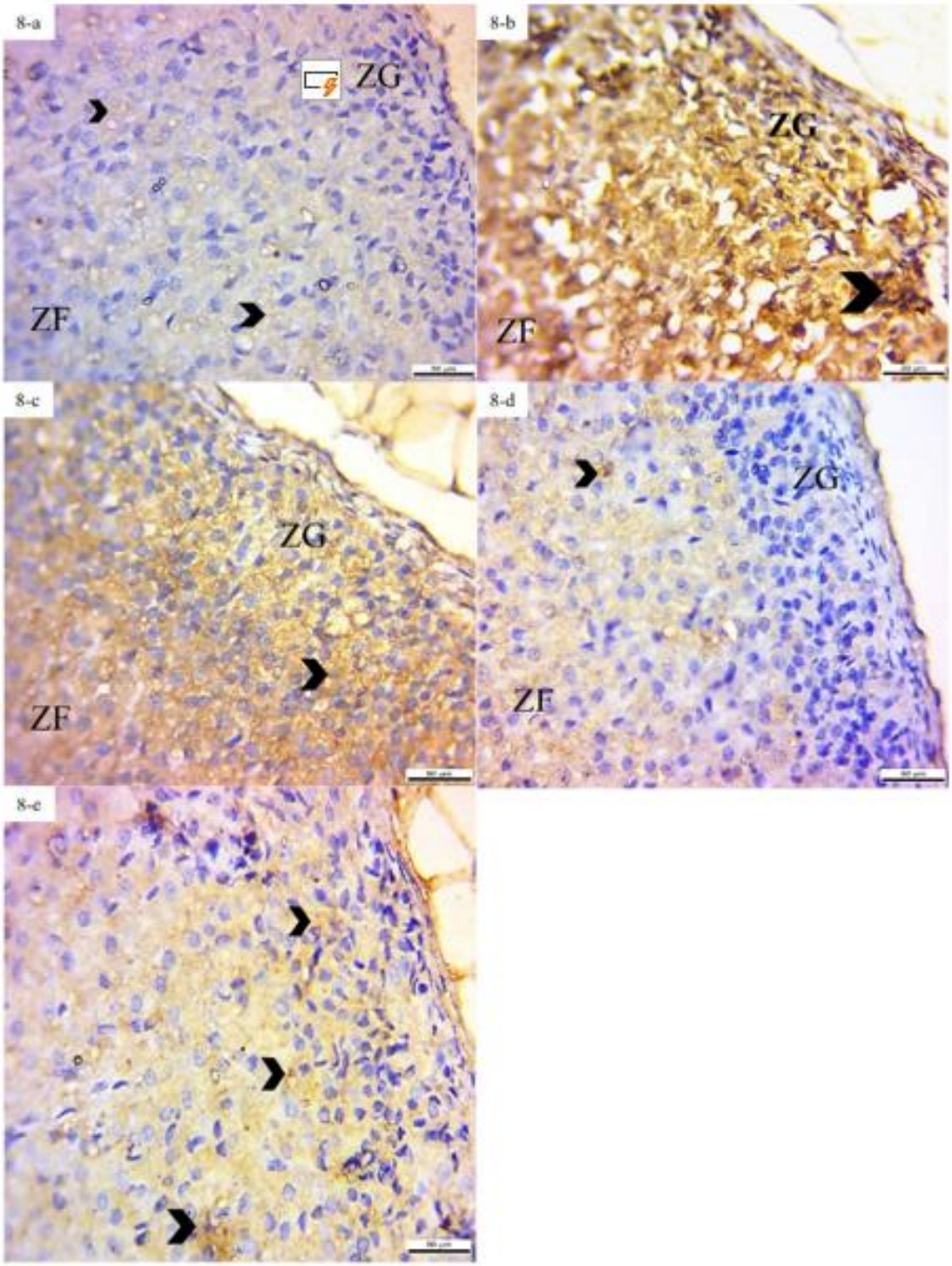

Fig. 8 The caspase 3 immune expression in the adrenal cortex in various groups: Fig. 8a—The control group shows weak caspase 3 immune expression in the ZG and ZF in the form of very light brown coloration (arrowheads). Fig. 8b—The BPA group shows marked caspase 3 immune expression (arrowhead) in the ZG and ZF. Figs. c and d—The selenium group exhibits a mild to moderate immune reaction for caspase 3 (arrowhead) in the ZF, which is more pronounced than in the ZG. The scale bar measures 50 m, Caspase-3

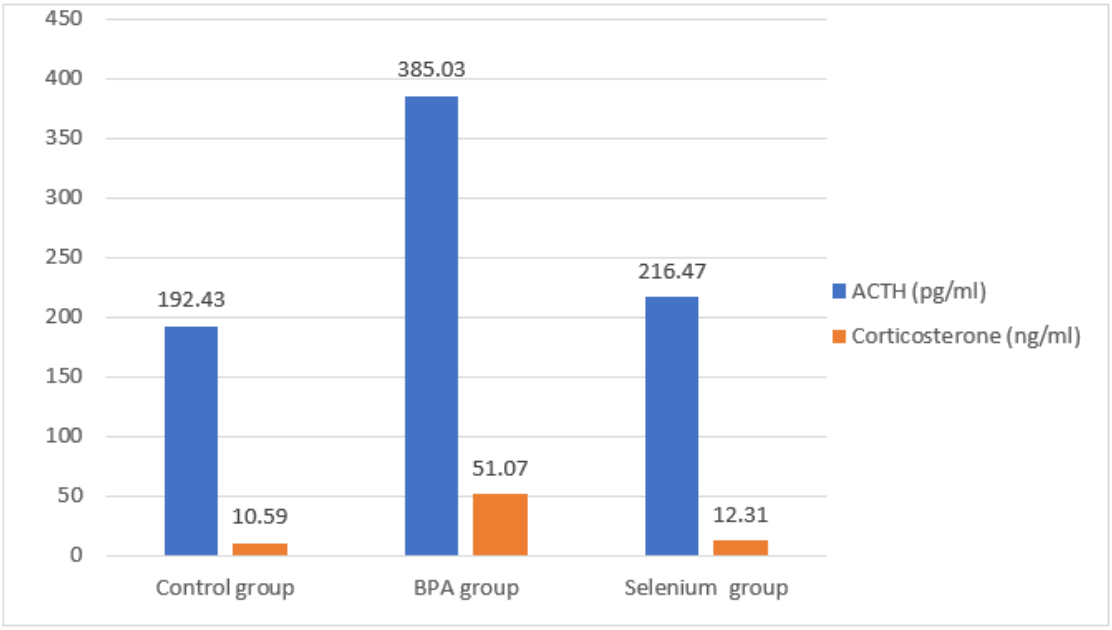

Fig. 9 Bar chart shows the mean serum ACTH and corticosterone levels in all groups

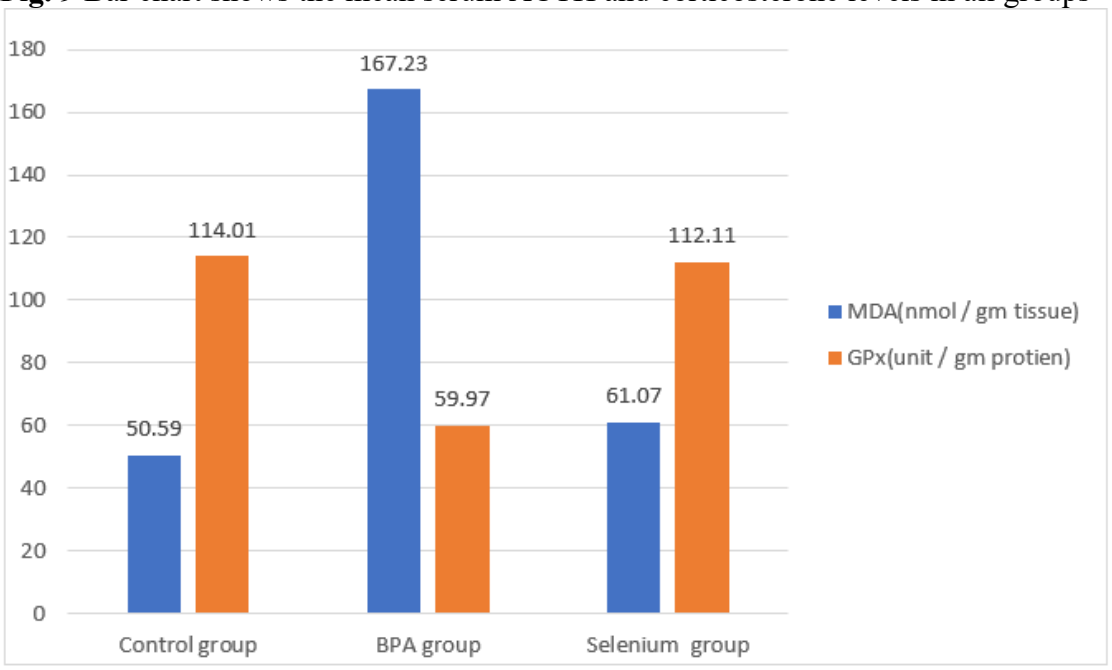

Fig 10. Bar chart shows the mean tissue levels of MDA and GPx in the different groups

DISCUSSION

In this study, BPA exposure produced significant histological, biochemical, and immunohistochemical alterations in the adrenal cortex of adult male albino rats. These changes included distortion of the normal zonal organization, cytoplasmic vacuolations, capsular thickening with lamellar separation of collagen fibers, and increased caspase-3 immunoreactivity. Collectively, these findings confirm that BPA induces oxidative stress–mediated structural damage and apoptosis within the adrenal cortex. Se supplementation effectively ameliorated these alterations, highlighting its protective, antioxidant, and anti-apoptotic properties.

Adrenocortical Structural Changes:

BPA disrupted the normal arrangement of cells in all cortical zones (ZG, ZF, and ZR), with nuclear pyknosis, vacuolation, and karyolysis, consistent with apoptotic cell death. These findings agree with previous studies reporting that oxidative stress induced by BPA promotes adrenal cell degeneration and apoptosis. Vacuolation observed in the ZG and ZF likely represents an early stage of degeneration, as described by

Laast *et al.* (2014). Congested and dilated blood sinusoids, observed in the BPA group, may be explained by excessive ACTH release and prostaglandin-mediated vascular changes, in line with Zidan and Elnegris (2013). Se co-treatment reduced both vacuolation and congestion, supporting its protective role reported by Hassan *et al.* (2016) and Abdallah (2018).

Capsular and Fibrotic Alterations:

One of the remarkable findings was capsular thickening and irregular collagen deposition after BPA exposure, which mirrors previous reports on endocrine disruptor–induced fibrosis (Bushra & Hassanin, 2023). Since the adrenal capsule plays a critical role in cell renewal and zonation (Vidal *et al.,* 2016), its disruption may impair cortical regeneration. Se administration restored normal capsule architecture, with thin parallel collagen fibers, suggesting an antifibrotic effect. This aligns with recent reports on Se's ability to inhibit fibroblast activation and multi-organ fibrosis (Xiao *et al.,* 2025).

Apoptotic Pathways:

Immunohistochemical analysis showed a marked increase in caspase-3 expression following BPA exposure, reflecting activation of the intrinsic apoptotic pathway. Similar findings were reported by Jiang *et al.* (2020) and Omran *et al.* (2017), confirming BPA's ability to trigger apoptosis through excessive ROS generation. Se treatment significantly attenuated caspase-3 expression, consistent with its known anti-apoptotic effects (Albrakati *et al.,* 2021; Mehanna *et al.,* 2022; Zhang *et al.,* 2024). These results reinforce the concept that Se confers cellular protection by limiting oxidative stress and blocking downstream apoptotic signaling.

Biochemical Findings:

The biochemical assays further support the histological observations. BPA exposure significantly elevated MDA levels, indicating enhanced lipid peroxidation, and reduced GPx activity, reflecting impaired antioxidant defenses. These findings are consistent with earlier studies demonstrating oxidative degradation of adrenal phospholipids by BPA (Yiin *et al.,* 2000; Anjum *et al.,* 2011). Se supplementation reversed these changes by lowering MDA levels and enhancing GPx activity, in agreement with previous reports of its ROS-scavenging and enzyme-stabilizing effects (Zaki *et al.,* 2020; Ahmed Zaki *et al.,* 2021).

Hormonal Dysregulation:

BPA exposure was associated with elevated serum ACTH and corticosterone levels, reflecting hypothalamic–pituitary–adrenal (HPA) axis hyperactivation. Similar results were described by Olukole *et al.* (2019), who attributed such changes to disruption of adrenal steroidogenesis. Se treatment normalized these hormone levels, suggesting a modulatory effect on HPA axis regulation. These findings align with previous studies demonstrating Se's ability to attenuate stress responses and endocrine disruption (Al-Amoudi, 2018; Khalaf *et al.,* 2019).

Study Implications and Limitations:

Taken together, the present results provide evidence that Se mitigates BPA-induced adrenal toxicity by reducing oxidative stress, preventing capsular fibrosis, downregulating caspase-3–mediated apoptosis, and restoring hormonal balance. This highlights Se as a promising protective agent against endocrine disruptor–related adrenal injury.

However, the study has limitations: the sample size was small, long-term effects were not assessed, and mechanistic pathways beyond oxidative stress and apoptosis (such as mitochondrial function or signaling molecules) were not investigated. Moreover, extrapolation to humans should be cautious, as BPA exposure levels, Se bioavailability, and adrenal physiology differ across species.

Conclusion

This study demonstrates that BPA induces significant structural and functional injury in the adrenal cortex

through oxidative stress, fibrosis, and caspase-3–mediated apoptosis. Supplementation effectively attenuated these alterations by restoring cortical architecture, reducing collagen deposition, normalizing oxidative stress markers, and modulating ACTH and corticosterone levels.

These findings suggest that Se confers a protective effect against BPA-induced adrenal toxicity and may represent a potential adjunct in mitigating endocrine disruptor–related oxidative damage. However, translation to clinical practice requires further investigation, particularly regarding dose optimization, long-term safety, and pharmacokinetic profiles in humans.

These experimental findings may pave the way for translational studies evaluating Se as an adjunctive protective strategy in humans exposed to endocrine disruptors.

List of Abbreviations

ACTH	Adrenocorticotropic Hormone
BPA	Bisphenol A
CORT	Corticosterone
EDC	Endocrine Disrupting Chemical
GPx	Glutathione Peroxidase
HPA	Hypothalamic-Pituitary-Adrenal
MDA	Malondialdehyde
NOAEL	no-observed-adverse-effect level
ROS	Reactive Oxygen Species
Se	Selenium
SS	Sodium Selenite
ZF	Zona Fasciculata
ZG	Zona Glomerulosa
ZR	Zona Reticularis
ZU	Zone of Undifferentiated cells (Zona intermedia)

Declarations:

Ethics Approval: The study was conducted in accordance with ethical standards for animal research and approved by the Institutional Animal Care and Use Committee (IACUC) under protocol number CU-III-F-2-23.

Competing Interests: The authors declare that they have no competing interests

Author contribution: All authors contributed equally to this work and approved the final version of the manuscript.

Data Availability Statement: Data supporting the findings of this study are available from the corresponding author upon reasonable request

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