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The role of chitosan in mitigating tert-butylhydroquinone induced cognitive impairment and neurotoxicity in a female rat model

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Abstract. – OBJECTIVE: This study aimed to investigate the impact of tert-butylhydroquinone (TBHQ), chitosan, and their combination on memory and neurobiochemical parameters in a rat model. The primary objectives were to assess the cognitive effects of TBHQ, explore the cognitive-enhancing properties of chitosan, and evaluate the combined effects of these substances.

MATERIALS AND METHODS: A rat model was employed for behavioral tests, biochemical analyses, and histological examinations. Rats were exposed to TBHQ, chitosan, or a combination of both, and cognitive function was assessed through behavioral tests. Biochemical analyses focused on neurobiochemical parameters associated with memory and oxidative stress. Histological examinations were conducted to observe any structural changes in the brain.

RESULTS: TBHQ exposure was associated with memory impairments and increased oxidative stress, indicating potential neurotoxic effects. Chitosan supplementation demonstrated cognitive-enhancing effects and showed promise in mitigating the memory impairments and oxidative stress induced by TBHQ. The combination of chitosan and TBHQ presented a potential protective effect on neurological health.

CONCLUSIONS: Chitosan supplementation alongside TBHQ may mitigate memory impairments and oxidative stress associated with TBHQ exposure in a rat model. The study provides valuable insights into the cognitive effects of TBHQ and the neuroprotective potential of chitosan, highlighting the need for further research to elucidate molecular pathways and clinical implications. These findings contribute to understanding chitosan's role in safeguarding neurological health in conditions where TBHQ exposure is a concern, warranting further investigations for translational applications in human health.

Key Words:

TBHQ, Tert-butylhydroquinone, Chitosan, Neurotoxicity, Memory, Cognitive enhancement.

Introduction

Preservatives are essential in food and cosmetics to prevent microbial growth and maintain product quality. However, the presence of certain preservatives, particularly artificial ones like tert-butylhydroquinone (TBHQ), can pose health risks when consumed regularly. TBHQ, widely used as an antioxidant, helps to prevent rancidity in various products[1]. Nonetheless, its extensive use and potential toxicity have raised concerns about its impact on human health. Tert-butylhydroquinone (TBHQ) is an antioxidant in food and cosmetic products, preventing rancidity and extending shelf life[2]. Despite its widespread use, TBHQ is not without potential adverse effects on human health, particularly with regular consumption of artificial preservatives. These substances can lead to hyperactivity and pose toxic risks. Moreover, the availability of TBHQ in various products necessitates accurate detection methods to maintain safety standards[3,4].

In the quest for environmentally friendly solutions, the scientific community is turning to biopolymers, like chitosan, to address various challenges in terms of sustainability, circularity, and energy efficiency. Chitosan, derived from chitin, is a biopolymer with numerous applications in the food industry, cosmetics, biomedicine, and pharmaceuticals. Its biodegradability, biological compatibility, antimicrobial properties, and antioxidant activity make it an attractive material for various purposes. Chitosan, a natural preservative, is gaining attention for its potential to preserve seafood quality by inhibiting microbial growth, enhancing oxidative stability, and safeguarding sensory properties. Additionally, chitosan offers health promotion benefits, including antimicrobial activities and high antioxidant[5-10]. This study is driven by the need to assess the po-

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tential of chitosan to mitigate the neurobehavioral and toxic effects induced by TBHQ, focusing on the brain tissue. The study aims to evaluate behavioral changes using the Morris water maze and passive avoidance tests, examine histopathological changes in brain tissue, and analyze the neurotoxic effects of TBHQ. This research intends to shed light on the efficacy of chitosan as an antioxidant agent in alleviating TBHQ-induced toxicity in the brain, which can have implications for food safety and human health.

Materials and Methods

Chemicals

Tert-butylhydroquinone (TBHQ), high molecular weight chitosan, and carboxymethylcellulose (CMC) were procured from Sigma Aldrich (Molekula, Alton Pkwy Ste C, Irvine, CA, USA). All drugs were dissolved in a 5% CMC solution to achieve the required experimental concentrations.

Experimental Animals

Thirty-two adult female Wistar rats (10 weeks, 100-150 g) were acquired from the Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia. The animals were acclimated for one week under controlled environmental conditions (25 ± 1°C temperature, 50 ± 10% humidity, and 12/12 hr light/dark cycles). All animal protocols were approved by the Animal Use and Care Committee KSU (KSU-SE-22-88).

Experimental Design

The experimental design involved the division of rats into four distinct groups, each comprising eight individuals (n = 8). The first group, designated as the Control Group, received the vehicle, specifically 5% carboxymethyl cellulose (CMC) (Sigma Aldrich, Molekula, Alton Pkwy Ste C, Irvine, CA, USA), administered daily for 3 weeks. In the second group, referred to as the Chitosan-treated Group, rats were orally administered chitosan at a dosage of 200 mg/kg per day for the same 3-week period. The third group, denoted as the TBHQ Model Group, underwent oral administration of an accumulative dose of tert-butylhydroquinone (TBHQ) (Sigma Aldrich, Molekula, Alton Pkwy Ste C, Irvine, CA, USA), amounting to 1,500 mg distributed over 3 weeks (500 mg/kg/week, administered once per week). Finally, the fourth group termed the chitosan + TBHQ-treated Group, followed the TBHQ administration protocol of the third group. In addition, rats in this group were concurrently subjected to daily oral administration of chitosan at a dose of 200 mg/kg for the entire 3-week period. This detailed experimental setup aimed to investigate and compare the effects of chitosan, TBHQ, and their combination on the subjects, providing a comprehensive understanding of the potential impact of these treatments on the observed outcomes.

Behavioral Tests

Morris Water Maze (MWM)

The MWM assesses spatial memory in rats. Rats were trained to find a hidden escape platform in a pool of opaque water over four days. On the fifth day, the platform was removed, and the number of times rats crossed the platform location was recorded[11-14].

Passive Learning Avoidance (PAL) fear test

This test measures rats' memory to avoid an electrical shock. During the exploration phase, rats were exposed to the shock after entering a dark room. In the test phase, the time taken to enter the dark room was recorded.

Histopathological investigation of the brain

Histological evaluations of brain samples were conducted, and the cerebral cortex and hippocampus were isolated. The samples were fixed in 10% buffered formalin and examined under a light microscope.

Preparation of brain homogenates

Brain tissues were homogenized by sonication in phosphate-buffered saline (PBS) (pH = 7.4) and centrifuged. The supernatants were stored at -80°C for biochemical analysis.

Tissue embedding and slide preparation

Tissue sections were obtained using a microtome, dehydrated, cleared, embedded in paraffin wax, and cut into (4 μm) thick sections. The sections were stained with hematoxylin and eosin (H&E).

Biochemical Analysis

Acetylcholine (Ach) and Choline Acetyltransferase (AchT) levels: Ach and AchT levels

in brain tissue homogenates were determined using ELISA kits following the manufacturer's (Eurogentec S.A., Seraing, Belgium) instructions.

Acetylcholinesterase (AChE) level: the activity of AChE in the brain tissue homogenates was determined using an assay kit following the manufacturer's instructions (Eurogentec S.A., Seraing, Belgium).

Antioxidant enzymes

Superoxide Dismutase (SOD): SOD levels in the brain and cerebral cortex were measured by ELISA kits following the manufacturer's (Eurogentec S.A., Seraing, Belgium) instructions.

Total Glutathione (GSH) levels: GSH levels in the brain and cerebral cortex were measured using ELISA kits following the manufacturer's instructions.

Oxidative Stress Biomarkers

Malondialdehyde (MDA) levels

MDA levels in the brain and cerebral cortex were measured using ELISA kits following the manufacturer's instructions.

Statistical Analysis

The data were subjected to analysis using GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). Normality was assessed, and statistical comparisons were performed utilizing appropriate non-parametric methods, given that ANOVA was not applicable in this context. Post-hoc Tukey's test was applied to evaluate statistical significance ($p \leq 0.05$). Results were presented as mean ± standard deviation (SD).

Results

Behavioral Tests Results

The Morris Water Maze (MWM) test was employed to assess memory functions and learning in rats. When analyzing the "Time required to find the hidden platform" (Escape Latency), it was observed that escape latency significantly decreased over the 4 days of the study in both the control and chitosan-treated groups. Importantly, there were no significant variations in escape time between these two groups throughout the study. Notably, the TBHQ-treated rats exhibited higher swimming latency on days 2, 3, and 4 compared to the control and chitosan-treated groups. Interestingly, the escape latency was significantly lower on day 4 in the TBHQ + chitosan-treated rats compared to the TBHQ-treated rats. However, this escape latency was still significantly higher compared to the control and chitosan-treated rats (Figure 1A). Regarding the "Path length traveled to find the hidden platform" (Escape Path Length), it also progressively declined in both the control and chitosan-treated groups over the 4 days, with no significant variations between them. However, TBHQ-treated rats exhibited significantly higher escape path length on days 2, 3, and 4 compared to the control and chitosan-treated rats. Notably, the escape path length significantly decreased on day 4 in the TBHQ + chitosan-treated rats compared to days 1, 2, and 3. Still, it remained significantly higher than the control and chitosan-treated rats (Figure 1B). No significant difference was observed in the crossing time when finding the hidden platform between the control and chitosan-treated rats. TBHQ-treated rats showed a significant decrease in crossing time compared to the control and chitosan-treated rats. This crossing time increased in TBHQ + chitosan-treated rats compared to TBHQ-treated rats but remained significantly lower compared to the control and chitosan control groups (Figure 1C).

Passive Learning Avoidance (PAL) Fear Test

The "required time to enter the dark area" during the passive avoidance test did not show significant differences between the control and chitosan-treated rats. In contrast, the time to enter the dark area was significantly decreased in the TBHQ-treated rats compared to the control and chitosan-treated groups. Importantly, the time to enter the dark area significantly increased in TBHQ + chitosan-treated rats compared to TBHQ-treated rats. Surprisingly, the time to enter the dark area was significantly lower in TBHQ + chitosan-treated rats compared to the control and chitosan control groups (Figure 1D).

Biochemical Analysis

Activity of acetylcholinesterase (AChE)

In the cerebral cortex, the activity of acetylcholinesterase (AChE) was significantly increased in the TBHQ-treated rats compared to

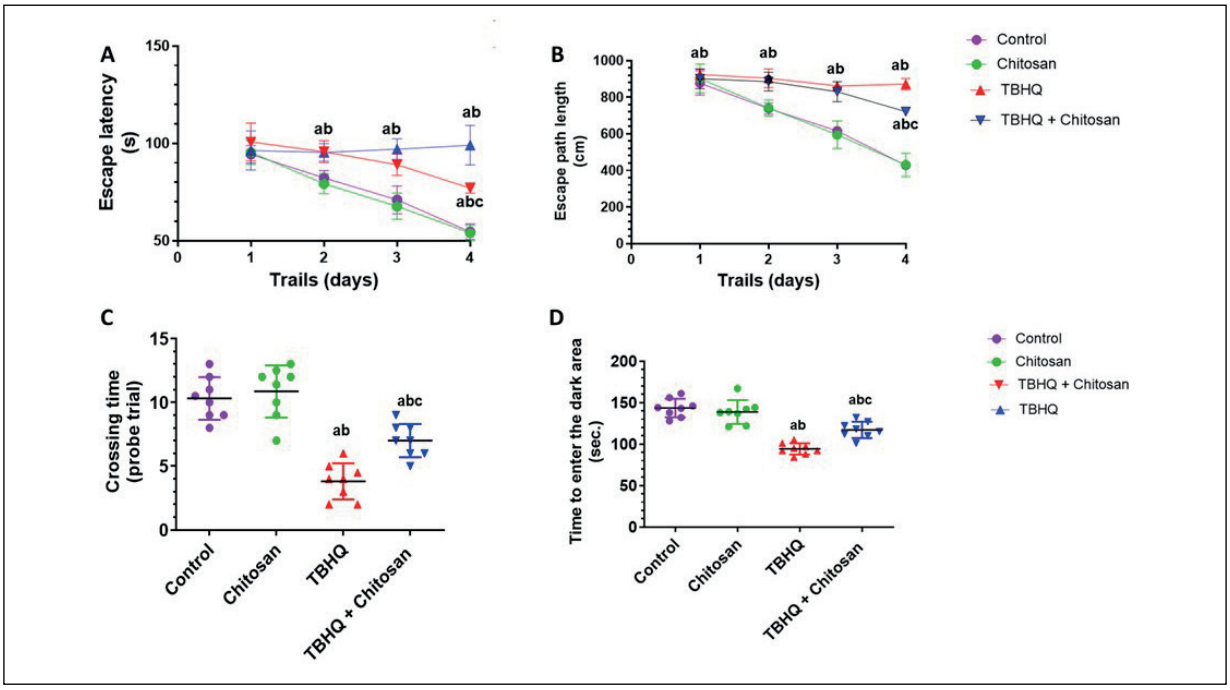

Figure 1. **A**, Time to find the hidden platform (escape latency) of rats during the Morris Water Maze (MWM) test over 4 consecutive days. **B**, The distance crossed to find the hidden platform (escape path length) during the Morris Water Maze (MWM) test over 4 consecutive days. **C**, The number of crosses over the hidden platform, which was removed during the probe trial on day 5 post the 4-day training of the Morris Water Maze (MWM) test over 4 consecutive days. **D**, Time to enter the dark area during the passive avoidance test (fear stress test). A reduction in time indicates impaired memory. Data were presented as mean ± SD of n = 8 rats/group. a: significantly different as compared to control rats, b: significantly different as compared to the chitosan-treated group, and c: significantly different as compared to the TBHQ-treated group.

control and chitosan-treated rats. In contrast, the activity of AChE significantly declined in the cerebral cortices of TBHQ + chitosan-treated rats compared to TBHQ-treated rats. Still, it remained significantly higher than the levels in the control and chitosan-treated rats (Figure 2A). However, the activities of AchE in the hippocampi of TBHQ-treated rats were significantly higher than control and chitosan-treated rats. Hippocampal activities of AchE significantly declined in TBHQ + chitosan-treated rats compared to TBHQ-treated rats but remained significantly higher than its levels in the control and chitosan-treated rats (Figure 2B). The levels of acetylcholine (Ach) in the cerebral cortices were significantly decreased in the TBHQ-treated rats compared to control and chitosan-treated rats. The levels of Ach increased in TBHQ + chitosan-treated rats compared to TBHQ-treated rats but were still significantly lower compared to control or chitosan-treated rats (Figure 2C). In the hippocampi, the activities of AchE were not significantly different between control and chitosan-treated rats (Figure 2D).

Choline acetyltransferase [AchT] level

The cerebral activities were not significantly different between the control and chitosan-treated rats but were significantly decreased in the cortices of TBHQ-treated rats compared to these groups. The activities of AchT have significantly increased in the cerebral cortices of TBHQ + chitosan-treated rats compared to TBHQ-treated rats. However, the cerebral activities of AchT measured in the TBHQ + chitosan-treated rats remained significantly lower than the levels measured in the control and chitosan-treated rats (Figure 3A). When the control rats were compared to chitosan-treated rats, no significant differences were seen in the hippocampal activities of AchT. The activities of AchT were significantly lower in the TBHQ-treated rats compared to control or chitosan-treated groups. The hippocampal activities of AchT have significantly increased in the cerebral cortices of TBHQ + chitosan-treated rats as compared to TBHQ-treated rats but remained significantly lower than the levels measured in the control or chitosan-treated rats (Figure 3B).

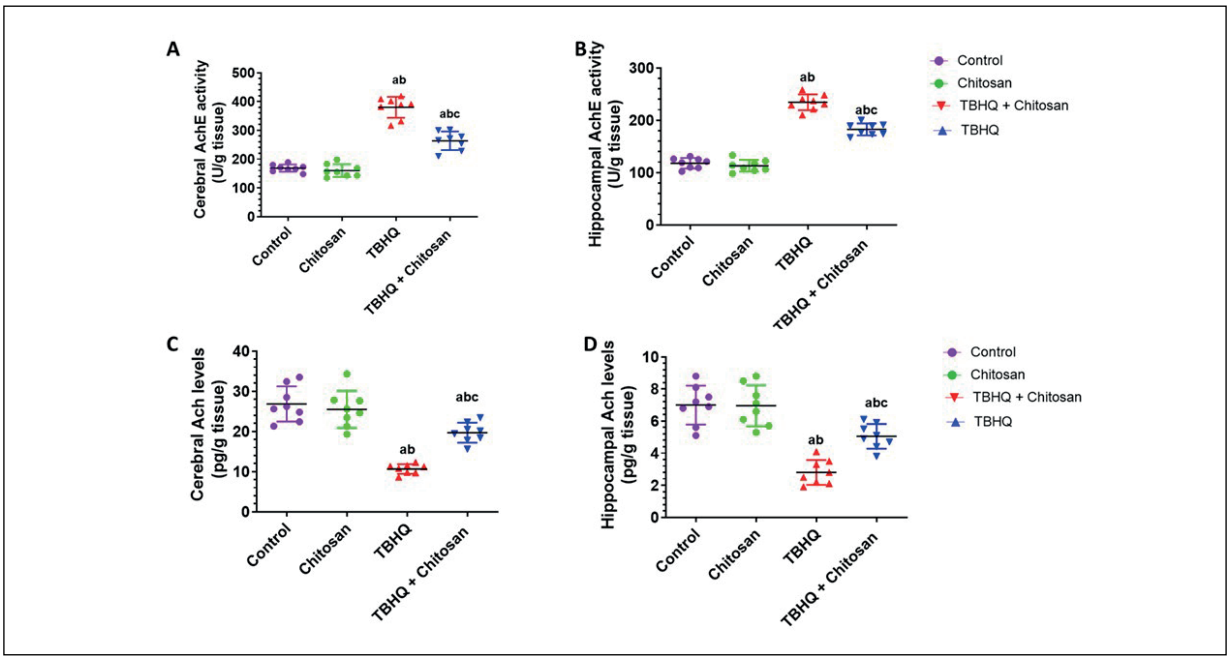

Figure 2. **A**, Activity of acetylcholine esterase (AchE) in the cerebral cortex of all groups of rats. **B**, AchE activity in the hippocampus of all groups of rats. **C**, Levels of acetylcholine (Ach) in the cerebral cortex of all groups of rats. **D**, Levels of acetylcholine (Ach) in the hippocampus of all groups of rats. Data were presented as mean ± SD of n = 8 rats/group. a: significantly different as compared to control rats, b: significantly different as compared to the chitosan-treated group, and c: significantly different as compared to the TBHQ-treated group.

Antioxidant Enzymes

The levels of glutathione (GSH) in the cerebral cortices of TBHQ-treated rats were significantly decreased compared to the control and chitosan-treated rats. The levels of GSH were significantly higher in the TBHQ + chitosan-treated rats than in the TBHQ-treated rats but were significantly lower than in control or chitosan-treated rats (Figure 4A). In the hippocampi, there was no significant difference in the levels of GSH between control and chitosan-treated rats. However, GSH levels were significantly lower in the TBHQ-treated rats compared to control or chitosan-treated rats, and they partially increased after treatment with chitosan (TBHQ + chitosan). Nevertheless, the levels of GSH in the hippocampi of TBHQ + chitosan-treated rats remained significantly

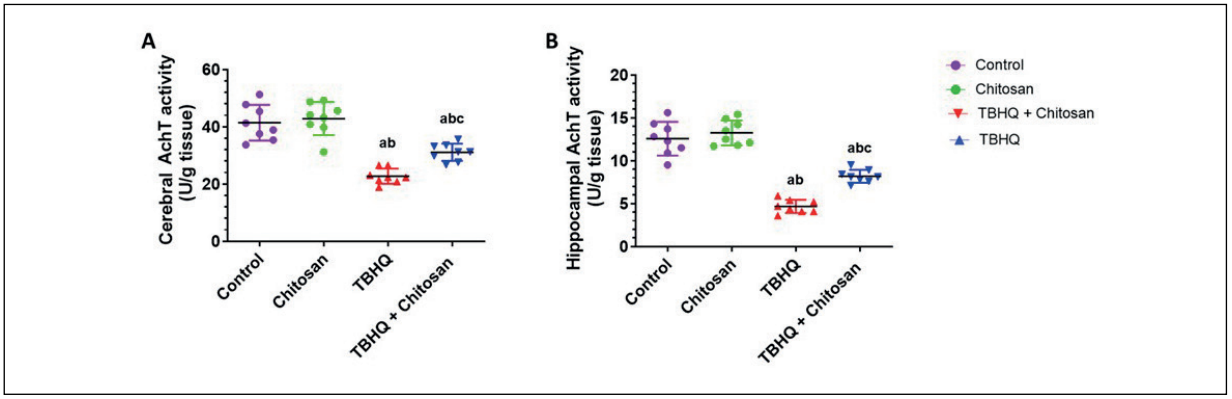

Figure 3. **A**, The activity of choline acetyltransferase (AchT) in the cerebral cortex of all groups of rats. **B**, The activity of choline acetyltransferase (AchT) in the hippocampus of all groups of rats.

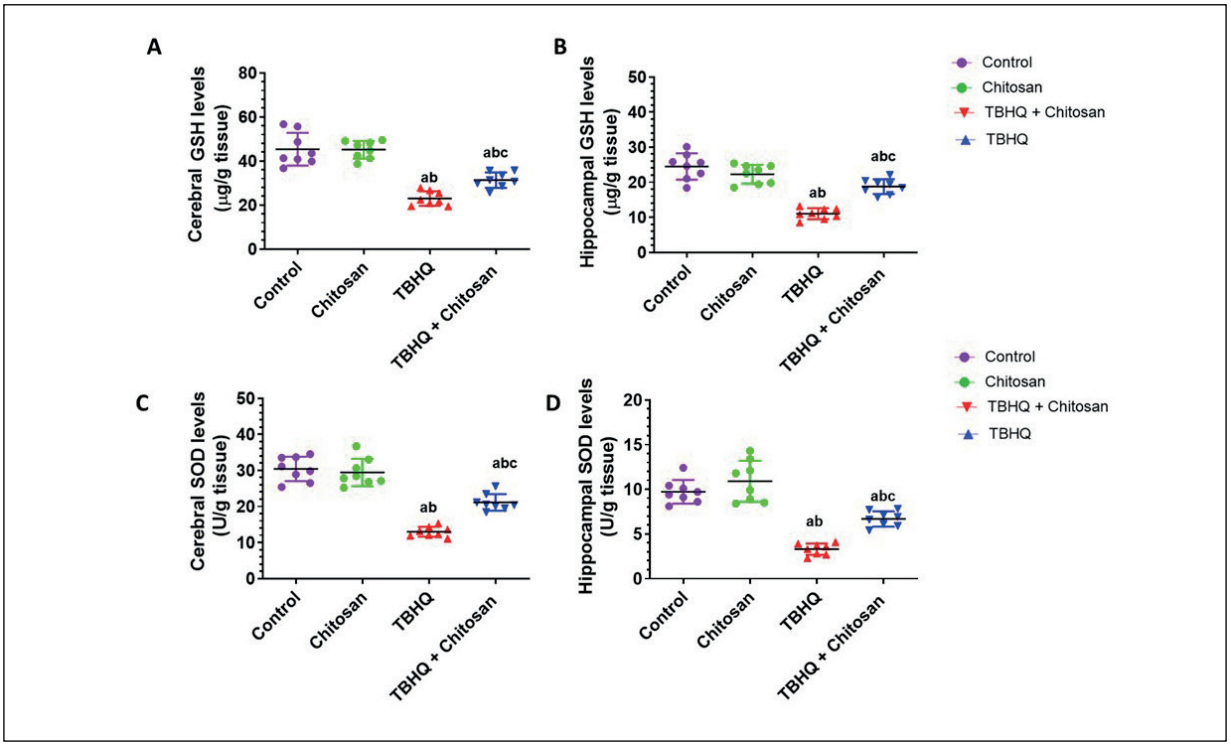

Figure 4. **A**, Cerebral glutathione levels (GSH). **B**, Hippocampal GSH levels. **C**, Cerebral superoxide dismutase (SOD) levels. **D**, Hippocampal SOD levels in all groups of rats. Data were presented as mean ± SD of n = 8 rats/group. a: significantly different as compared to control rats, b: significantly different as compared to the chitosan-treated group, and c: significantly different as compared to the TBHQ-treated group.

lower compared to control rats (Figure 4B). The activity of superoxide dismutase (SOD) in the cerebral cortices of TBHQ-treated rats showed a significant decrease compared to both the control and chitosan-treated groups. In the TBHQ + chitosan-treated rats, SOD activity was significantly higher than in the TBHQ-treated rats, although it remained significantly lower than in the control or chitosan-treated rats (Figure 4C). In the hippocampi, no significant difference in SOD activity was observed between the control and chitosan-treated rats. However, SOD activity was significantly lower in the TBHQ-treated rats compared to the control or chitosan-treated rats, with a partial increase after treatment with chitosan (TBHQ + chitosan). Nevertheless, SOD activity in the hippocampi of TBHQ + chitosan-treated rats remained significantly lower compared to control rats (Figure 4D).

Oxidative Stress Biomarkers

In the cerebral cortices, the levels of malondialdehyde (MDA) were significantly higher in TBHQ-treated rats compared to control or chitosan-treated rats. The levels of MDA in the TBHQ + chitosan-treated rats were significantly reduced compared to TBHQ-treated rats. Still, they were higher in the cerebral cortices compared to control or chitosan-treated rats (Figure 5A). In the hippocampi, no significant difference in the levels of MDA was observed between chitosan-treated and control rats. The levels of MDA were significantly higher in the hippocampi of TBHQ-treated rats compared to control or chitosan-treated rats, and they partially decreased after treatment with chitosan (TBHQ + chitosan). However, the levels of MDA in the hippocampi of TBHQ + chitosan-treated rats remained significantly higher compared to control rats (Figure 5B).

Histological Section

In the untreated control group, the cerebral cortex displayed a normal structure of pyramidal neurons, granulocytes, and glial cells. Similarly, animals treated with chitosan exhibited the same structure in the cerebral cortex. Conversely, animals treated with TBHQ showed signs of

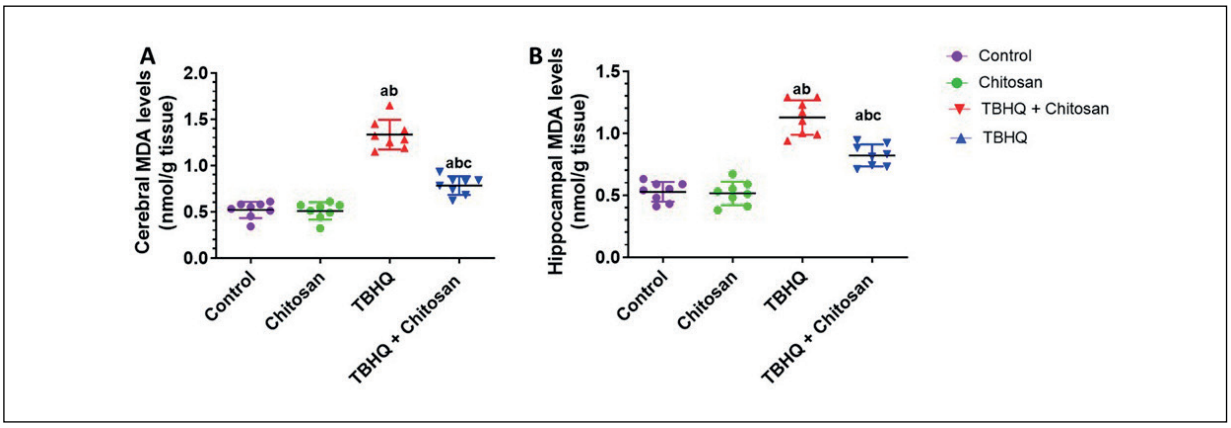

Figure 5. A, Cerebral malondialdehyde (MDA) levels and (**B**) hippocampal MDA levels in all groups of rats. Data were presented as mean ± SD of n = 8 rats/group. A: significantly different as compared to control rats, b: significantly different as compared to the chitosan-treated group, and c: significantly different as compared to the TBHQ-treated group.

neuronal distortion, faded ghost neurons, hemorrhage, inflammation, and aggregations of acidophilic and inflammatory cells in the cerebral cortex. However, animals treated with TBHQ + chitosan showed marked improvements with healthier-looking neurons and reduced acidophilic cell aggregations in the cerebral cortex. In the hippocampus, the untreated control group displayed a normal structure of the dentate gyrus zone. The dentate gyrus zone of rats treated with chitosan remained similar to the control. However, in rats treated with TBHQ, neurons in the sub-granular layer exhibited shrinkage and distortion. In contrast, rats treated with TBHQ + chitosan showed a healthy granular cell layer, except for some granulocytes that exhibited mild degeneration and fading. These histological results suggest that the combination of TBHQ and chitosan treatment had a positive impact on the structural integrity of both the cerebral cortex and hippocampus, ameliorating some of the detrimental effects observed with TBHQ treatment alone (Figures 6 and 7, **Supplementary Figures 1** and **2**).

Discussion

The present study aimed to investigate the effects of TBHQ, chitosan, and their combination on memory and neurobiochemical parameters in a rat model. The results obtained from a battery of behavioral tests, biochemical analyses, and histological examinations reveal several important findings that warrant thorough discussion.

Behavioral Outcomes

In the Morris Water Maze (MWM) test, escape latency and escape path length are key parameters reflecting spatial learning and memory. The observed decline in escape latency and escape path length in control and chitosan-treated rats across the four days of training suggests progressive improvement in spatial memory and learning. This finding is consistent with previous studies[15-18] highlighting the cognitive-enhancing potential of chitosan, possibly attributed to its neuroprotective and antioxidant properties. In contrast, TBHQ-treated rats exhibited increased escape latency and path length on days 2, 3, and 4. This could be indicative of memory impairment, potentially linked to the pro-oxidative properties of TBHQ, which have been associated with neurotoxicity[19,20]. However, on the fourth day, escape latency significantly decreased in the TBHQ + chitosan-treated group, which may suggest a restorative effect of chitosan when administered alongside TBHQ. Interestingly, the reduced crossing time in the probe trial, a measure of memory retention, was observed in TBHQ-treated rats compared to control and chitosan-treated groups. The improved retention of spatial memory in TBHQ-treated rats may raise questions about the compound's influence on specific memory phases. Conversely, the increased crossing time in TBHQ + chitosan-treated rats might indicate that the combination therapy enhances memory retention, possibly due to the neuroprotective effects of chitosan[19,21].

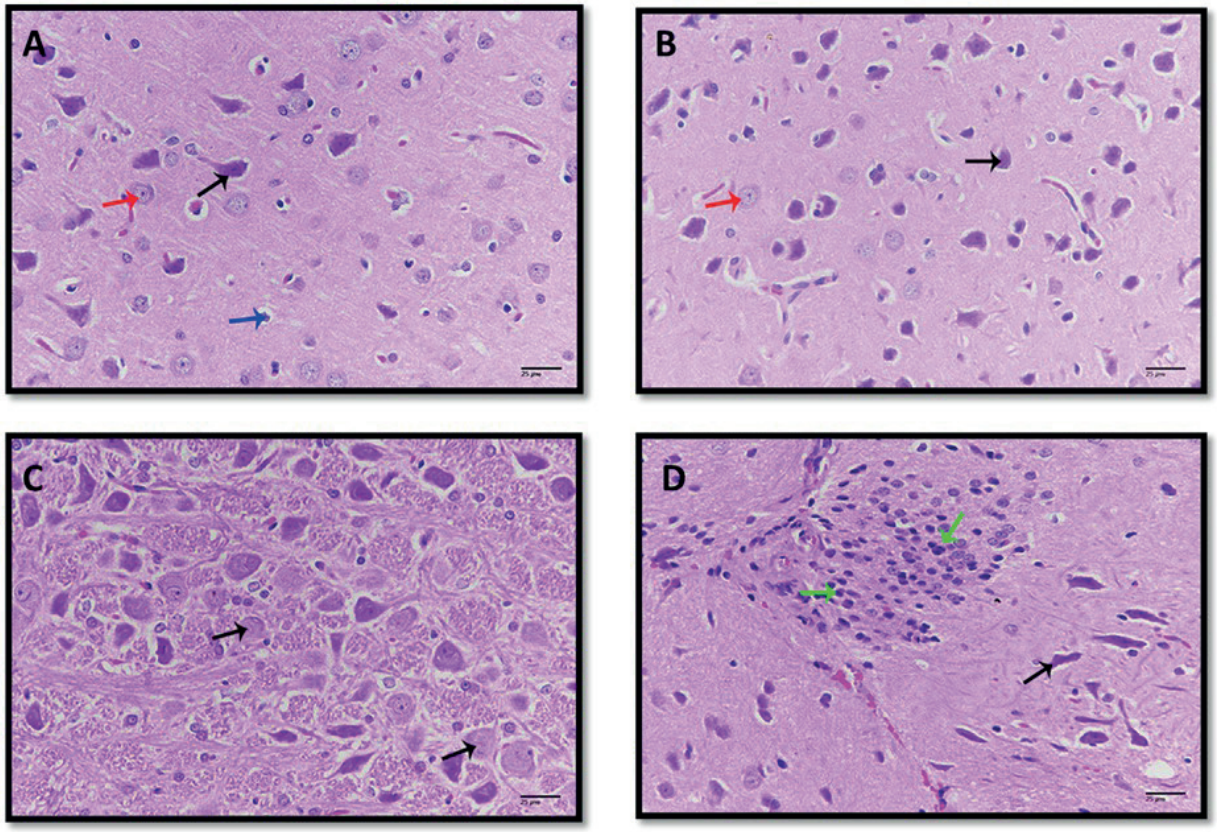

Figure 6. A, Photomicrograph of the cerebral cortex of the control group. **B**, Animal treated with chitosan revealing healthy cerebral cortex, pyramidal neurons (black arrow), granular cells (red arrow). **C**, Animal cerebral cortex treated with TBHQ exhibiting faded ghost neurons (black arrow). **D**,Animal cerebral cortex treated with TBHQ exhibits faded distorted neurons (black arrow), and inflammatory cells (green arr ow).

The Passive Learning Avoidance (PAL) fear test further evaluated the impact of these treatments on passive avoidance learning and memory. The significant reduction in time to enter the dark area in TBHQ-treated rats compared to control and chitosan-treated rats signifies impaired memory, suggesting that TBHQ may have adverse effects on aversive memory formation. However, TBHQ + chitosan-treated rats displayed significantly higher time to enter the dark area than TBHQ-treated rats and lower times than control and chitosan control groups. This result supports the hypothesis that chitosan supplementation alongside TBHQ may mitigate the memory impairments associated with TBHQ, potentially through its antioxidant properties[15,22].

Biochemical Parameters

The biochemical analysis provided insights into the impact of TBHQ, chitosan, and their combination on key neurobiochemical parameters, including AChE, Ach, AchT, GSH, SOD, and MDA. Notably, TBHQ significantly increased AChE activity in the cerebral cortex. This observation aligns with previous studies[22,23] suggesting that TBHQ can interfere with cholinergic neurotransmission. The concomitant increase in AChE activity and reduction in Ach levels in TBHQ-treated rats indicate an unfavorable cholinergic balance that could contribute to cognitive deficits. Chitosan supplementation, either alone or in combination with TBHQ, did not significantly impact AChE activity or Ach levels, suggesting that it does not directly influence cholinergic functioning. However, the combination of TBHQ and chitosan was associated with a decline in AChE activity compared to TBHQ treatment alone. This finding indicates that chitosan could potentially mitigate the TBHQ-induced increase in AChE activity. Furthermore, TBHQ led to significantly increased AChE activity in the hippocampus, contributing to altered cholinergic functioning. However, similar to the cortex, chitosan supplementation alone did not significantly affect

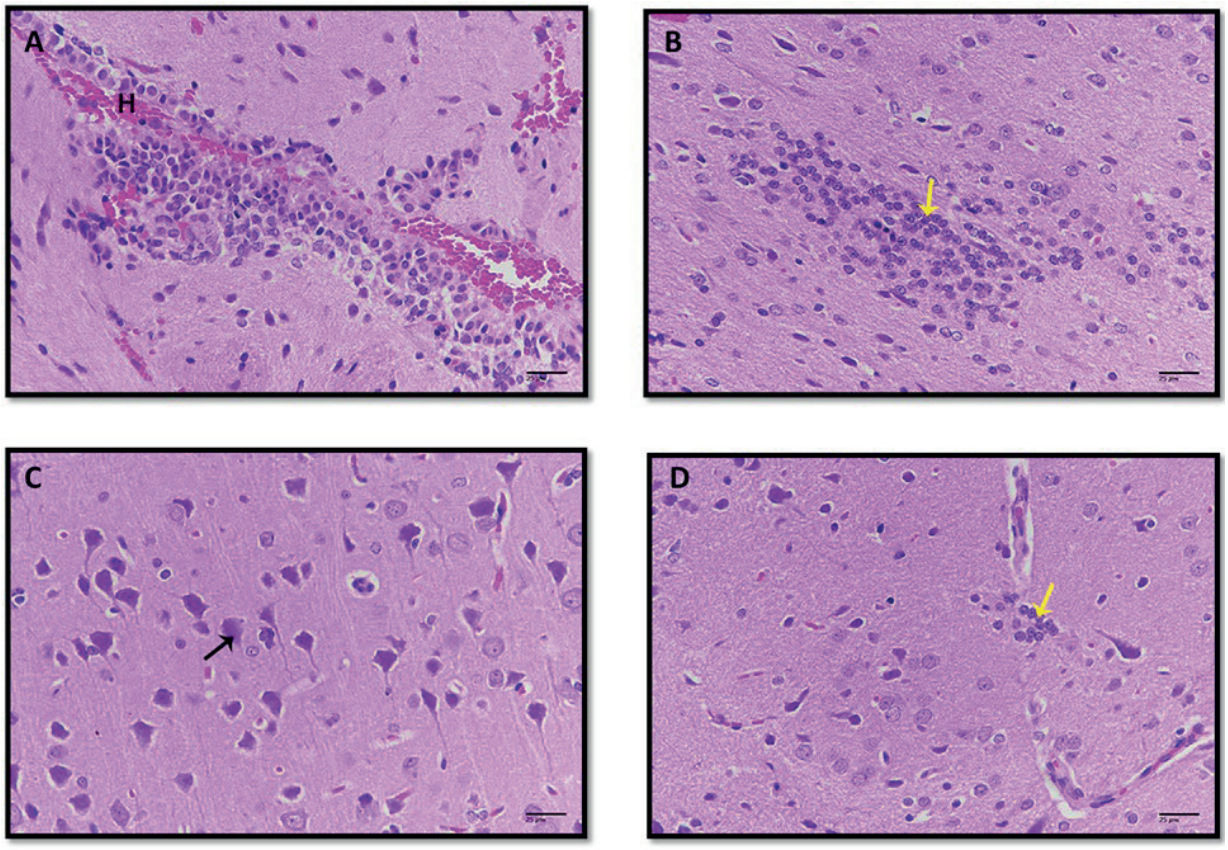

Figure 7. **A**, Photomicrograph of animal cerebral cortex treated with TBHQ exhibiting hemorrhage (H) surrounded by inflammation. **B**, Animal cerebral cortex treated with TBHQ exhibiting acidophilic cells (yellow arrow). **C**, Animal cerebral cortex treated with TBHQ + Chitosan displaying relative healthy neurons, others looked faded. **D**, Animal cerebral cortex treated with TBHQ + Chitosan showing less acidophilic cells (yellow arrow) (H&E-400X).

AChE activity or Ach levels in the hippocampus. The combination of TBHQ and chitosan resulted in a decline in AChE activity but remained significantly higher than controls. This pattern suggests that chitosan may partially attenuate the TBHQ-induced increases in AChE activity, particularly in the cortex. The activities of AchT, an enzyme involved in acetylcholine synthesis, were not significantly altered by chitosan alone, neither in the cortex nor the hippocampus. Interestingly, the combination of TBHQ and chitosan in the cortex led to increased AchT activity compared to TBHQ alone. This result implies that chitosan might exert a modulatory effect on the cholinergic system when administered in conjunction with TBHQ. GSH and SOD are crucial antioxidant enzymes involved in the protection against oxidative stress.

In the cerebral cortex, TBHQ significantly reduced GSH levels, indicative of increased oxidative stress, while chitosan alone did not affect GSH levels. The combination of TBHQ and chitosan significantly elevated GSH levels, suggesting a potential antioxidant effect of chitosan that counteracts TBHQ-induced oxidative stress. In the hippocampus, chitosan treatment did not impact GSH levels, but TBHQ significantly reduced GSH. Interestingly, the combination of TBHQ and chitosan led to a partial restoration of GSH levels. These results underscore the potential neuroprotective role of chitosan, which may counterbalance TBHQ-induced oxidative stress in the hippocampus. SOD levels were significantly reduced in the cortex of TBHQ-treated rats, signifying diminished antioxidant capacity and increased vulnerability to oxidative damage. The combination of TBHQ and chitosan was associated with increased SOD levels compared to TBHQ alone. This demonstrates a protective effect of chitosan against oxidative stress, particularly in the cortex. In the hippocampus, neither chitosan alone nor the combination with TBHQ significantly affected SOD levels. The

decline in SOD levels caused by TBHQ was partially reversed by the combination of TBHQ and chitosan. This demonstrates a protective effect of chitosan against oxidative stress, particularly in the cortex. Finally, MDA levels, an indicator of lipid peroxidation and oxidative stress, were significantly elevated in the cortex of TBHQ-treated rats. Chitosan treatment did not influence MDA levels in the cortex. However, the combination of TBHQ and chitosan resulted in a partial reduction of MDA levels. This suggests that chitosan may counteract the pro-oxidative impact of TBHQ in the cortex. In the hippocampus, chitosan alone did not influence MDA levels. TBHQ treatment led to significantly increased MDA levels in the hippocampus, indicating oxidative damage. The combination of TBHQ and chitosan mitigated this effect, underscoring chitosan's potential neuroprotective role against TBHQ-induced lipid peroxidation in the hippocampus.

The histological examination of the cerebral cortex revealed that TBHQ treatment resulted in distorted and faded neurons, hemorrhage, inflammation, and aggregations of acidophilic and inflammatory cells. This observation aligns with prior studies[24-26] suggesting that TBHQ has the potential to induce neuronal damage. Notably, the administration of TBHQ alongside chitosan led to marked improvements in the structural integrity of the cerebral cortex, characterized by healthier neurons and reduced acidophilic cell aggregations. In the hippocampus, TBHQ-treated rats displayed neuronal shrinkage and distortion in the sub-granular layer of the dentate gyrus. This is consistent with the hypothesis that TBHQ can induce hippocampal damage and memory impairment[22]. Interestingly, TBHQ + chitosan-treated rats showed a healthier granular cell layer, although mild degeneration and fading in some granulocytes were observed. This suggests that the combination of TBHQ and chitosan may partially mitigate the detrimental effects of TBHQ on hippocampal structural integrity.

Mechanisms of Action and Clinical Implications

The observed results collectively suggest that chitosan, when administered in conjunction with TBHQ, may have a mitigating influence on the cognitive and neurobiochemical consequences of TBHQ exposure in rats. The authors propose that chitosan's neuroprotective role is attributed to its antioxidant properties, countering oxidative stress and reducing lipid peroxidation and acetylcholinesterase (AChE) activity. TBHQ, a synthetic antioxidant, interacts with cells by neutralizing free radicals and preventing oxidative damage. Chitosan, a natural polysaccharide, may disrupt this interaction by scavenging free radicals, mitigating oxidative stress, and modulating AChE activity. This dual action could protect neuronal cells from TBHQ-induced oxidative damage and maintain proper neurotransmission. Overall, chitosan's antioxidant effects may divert or prevent the detrimental cellular interactions associated with TBHQ, contributing to its neuroprotective benefits[27,28].

Conclusions

These findings raise the intriguing possibility that chitosan supplementation could be explored further as a therapeutic strategy to counteract neurotoxicity associated with TBHQ exposure. In addition to the cited studies, it is important to acknowledge the potential implications of chitosan and TBHQ exposure in the context of food safety regulations and public health. This study highlights the importance of exploring chitosan further as a potential neuroprotective agent in conditions where TBHQ exposure may be a concern, shedding light on potential strategies to safeguard neurological health.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Approval

This research was approved by the Ethical Review Board of King Saud University, Riyadh, Saudi Arabia (Number KSU-SE-22-88).

Availability of Data and Materials

Data are available from the corresponding author upon request.

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Authors' Contributions

Conceptualization: MA, PV. Data curation, formal analysis, investigation, methodology, funding acquisition, project administration, resources, software, validation, visualization, and writing – review and editing: MA, PV, KE, WA; Writing – original draft and corresponding author: GA.

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