IJMA

INTERNATIONAL JOURNAL OF MEDICAL ARTS

VOLUME 6, ISSUE 12, December 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780

Available online at Journal Website
https://ijma.journals.ekb.eg/
Main Subject [Basic Sciences]

Original Article

Neuroprotective Potential of Sericin Against Bisphenol A-Induced Neurotoxicity in Male Rats: Insights into Oxidative Stress and Neuro-Inflammatory Mechanisms

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Article information

Received: 20-10-2024

Accepted: 6-12-2024

DOI: 10.21608/ijma.2024.329937.2058.

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Citation: Hassan EH, El-Samad LM. Neuroprotective Potential of Sericin Against Bisphenol A-Induced Neurotoxicity in Male Rats: Insights into Oxidative Stress and Neuro-Inflammatory. IJMA 2024 Dec; 6 [12]: 5178-5188 doi: 10.21608/ijma.2024.329937.2058.

Abstract

Background: Bisphenol A [BPA] is known to negatively impact brain function by inducing oxidative stress in brain tissues. Sericin, a silk-derived protein, may offer neuroprotective effects by mitigating BPA-induced physiological stress and neurotoxicity.

Patients and Methods: Adult male albino rats were divided into four groups: Control, BPA, Sericin + BPA, and Sericin, with five rats per group. Over a 60-day treatment period, physiological assessments were conducted to measure antioxidants, inflammatory markers, and neurotransmitters in brain tissues.

Results: Sericin significantly reduced oxidative stress, as well as pro-inflammatory markers TNF-α, IL-6, and β-amyloid 1-42, compared to the BPA group. It also improves brain function by enhancing antioxidant enzyme activity and increasing the levels of serotonin, norepinephrine, and GABA. Additionally, sericin treatment lowered brain ATPase and Na/K-ATPase activity, while downregulating the expression of p53 and caspase 3 genes, both of which are involved in apoptosis.

Conclusion: Sericin exerts a neuroprotective effect by reducing BPA-induced oxidative stress, neuroinflammation, and neurotoxicity. Sericin's antioxidant and anti-inflammatory properties suggest it may have potential as a novel therapeutic agent for preventing neurotoxicity associated with environmental toxins like BPA.

Keywords: Bisphenol A; Neurotoxicity; Sericin; Oxidative Stress; TNF-α; IL-6

INTRODUCTION

Neurotoxicity refers to the potential of a substance to harm the central nervous system, peripheral nerves, or sensory organs [1].

A substance is considered neurotoxic if it alters the chemistry or structure of the nervous system, leading to a consistent pattern of brain dysfunction [2]. The manifestations of neurotoxicity vary depending on the type of chemical, dosage, duration of exposure, and the characteristics of the individual exposed [3].

Neurotoxins may enter the body through injection, ingestion, skin contact, or inhalation, leading to neuronal dysfunction or interference with interneuronal communication, which can result in both short- and long-term effects [4]. Monomers, a broad class of reactive substances, are widely used in the chemical synthesis of polymers [5], resins [6], and plastics [7].

Occupational exposure to neurotoxic monomers is common in industries involved in the production, transportation, and use of plastic and chemical products [8]. One of the most widely used synthetic monomers is Bisphenol A [BPA; IUPAC name: 4,4′-[propane-2,2-diyl] diphenol], which forms epoxy resins and polycarbonate plastics upon polymerization. BPA is a significant contributor to the pervasive plastic pollution in the modern environment [9]. BPA has been shown to cause neurotoxicity by impairing synaptic plasticity, inhibiting neurogenesis, inducing oxidative stress, and promoting autophagy and apoptosis [10]. Additionally, BPA exposure has been linked to inflammation, oxidative stress, and insulin resistance [11].

Although the full extent of BPA's effects on the nervous system remains poorly understood, environmental exposure to BPA is known to influence brain physiology and development by compromising endogenous defence mechanisms [12].

BPA exposure has also been associated with an increased risk of stroke [10, 12]. In recent years, the use of natural products has gained attention due to their diverse biological benefits [13]. One such natural product is sericin, a protein secreted by the silk gland of silkworms [*Bombyx mori*]. Sericin, a hydrophilic polymer, is rich in polar side groups [hydroxyl, carboxyl, and amino groups] and contains high concentrations of serine, aspartic acid, and glycine [14].

Notably, sericin has demonstrated antioxidant properties, preventing lipid peroxidation in rat brain homogenates[15]. Furthermore, sericin is non-allergenic [16] and serves as a promising foundation for the development of anti-inflammatory compounds [17].

The aim of this study was to evaluate the potential of sericin as a therapeutic agent to mitigate neurological damage induced by Bisphenol A.

METHODOLOGY

Reagents: Bisphenol A [BPA] was obtained from Loba Chemie [India] as white crystals with a purity of 98%, intended for fine chemicals and laboratory reagents. Sericin was supplied by Sigma Aldrich [Germany].

Exposure Techniques and Animals: Twenty adult male Wistar albino rats, each weighing between 130 and 140 grams, were procured from the vivarium of the Faculty of Medicine at Alexandria University, Egypt. The rats were accommodated in stainless steel enclosures with unimpeded access to tap water and conventional rodent feed. The subjects were kept under regulated conditions: 25 ± 5 °C, 50–60% relative humidity, and a 12-hour light/dark cycle. Following a two-week acclimatization phase, the rats were randomly assigned to four groups, each consisting of five individuals.

1. Control group: Received 0.5 ml of pure water.

2. The BPA group received 0.5 ml of BPA dissolved in maize oil at a dosage of 50 mg/kg body weight each day [18].

3.The Sericin + BPA group received 0.5 ml of Sericin dissolved in distilled water at a dosage of 1 g/kg body weight per day and 0.5 ml of BPA dissolved in maize oil at a dosage of 50 mg/kg body weight per day for a duration of 60 days [19].

4. Sericin group administered sericin [1 g/kg BW/day] dissolved in distilled water, accompanied with weekly intraperitoneal saline injections. Sericin was solubilized in deionized water and vortexed for five minutes at ambient temperature. The rats were weighed every five days to calibrate the doses of BPA and sericin according to their body weight. Daily preparations of fresh BPA and sericin solutions were made, with dosages estimated based on the rats' latest body weight. The experimental design and techniques received approval from the Institutional Animal Care and Use Committee [IACUC] of Alexandria University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Measures were implemented to alleviate the animals' discomfort during the study [20].

Blood Collection and Tissue Preparation

At the end of the 60-day experiment, the rats underwent a 12-hour fast before being killed. Four milliliters of blood were obtained by heart puncture utilizing sterile syringes. The blood was centrifuged at 1000 × g for 10 minutes at 25 °C to isolate serum, which was subsequently kept at −20 °C for lipid profile analysis [21]. The brain tissues were promptly excised, and the connective tissues were eliminated. Approximately 50% of the cerebral tissue was rapidly frozen in liquid nitrogen and preserved at −80 °C for molecular examination. The residual brain tissue was homogenized [10%, w/v] in ice-cold phosphate buffer [0.01 M, pH 7.4] with 1.15% KCl. The homogenate underwent centrifugation at 10,000 × g for 20 minutes at 4 °C using a Hitachi Ltd. model EBA 12R. Aliquots of the supernatant were preserved at −80 °C for future biological investigations.

Lipid Profile Assessment:

The serum lipid profile, including total cholesterol, triglycerides, and HDL, was measured using a diagnostic analyzer [Dimension RXL, Siemens, Germany] and commercial kits specific to each analyte. LDL was calculated using the Friedewald equation [22].

Measurement of Proinflammatory Cytokines and β-Amyloid: The levels of TNF-α, IL-6, and β-amyloid 1-42 in brain homogenates were measured using enzyme-linked immunosorbent assay [ELISA] kits, following the protocols of **Hedayati *et al.*** [23].

Oxidative Stress Biomarkers: Total protein concentration in brain tissue [mg/mg tissue] was determined using the Lowry et al.

method [24]. Lipid peroxidation, as indicated by malondialdehyde [MDA] and nitric oxide [NO] levels, was measured using the Ohkawa et al. protocol [25].

Assessment of Antioxidant and Detoxifying Activities in Brain Tissue: The activity of superoxide dismutase [SOD] was assessed utilizing a modified method from Misra and Fridovich [26], which relies on the enzyme's capacity to inhibit the auto-oxidation of adrenaline in an alkaline solution [pH 10.2]. Catalase [CAT] activity was evaluated utilizing the Aebi method [27], whereas glutathione-S-transferase [GST] and reduced glutathione [GSH] activities were quantified following the protocols established by **Habig *et al.*** [28] and **Jollow *et al.*** [29], respectively. The activity of glutathione peroxidase [GPx] was assessed utilizing the methodology established by **Mohandas *et al.*** [30].

Gene Expression and Molecular Analysis:

RNA was extracted from brain tissues, followed by cDNA synthesis. Caspase-3 gene expression was analyzed using real-time polymerase chain reaction [RT-PCR] [31-32].

mRNA Expression of p53: p53 mRNA levels were quantified by reverse-transcriptase PCR. Specific primers were employed for cDNA synthesis and target gene amplification, adhering to the Maxime RT-PCR Premix Kit procedure [Qiagen, Germany]. The primers for p53 were as follows:

- Forward: 5′-GTATTTCACCCTCAAGATCC-3′
- Reverse: 5′-TGGGCATCCTTTAACTCTA-3′

Neuro-Specific Biomarkers: The activities of total ATPase and Na+/K+-ATPase in brain supernatants were quantified calorimetrically utilizing ELISA kits [ImmunoWay Biotechnology, USA, and MyBioSource, USA], in accordance with the manufacturer's guidelines. The activities were quantified in µmol/min/mg protein. Dopamine [DA] and serotonin concentrations in cerebral tissue were measured utilizing solid-phase ELISA kits [GenWay Biotech, USA].

Neurotransmitter Assessment: Neurotransmitter levels, such as serotonin, glutamate, GABA, and norepinephrine, were quantified in brain homogenates utilizing solid-phase ELISA kits, following the manufacturer's guidelines [ImmunoWay, USA; SunRed, Shanghai, China].

Statistical Analysis: All data were presented as mean ± standard deviation [SD]. Statistical analysis was conducted with GraphPad Prism 5 software [San Diego, California]. One-way analysis of variance [ANOVA] was employed for group comparisons, succeeded by Tukey's post hoc test. Statistical significance was established at $p < 0.05$.

RESULTS

Lipid Profile Response: Table [1] delineates the amounts of total cholesterol [TC], triglycerides [TG], LDL-C, and HDL-C in the brain tissues across various ethnicities. BPA therapy, relative to the control group, led to a considerable [$P < 0.05$] elevation in TC, TG, and LDL-C levels, as well as a significant [$P < 0.05$] decrease in HDL-C. In the Sericin + BPA group, there was a significant [$P < 0.05$] decrease in TC, TG, and LDL-C levels, alongside a substantial [$P < 0.05$] increase in HDL-C, suggesting that the oral administration of sericin prior to BPA exposure enhanced the lipid profile. Moreover, the administration of sericin alone considerably [$P < 0.05$] improved the lipid profile in comparison to both the control and BPA groups.

Antioxidant and Oxidative Stress Parameters: Figures 1[a-b], 2[a-b], and 7[a-b] illustrate the impact of BPA and sericin on antioxidant and oxidative stress metrics. Rats subjected to BPA demonstrated markedly elevated concentrations of malondialdehyde [MDA] and nitric oxide [NO] in their brains relative to the control group [$P < 0.05$]. BPA exposure resulted in a significant [$P < 0.05$] reduction in the levels of reduced glutathione [GSH], total thiol, and the activity of essential antioxidant enzymes—glutathione peroxidase [GPx], superoxide dismutase [SOD], and glutathione-S-transferase [GST]—relative to the control group. Pre-treatment with sericin in the Sericin + BPA group considerably mitigated oxidative stress, as indicated by a substantial rise [$P < 0.05$] in GSH, total thiol, GPx, SOD, and GST activities, alongside a notable drop [$P < 0.05$] in MDA and NO levels relative to the BPA group. Furthermore, the administration of sericin alone resulted in either enhancements compared to control values or a return to control levels, signifying a protective effect of sericin.

Evaluation of Inflammatory Biomarkers: The effects of prolonged BPA exposure, both alone and in combination with Sericin treatment, on the activity of TNF-α, IL-6, and β-amyloid 1-42 in brain cells were assessed. The results are presented in Figures 3 [a-b] and 7 [c]. Rats' Brain Levels of Neurotransmitters in Relation to BPA and/or Sericin. The levels of serotonin, norepinephrine, glutamate, and GABA in the brains of the experimental groups are summarized in Figures 4 [a–b] and 7 [d]. The results indicated that BPA-treated rats exhibited significantly higher levels of glutamate [$P < 0.05$] and significantly lower levels of norepinephrine, GABA, and serotonin compared to control rats. In contrast, the Sericin + BPA group, which received Sericin prior to BPA exposure, showed a significant increase [$P < 0.05$] in norepinephrine, GABA, and serotonin levels, along with a decrease in glutamate levels, compared to the BPA group. Furthermore, significant differences in dopamine and serotonin levels were observed between the Sericin group and the control group.

Rat Brain Total ATPase and Na/K-ATPase Activity in Relation to BPA and/or Sericin: The activity of Na/K-ATPase and total ATPase in the BPA-treated group were considerably diminished [$P < 0.05$] relative to the control group. Oral administration of sericin, whether either alone or before BPA exposure, significantly [$P < 0.05$] enhanced the activities of Na/K-ATPase and total ATPase, as illustrated in Figures 5 [a–b] and 7 [e].

Effects of Sericin and/or BPA on the Levels of Gene Expression in the Brains of Rats: Caspase 3 and p53: Figures 6 [a–b] and 7 [f] illustrate the impact of prolonged BPA exposure, either alone or following Sericin administration, on the expression of the Caspase 3 and p53 genes in brain cells across the treatment groups, with β-actin as the reference gene. BPA treatment [lane 2] significantly [$P < 0.05$] increased the expression of p53 and Caspase 3 compared to the control group [lane 1]. However, in both the Sericin-treated group [Sericin group] and the Sericin + BPA group, the oral administration of sericin resulted in a significant [$P < 0.05$] reduction in the expression of Caspase 3 and p53 mRNA in the brain [lanes 3 and 4]. In these groups, the expression levels of Caspase 3 and p53 were nearly undetectable and did not statistically differ from those observed in the control group.

Table [1]: Lipid Profile Parameters

Parameters	Control	Bisphenol A	Bisphenol A + Sericin	Sericin	F[P]
Total Cholesterol [TC] [mg/dL]	183.41 ± 10.27	393.20 ± 15.19	219.78 ± 15.50	167.76 ± 12.38	295.14 [<0.001]*
Triglycerides [TG] [mg/dL]	135.18 ± 6.58	223.64 ± 13.48	175.96 ± 8.59	131.22 ± 6.22	110.27 [<0.001]*
LDL-C [mg/dL]	81.92 ± 9.53	156.00 ± 7.00	121.80 ± 8.47	67.00 ± 7.91	117.98[<0.001]*
HDL-C [mg/dL]	64.66 ± 8.03	44.62 ± 8.22	57.78 ± 8.90	75.76 ± 7.62	12.61 [<0.001]*

n = 5, SD = Standard Deviation, F = One-Way ANOVA, *P < 0.05

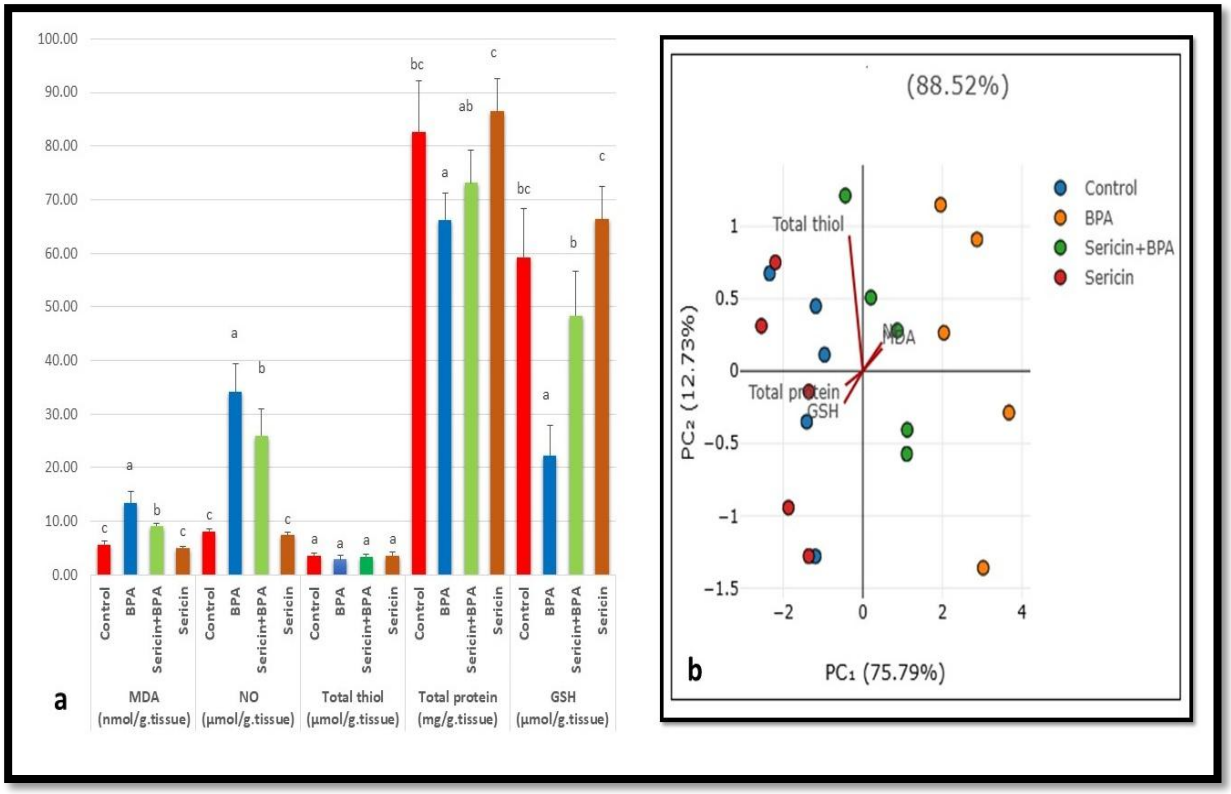

Figure [1]: [a] Concentrations of oxidative stress markers and antioxidant parameters across the four experimental groups: Control, BPA, Sericin + BPA, and Sericin. [b] Principal Component Analysis [PCA] illustrating the similarities and variations among the oxidative stress and antioxidant biomarkers measured in the Control, BPA, Sericin + BPA, and Sericin groups.

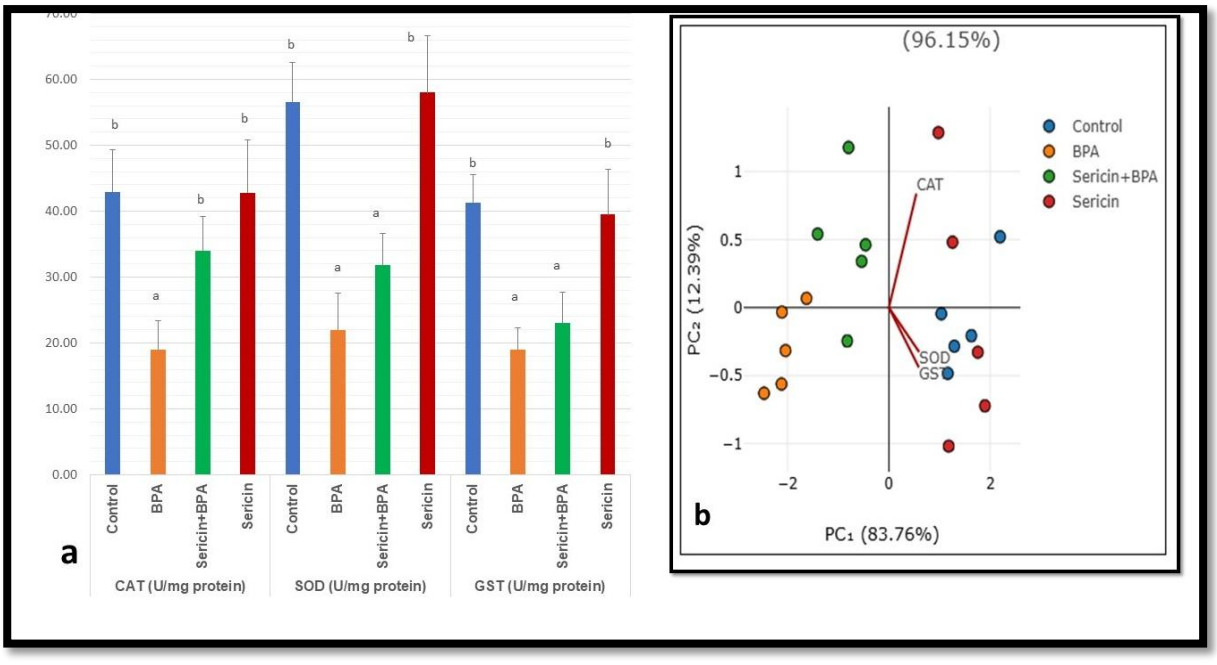

Figure [2]: [a] Concentrations of antioxidant parameters in the Control, BPA, Sericin + BPA, and Sericin groups. [b] Principal Component Analysis [PCA] illustrating the similarities and differences among the antioxidant biomarkers measured in the Control, BPA, Sericin + BPA, and Sericin groups.

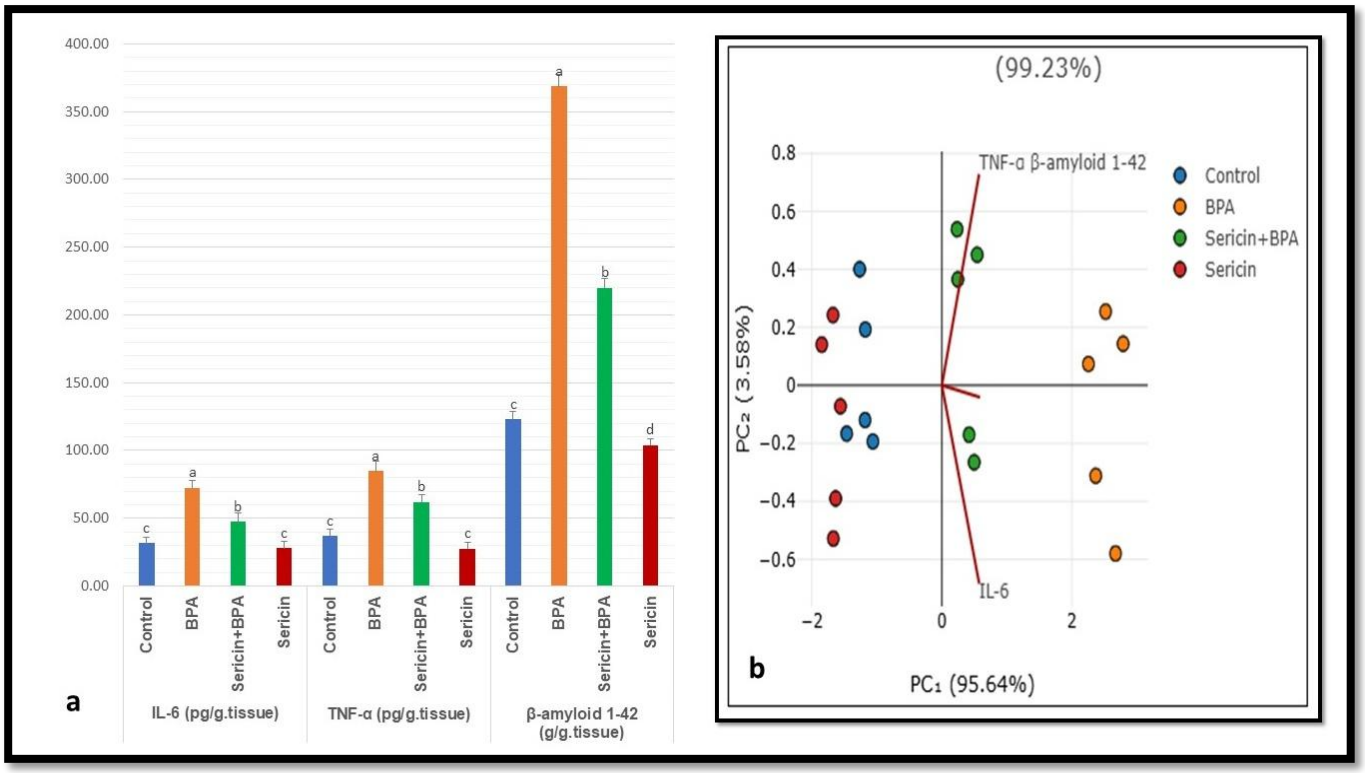

Figure [3]: [a] The activity of inflammatory markers [TNF-α, IL-6, and β-amyloid 1-42] in the Control, BPA, Sericin + BPA, and Sericin groups. [b] Principal Component Analysis [PCA] illustrating the similarities and differences among the inflammatory markers measured in the Control, BPA, Sericin + BPA, and Sericin groups.

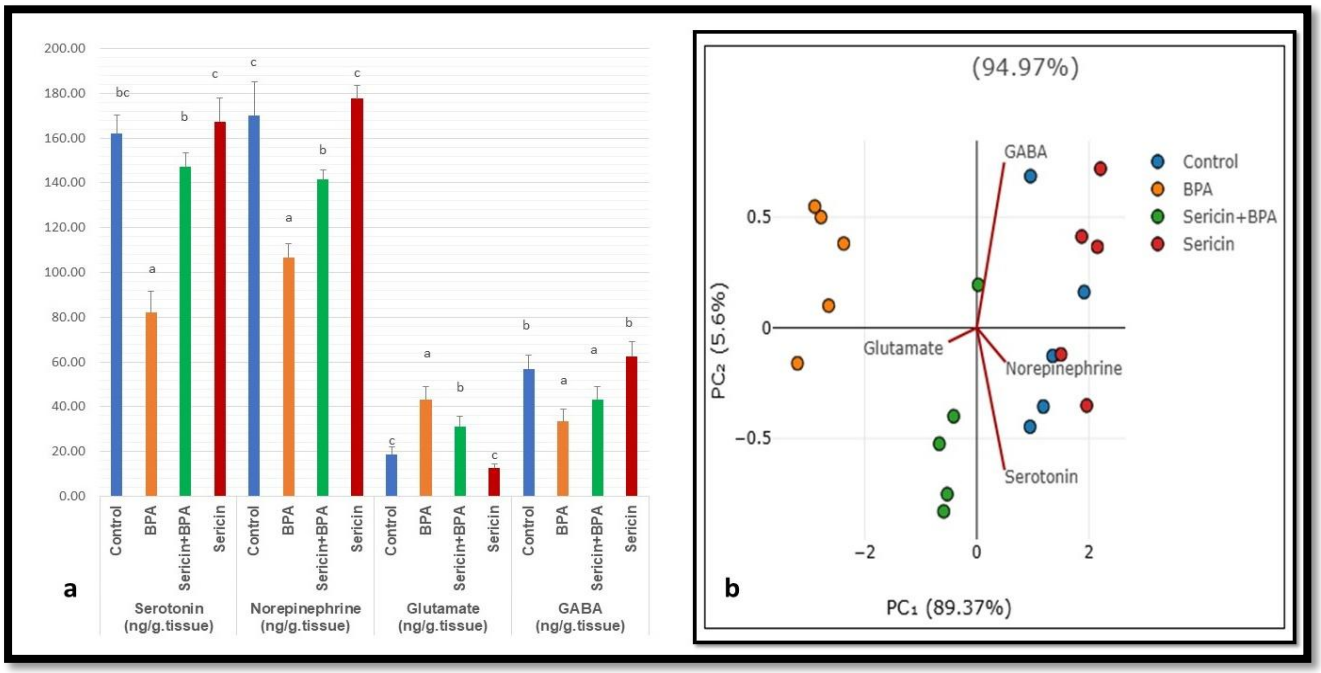

Figure [4]: [a] Concentrations of neurotransmitters [serotonin, norepinephrine, glutamate, and GABA] in the Control, BPA, Sericin + BPA, and Sericin groups. [b] Principal Component Analysis [PCA] illustrating the similarities and differences among the neurotransmitter markers measured in the Control, BPA, Sericin + BPA, and Sericin groups.

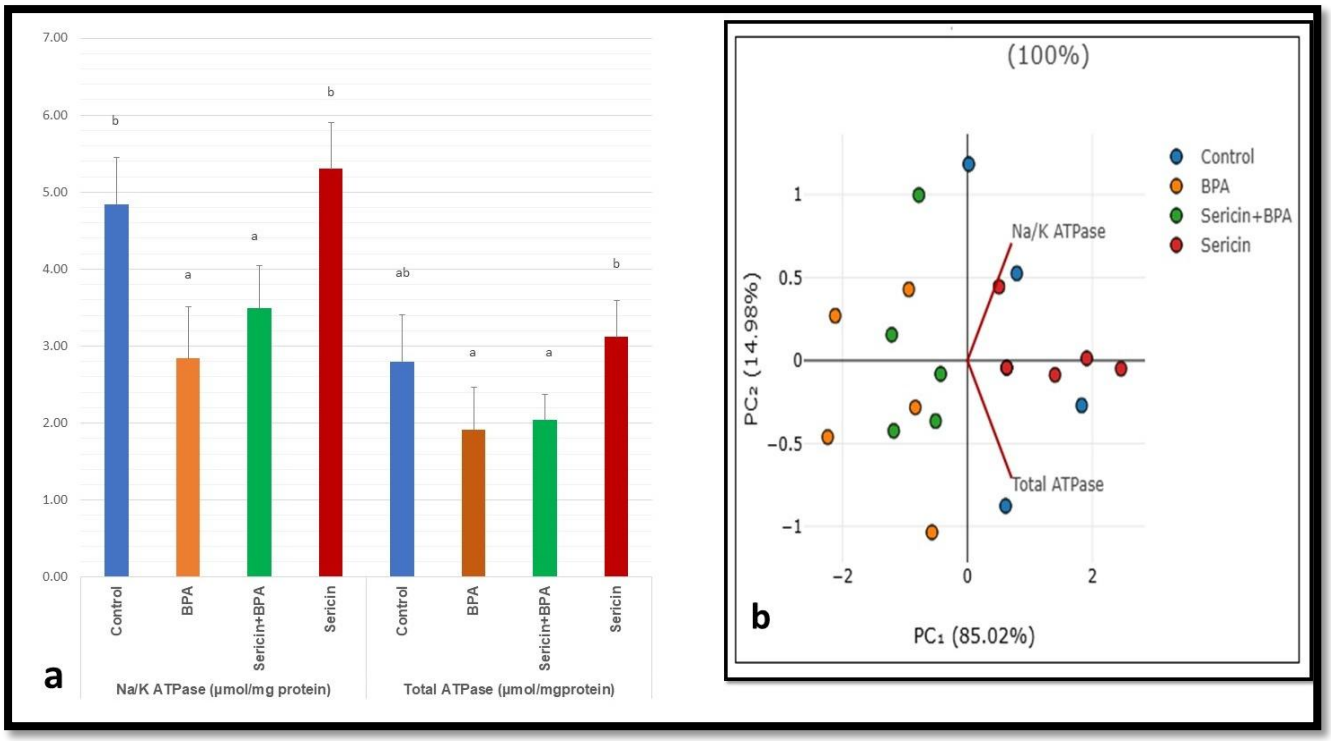

Figure [5]: [a] Activities of Na/K-ATPase and Total ATPase in the Control, BPA, Sericin + BPA, and Sericin groups. [b] Principal Component Analysis [PCA] illustrating the similarities and differences among Na/K-ATPase and Total ATPase markers measured in the Control, BPA, Sericin + BPA, and Sericin groups.

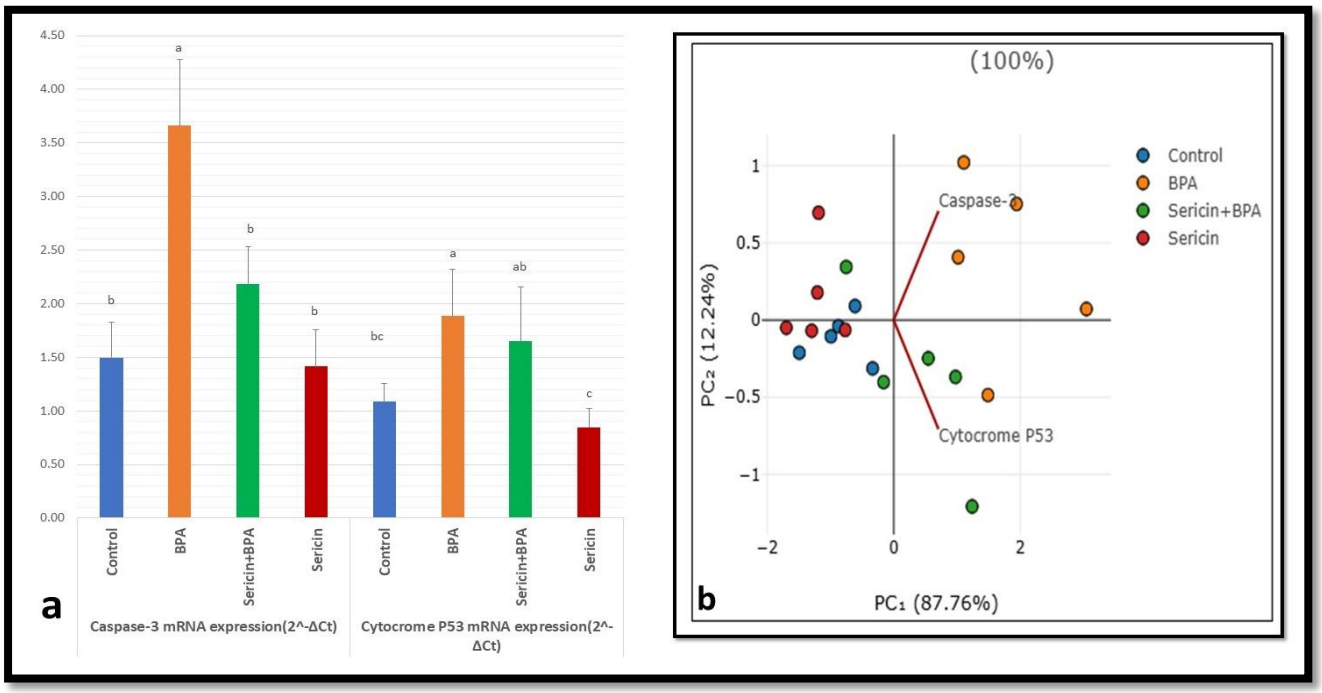

Figure [6]: [a] Gene expression levels of Caspase 3 and p53 in the Control, BPA, Sericin + BPA, and Sericin groups. [b] Principal Component Analysis [PCA] illustrating the similarities and differences in Caspase 3 and p53 gene expression among the Control, BPA, Sericin + BPA, and Sericin groups.

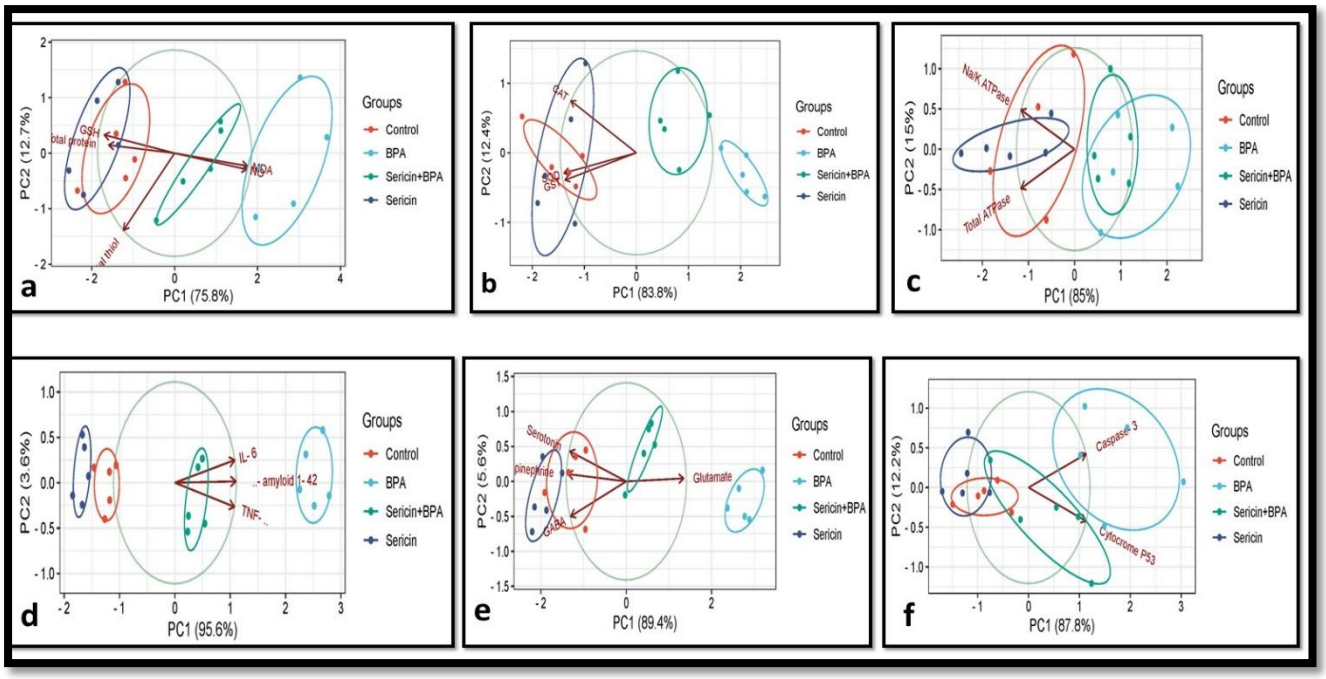

Figure [7]: [a] 2D Principal Component Analysis [PCA] plot of oxidative stress parameters in the Control, BPA, Sericin + BPA, and Sericin groups. [b] 2D PCA plot of antioxidant parameters in the Control, BPA, Sericin + BPA, and Sericin groups. [c] 2D PCA plot of inflammatory markers in the Control, BPA, Sericin + BPA, and Sericin groups. [d] 2D PCA plot of neurotransmitter markers in the Control, BPA, Sericin + BPA, and Sericin groups. [e] 2D PCA plot of Na/K-ATPase and Total ATPase markers in the Control, BPA, Sericin + BPA, and Sericin groups. [f] 2D PCA plot of Caspase 3 and p53 gene expression in the Control, BPA, Sericin + BPA, and Sericin groups.

DISCUSSION

Our analysis of the lipid profile measurements showed a significant increase in triglycerides [TG], LDL-C, and total cholesterol [TC] levels in the BPA-treated group, with notable variations observed when compared to the control and Sericin + BPA groups.

These findings align with **Kakihara *et al.*** [33], who also reported increased lipid profile values due to BPA exposure. BPA has been shown to alter the expression of genes related to lipid production in experimental rats [34].

Early-life exposure to BPA has been demonstrated to raise circulating levels of TG, LDL-C, and TC, while reducing HDL-C levels [35-36]. Based on the available data, we hypothesize that chronic exposure to BPA disrupts lipid homeostasis and contributes to the development of dyslipidaemia [37].

In our study, rats exposed to BPA exhibited increased oxidative stress in their brains, as evidenced by elevated levels of malondialdehyde [MDA], total protein, total thiol, and nitric oxide. Concurrently, antioxidant markers such as catalase [CAT], superoxide dismutase [SOD], and glutathione-S-transferase [GST] were significantly decreased compared to the control group. BPA's toxic effects may result from its molecular characteristics, including interactions with hormone receptors and its ability to bind to various membrane and nuclear receptors [38].

BPA exposure has been linked to elevated levels of reactive oxygen species [ROS] and MDA, coupled with a reduction in SOD activity, which can accelerate apoptosis and impair neuronal viability[39].

These results are consistent with recent studies demonstrating that BPA toxicity is closely linked to oxidative stress and its associated markers in various experimental models [40-41].

Oxidative stress and inflammation are interrelated, and both contribute to cellular damage. Chronic inflammation, induced by ROS and nitrogen species produced by immune cells like neutrophils and macrophages, can exacerbate oxidative stress [42].

Our study also observed a decline in SOD, GST, and GSH levels, similar to findings by **Tsen *et al.*** [43], who reported elevated H_2O_2 and lipid peroxidation levels in offspring of dams exposed to BPA, alongside a depletion of antioxidant defence mechanisms.

Gaber *et al.* [44] also highlighted how polyunsaturated lipid peroxidation in the endoplasmic reticulum reduces GST levels and diminishes antioxidant enzyme efficacy.

Our findings also demonstrated a significant rise in inflammatory markers, including IL-6, TNF-α, and β-amyloid 1-42, in the brains of BPA-treated rats. Additionally, BPA exposure elevated p53 gene expression, consistent with findings by **Alizadeh-Fanalou *et al.*** [45], who reported dose-dependent increases in TNF-α, IL-1β, and IL-6 in BPA-exposed mice.

Peinado *et al.* [46] similarly observed increased production of proinflammatory cytokines, such as TNF-α and IL-1β, following BPA exposure. BPA's ability to activate proinflammatory transcription factors further contributes to its harmful effects [47].

The overproduction of ROS and reactive nitrogen species [RNS] can promote the excessive release of proinflammatory cytokines, including TNF-α and IL-6, as well as increase p53 expression [48].

TNF-α can upregulate COX-2, which leads to the overproduction of free radicals and may contribute to neuronal necrosis [49].

Liu and Xu [50] noted that p53 exhibits prooxidative activity, resulting in cell death when antioxidant defences are overwhelmed.

Our data indicated that subchronic high-dose BPA exposure resulted in diminished Na+/K+-ATPase and total ATPase activity in neuronal cells, potentially causing intracellular acidification, calcium overload, and neuronal damage by modifying sodium-calcium exchanger activity [51]. The noted decrease in ATPase activity may result from oxidative damage caused by BPA exposure.

Early-life exposure to BPA has been correlated with neurodevelopmental impairments and congenital anomalies [52-53], and research increasingly associates environmental BPA exposure with neurodegenerative disorders such as Parkinson's, Alzheimer's, and schizophrenia [54-57]. BPA is associated with heightened risks of oxidative stress, hepatotoxicity, developmental toxicity, and reproductive toxicity [58-60].

Our study also found that BPA exposure increased the levels of neurotransmitters such as dopamine, norepinephrine, GABA, and serotonin in the brain.

This is consistent with **Wang *et al.*** [1]**,** who suggested that abnormal neurotransmitter levels, including serotonin and dopamine, may be linked to BPA-induced neurobehavioral damage. The altered levels of dopamine and serotonin may result from BPA's effects on monoamine oxidase [MAO] activity, serotonin release, and dopamine reuptake.

Monzani *et al.* [61] proposed that increased dopamine levels could undergo autoxidation, producing neurotoxic byproducts like hydrogen peroxide.

The administration of sericin demonstrated significant neuroprotective effects against BPA-induced brain damage. Pre-treatment with sericin significantly reduced MDA and NO levels, while increasing GSH, total thiol concentrations, and GPx activity in brain cells, consistent with sericin's antioxidant potential [62-63].

Sericin's hydroxyl groups, which can chelate trace metals like iron and copper, may contribute to its ability to counteract oxidative stress [64-65].

Furthermore, sericin's antioxidant activity increases with smaller molecular size [66]. Sericin's antioxidant properties also stem from its polyphenols and flavonoids, which are effective in scavenging free radicals [67-69].

The pre-treatment of BPA-exposed rats with sericin also alleviated dyslipidemia by reducing TC, TG, and LDL-C levels while raising HDL-C.

These findings are consistent with **Wang *et al.*** [69]**,** who demonstrated sericin's hypocholesterolemic effects by inhibiting cholesterol absorption through decreased bile acid binding and micelle formation. Sericin has also shown promise in restoring total ATPase and Na+/K+-ATPase activity in brain tissues, which further supports its neuroprotective role.

Overall, sericin treatment reduced the expression of caspase-3 and p53, along with the levels of inflammatory markers such as β-amyloid 1-42, TNF-α, and IL-6. These results corroborate previous studies showing that sericin modulates inflammation by inhibiting cytokine production [70-74]. Therefore, sericin may offer a potential therapeutic option for treating neurotoxicity, neuroinflammation, and neurodegenerative diseases.

Conclusion

This study demonstrated the neurotoxic effects of Bisphenol A [BPA] on brain tissues, evidenced by significant alterations in neurotransmitter concentrations, inflammatory markers, and antioxidant activity. Conversely, treatment with sericin effectively mitigated or neutralized the oxidative stress and neurotoxic effects induced by BPA, highlighting its potential as a neuroprotective agent.

Recommendations

Sericin presents a promising application in the development of environmentally friendly food packaging materials. The cost-effective and sustainable production of sericin-based films could be utilized for food packaging, as sericin's properties may help extend food shelf life and reduce food waste.

Ethics Approval

Animal procedures were conducted with the approval of the relevant ethics committee [Approval number: AU1024627301].

Consent for Publication

All authors have reviewed and approved the manuscript for publication.

Disclosure Statement

The authors declare no conflicts of interest.

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IJMA

INTERNATIONAL JOURNAL OF MEDICAL ARTS

VOLUME 6, ISSUE 12, December 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780