The Effect of Sodium Benzoate on the Cerebellum of the Adult Male Albino Rats and the Ameliorative Effect of its Withdrawal and Zinc Administration:

Histological and Immunohistochemical Study

Original Article

Eman A. El-Sawaf[1] and Noha O. Shawky[2]

[1]Department of Anatomy and Embryology, [2]Department of Physiology, Faculty of Medicine, Helwan University, Cairo, Egypt

ABSTRACT

Background: Sodium benzoate (NaB) is widely used as food additive in nutritional industries and many personal care products. Long-term consumption of food additives may contribute to various health issues.

Aim of the Work: This study aims to investigate the hazards of sodium benzoate (NaB) on the cerebellar cortex in male rats and to assess the potential ameliorative impact of drug withdrawal and zinc (Zn) administration.

Material and Methods: Forty adult male albinos' rats were divided into five groups (Gps): Gp I (Control), Gp II (Zinc), Gp III (Sodium benzoate), Gp IV (Sodium benzoate withdrawal) and Gp V (Sodium benzoate + Zinc). Blood samples were collected to measure the brain-derived neurotrophic factor (BDNF) levels. The cerebellum was processed for histological & immunohistochemical analysis.

Results: Inspection of specimens from the cerebellum of NaB group detected cerebellar histological alterations. Purkinje cells exhibited loss of their distinctive pyriform outline, with distorted nuclei. Cellular clumping was observed in the granular layer. A strong positive GFAP immunoreaction was observed in this group.

Withdrawal from NaB led to partial histological improvement and moderate GFAP immunoreactivity. Co-administration of Zn mitigated histological changes with moderate GFAP immunoreactivity.

NaB significantly reduced BDNF levels, while withdrawal partially restored them. Zn supplementation helped maintain BDNF levels.

Conclusion: This study demonstrates that NaB a commonly used food preservative induces structural damage in the cerebellar cortex. Partial improvement was observed following NaB withdrawal. Co-administration of zinc mitigated the deleterious effects of NaB on the cerebellar cortex.

Key Words: Food additives, nervous, protective.

Received: 29 December 2024, **Accepted:** 5 February 2025.

Corresponding Author: Eman A. El-Sawaf, Department of Anatomy and Embryology, Faculty of Medicine, Helwan University, Helwan, Egypt, **Tel.:** 01226659353, **E-mail**: eman.elsawaf@med.helwan.edu.eg

ISSN: 2735-3540, Vol. 76, No. 1, March 2025.

INTRODUCTION

Due to the massive advancements in technology and daily living, a lot of people now prefer to eat fast food and prepackaged foods from stores rather than cooking their own food at home, often without thinking about the additional ingredients that may alter or damage the food's quality or worsen existing health issues[1].

Food additives are substances primarily added to processed food that add no nutritional value in order to prevent it from spoiling and rises its appeal to consumers. It also ameliorates the food taste, smell and color. There are many varied types of food additives, such as food dyes, which give food color or improve its appearance and composition, preservatives, which prevent food spoilage, chemical and biological corruption and flavors, which are added to give food a particular flavor[2-4].

Sodium benzoate (NaB) was defined as a salt of benzoic acid, it exhibits high solubility in water, white in color, tasteless and odorless. It also has a remarkable bactericidal and fungicidal activities[5]. It is commonly applied in nutritional manufacturing for preservation of drinks, fruit products, spices, different types of cheeses and in countless cosmetics[6].

DOI: 10.21608/ASMJ.2025.348488.1357

NaB was admitted by the Food and Drug Administration (FDA) as a primary food preservative. The permissible intake quantity during the day is 0–5 mg / kg body weight[7]. Moreover, because NaB has so many uses, individuals are unintentionally consuming more NaB than is suggested, which exposes them to levels above what is recommended.

According to certain researches, using food additives over an extended period can lead to a number of illnesses, including cancer, conditions affecting the liver, kidneys and spleen. It also causes digestive illnesses, growth delay, and cerebral neuronal damage[8-10]. NaB increased free radicals, oxidative stress[11].

Experimental studies suggest that overdose of NaB could produce adverse effects on vital organs like liver, kidney[12,13], reproductive organs[14], brain and causes intellectual disability and problems associated with learning and memory[15]. Studies report that the intake of NaB has genotoxic effects on erythrocytes[16] and can elicit damage to mitochondrial DNA[17] and it was associated with changes in serum clinical parameters[15].

Zinc (Zn) is the second most plentiful metal. It is necessary for more than 200 enzymes in the body. It has a significant role in preserving human health. It is involved in many physiological processes, particularly those related to immunity, proliferation, proteostasis, apoptosis and antioxidant defense against oxidative stress[18,19]. Zinc homeostasis starts after its absorption. It reaches all body parts via the blood stream. At the cellular level, Zn is further transported between organelles. Any change in the intracellular concentrations is highly related to extracellular Zn supply, Zn homeostasis needs to be highly controlled to prevent its hazards[19]. There is a convincing evidence that Zn supplementation minimizes the organ toxicity[20].

Zinc is a trace element needed for brain functions. It is a neurotransmitter that promotes memory and cognition. Zn deficiency is accompanied with nerve degeneration, brain abnormalities and lack of cognition[21,22].

AIM OF THE WORK

This study aims to investigate the hazards of sodium benzoate (NaB) on the cerebellar cortex in male rats and to assess the potential ameliorative impact of drug withdrawal and zinc (Zn) administration.

ETHICAL CONSIDERATION

Ethical approval on this research was received by the Research Ethics Committee (REC) at the college of Medicine, Helwan University, Cairo Egypt, serial: 15-2024.

MATERIAL AND METHODS

Chemicals

All chemicals were supplied by Alpha Chemical Group, 6 Industrial Zone 6 of October, Cairo Egypt:

- Sodium benzoate powder (99% purity) was dissolved in distilled water (ACG.NO.8046321).
- Zinc chloride powder (97% purity) was dissolved in normal saline (ACG.NO.2049821).

Animals

Forty adult male albinos' rats' weight about 180-200 grams were used in this research. The animals were saved in medium sized cages at physiology department, Faculty of Medicine, Helwan University. The rats were saved under the prevailing atmospheric conditions and they had an open access to water and standard rat diet.

Experimental design:

The animals were sub grouped into 5 groups (8 rats / group):

Group I (control Gp):

The rats fed a normal diet with no treatment.

Group II (zinc Gp):

The rats were injected intraperitoneally (I.P.) by zinc chloride dissolved in normal saline (5 mg/kg/day) for 28 days[23].

Group III (sodium benzoate Gp):

The rats received NaB dissolved in distilled water orally (200 mg/kg/day) for 28 days[13].

Group IV (sodium benzoate withdrawal Gp):

The rats received NaB dissolved in distilled water orally (200 mg/kg/day) for 28 days and then we stopped the NaB administration in the next 28 days[13].

Group V (sodium benzoate + zinc Gp):

The rats received NaB dissolved in distilled water orally (200 mg/kg/day) with coadministration of zinc chloride dissolved in normal saline intraperitoneally (I.P.) (5 mg/kg/day) for 28 days[13,23].

By the end of the experiment:

A. Biochemical analysis:

The retrobulbar vein was used to collect the blood samples from various groups, the serum was isolated to measure the BDNF.

B. Histopathological analysis

The animals were anesthetized by inhaling ether and then sacrificed. The cerebellum was separated carefully, fixation was done by 10% formalin and five micrometers (μm) thick paraffin sections were prepared for the following:

1. Hematoxylin and Eosin stain (H&E): to demonstrate the cerebellar structures[24].
2. Toluidine blue stain: to inspect the Nissl's granules[24].
3. Immunohistochemical stain: Glial fibrillary acidic protein (GFAP). For astrocytes detection in the granular layer[25].

Specimens were analyzed by light microscopy and photographed at ×400 & ×1000 magnification.

C. Morphometric measurement

GFAP positive expression area percentage was measured in the granular layer by Image-J software 1.46r (NIH, USA) in three images per group after converting into greyscale and adjusting threshold[26].

D. Statistical analysis

Statistical analysis was directed using ANOVA test to compare quantitative data within the same group at different times to estimate the mean and standard deviation. (IBM SPSS Statistical program for Windows. Version 20.0. Armonk, NY: IBM Corp.)[26].

RESULTS

Histological results

Hematoxyline and eosin stain:

Inspection of cerebellar cortex specimens of Gp I (Control) revealed the three layers. The molecular layer included basket and stellate cells. The Purkinje cell layer was characterized by a single row of pyriform shape Purkinje cells, with vesicular nuclei and prominent nucleoli. The granular layer was characterized by numerus small rounded dense cells with intervening cerebellar islands (Fig.1). These findings were the same in Gp II (Zn) (Fig.2).

Specimens from the cerebellar cortex of Gp III (NaB) appeared with few, irregular, Purkinje cells, their distinctive pyriform appearance were lost with empty spaces surrounding and in between them and their nuclei appeared distorted. Clumping of granular cells was observed (Fig.3).

In Gp IV (NaB withdrawal), specimens from the cerebellar cortex revealed the Purkinje cells which appeared nearly pyriform in shape, with distorted nuclei and empty spaces surrounding and in between them. Clumping of some granular cells was observed (Fig.4).

Inspection of cerebellar cortex specimens of Gp V (NaB and Zn) revealed some Purkinje cells which resumed their normal appearance. Other Purkinje cells appeared irregular with distorted nuclei. Less clumping of granular cells was observed (Fig.5).

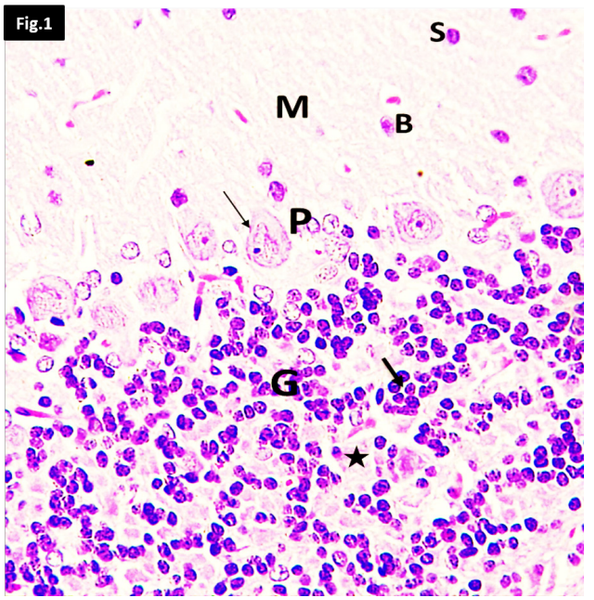

Fig.1: Photomicrograph of specimen from the cerebellar cortex of Gp. I (Control) showing: The molecular layer (M) composed of basket (B) and stellate cells (S). The Purkinje cell layer (P) is characterized by a single row of pyriform shape Purkinje cells, with vesicular nuclei and prominent nucleoli (thin arrow). The granular layer (G) is characterized by numerus small rounded dense cells (thick arrow) with intervening cerebellar islands (star).

(H&E x400)

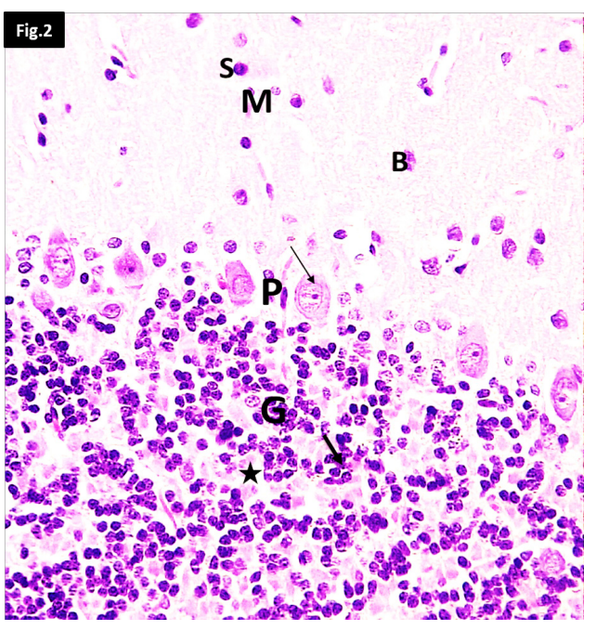

Fig.2: Photomicrograph of specimen from the cerebellar cortex of Gp. II (Zn) showing the same results as group I; The molecular layer (M) composed of basket (B) and stellate cells (S). The Purkinje cell layer (P) is characterized by a single row of pyriform shape Purkinje cells, with vesicular nuclei and prominent nucleoli (thin arrow). The granular layer (G) is characterized by numerus small rounded dense cells (thick arrow) with intervening cerebellar islands (star).

(H&E x400)

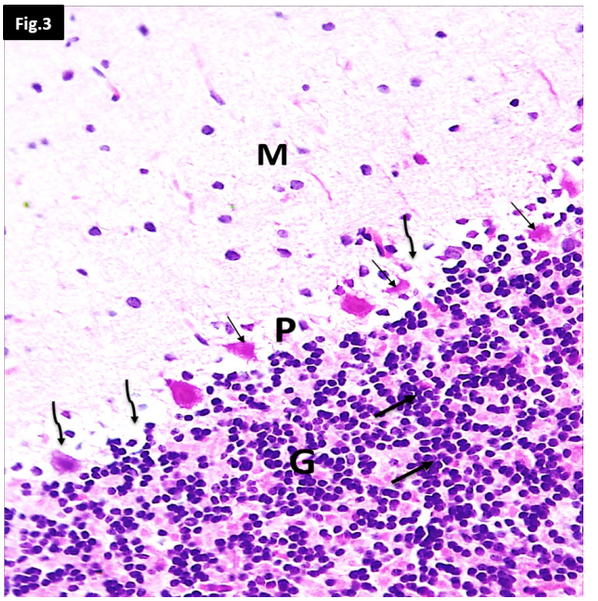

Fig.3: Photomicrograph of specimen from the cerebellar cortex of Gp. III (NaB) showing few, irregular Purkinje cells, their distinctive pyriform appearance are lost with distorted nuclei (thin arrows) and empty spaces surrounding and in between them (wavy arrows). The granular cell layer (G) appears with clumping of their cells (thick arrows). The molecular layer (M) appears normal.

(H&E x400)

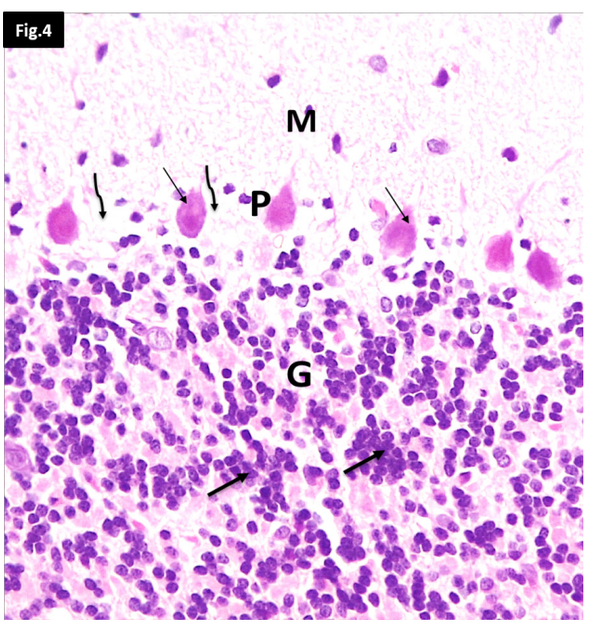

Fig.4: Photomicrograph of specimen from the cerebellar cortex of Gp. IV (NaB withdrawal) showing the Purkinje cells which appear nearly pyriform in shape with distorted nuclei (thin arrows) and empty spaces surrounding and in between them (wavy arrows). The granular cell layer (G) shows some clumping of their cells (thick arrows). The molecular layer (M) appears normal.

(H&E x400)

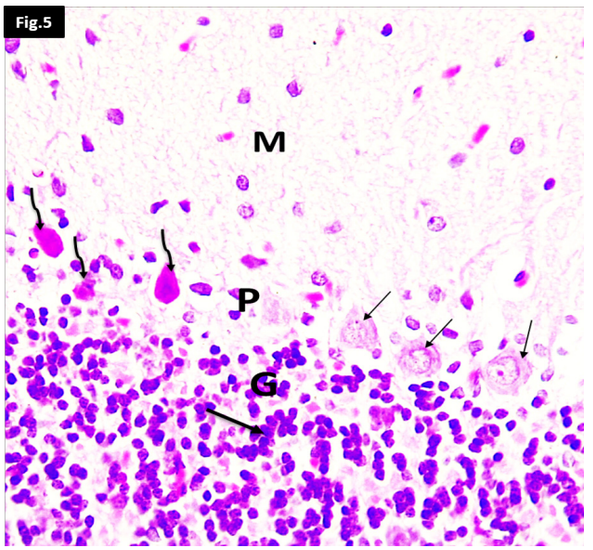

Fig.5: Photomicrograph of specimen from the cerebellar cortex of Gp. V (NaB + Zn) showing some Purkinje cells which resumed their normal appearance (thin arrows). Other Purkinje cells appear irregular with distorted nuclei (wavy arrows). The granular cell layer (G) shows less clumping of their cells (thick arrow). The molecular layer (M) appears normal.

(H&E x400)

Toluidine blue stain:

Toluidine blue was done to investigate the Nissl granules in the cytoplasm. Inspection of specimens from the cerebellar cortex of Gp I (Control) revealed the three layers. The Purkinje cells appeared pyriform in shape, Nissl granules appeared in their cytoplasm with large central vesicular nucleus and prominent nucleolus (Fig.6). These findings were the same in Gp II (Zn) (Fig.7).

Inspection of specimens from the cerebellar cortex of Gp III (NaB) revealed irregular, distorted Purkinje cells with darkly stained cytoplasm, hardly identified Nissl granules and invisible nucleus. Some Purkinje cells appeared degenerated (Fig.8).

In Gp IV (NaB withdrawal), inspection of cerebellar cortex specimens revealed irregular Purkinje cells, the cytoplasm appeared darkly stained, with some visible Nissl granules and shrunken nucleus. Some Purkinje cells appeared degenerated (Fig.9).

Inspection of cerebellar cortex specimens of Gp V (NaB and Zn) revealed some Purkinje cells which look nearly normal with visible Nissl granules in the cytoplasm and the nucleus looked with prominent nucleolus. Other Purkinje cells appeared irregular and distorted with invisible nucleus (Fig.10).

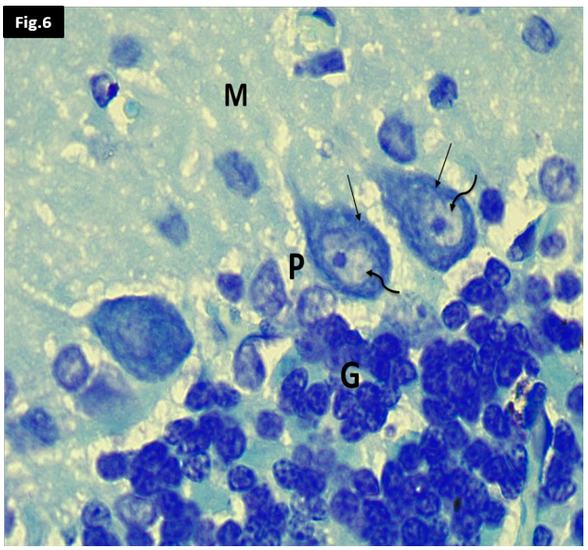

Fig.6: Photomicrograph of specimen from the cerebellar cortex of Gp. I (Control) showing: Pyriform shape cells of Purkinje cell layer (P), the cytoplasm shows the Nissl granules (arrows) and the nucleus appears large central vesicular with prominent nucleolus (wavy arrows). The molecular layer (M) and granular layer (G) can be noticed.

(Toluidine blue x1000)

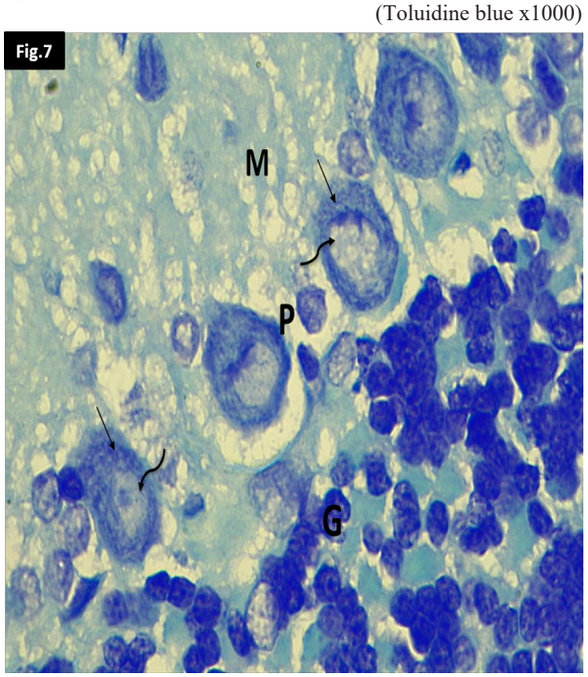

Fig.7: Photomicrograph of specimen from the cerebellar cortex of Gp. II (Zn) showing the same results as group I: Pyriform shape cells of Purkinje cell layer (P), the cytoplasm shows the Nissl granules (arrows) and the nucleus appears large central vesicular with prominent nucleolus (wavy arrows). The molecular layer (M) and granular layer (G) can be noticed.

(Toluidine blue x1000)

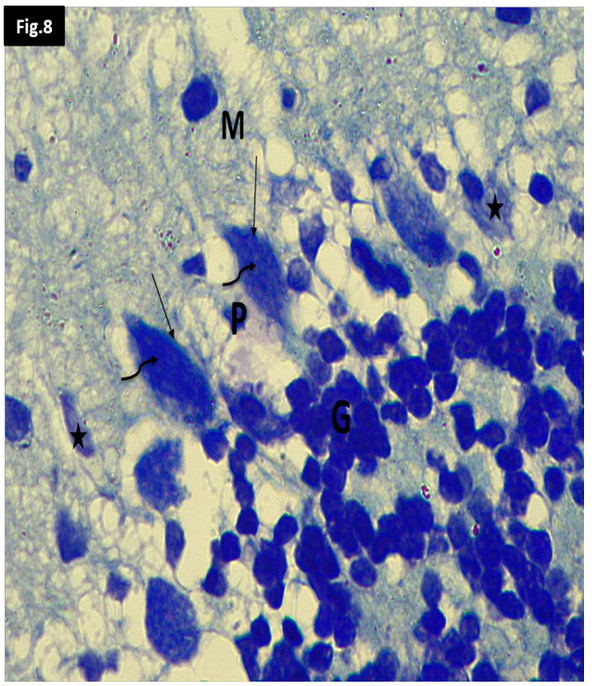

Fig.8: Photomicrograph of specimen from the cerebellar cortex of Gp. III (NaB) showing irregular distorted Purkinje cells (arrows), the cytoplasm appears darkly stained with hardly identified Nissl granules and invisible nucleus (wavy arrows). Some Purkinje cells appear degenerated (stars). The molecular layer (M) and granular layer (G) can be noticed.

(Toluidine blue x1000)

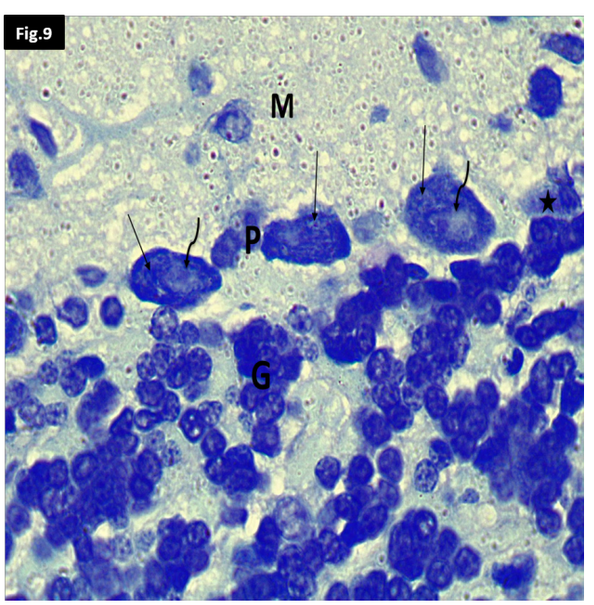

Fig.9: Photomicrograph of specimen from the cerebellar cortex of Gp. IV (NaB withdrawal) showing irregular Purkinje cells, the cytoplasm appears darkly stained with some visible Nissl granules (arrows) and the nucleus appears shrunken (wavy arrows). Some Purkinje cells appear degenerated (star). The molecular layer (M) and granular layer (G) can be noticed.

(Toluidine blue x1000)

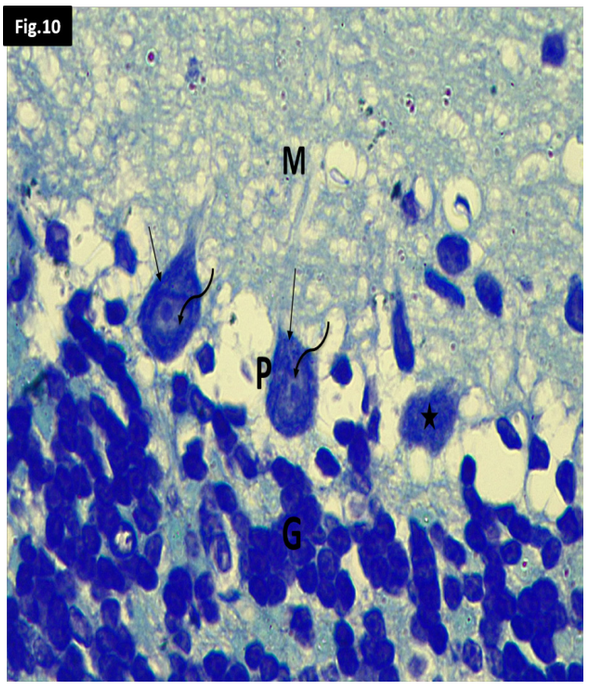

Fig.10: Photomicrograph of specimen from the cerebellar cortex of Gp. V (NaB + Zn) showing some Purkinje cells looking nearly normal with visible Nissl granules in the cytoplasm (arrows) and the nucleus appears with prominent nucleolus (wavy arrows). Other Purkinje cells appears irregular and distorted with invisible nucleus (star). The molecular layer (M) and granular layer (G) can be noticed.

(Toluidine blue x1000)

Immunohistochemical stain (GFAP):

GFAP immunohistochemical stain was done to demonstrate the granular layer astrocytes. Inspection of cerebellar cortex specimens of Gp I (Control) revealed star-shaped astrocytes with mild positive immune-reaction for GFAP (Fig.11). These findings were the same in Gp II (Zn) (Fig.12).

Inspection of cerebellar cortex specimens of Gp III (NaB) revealed abundant astrocytes with strong positive immunoreaction for GFAP (Fig.13).

In Gp IV (NaB withdrawal) inspection of cerebellar cortex specimens revealed moderate GFAP immunoreaction (Fig.14).

Inspection of cerebellar cortex specimens of Gp V (NaB and Zn) expressed moderate GFAP immunoreaction but less intense compared with group IV (Fig.15).

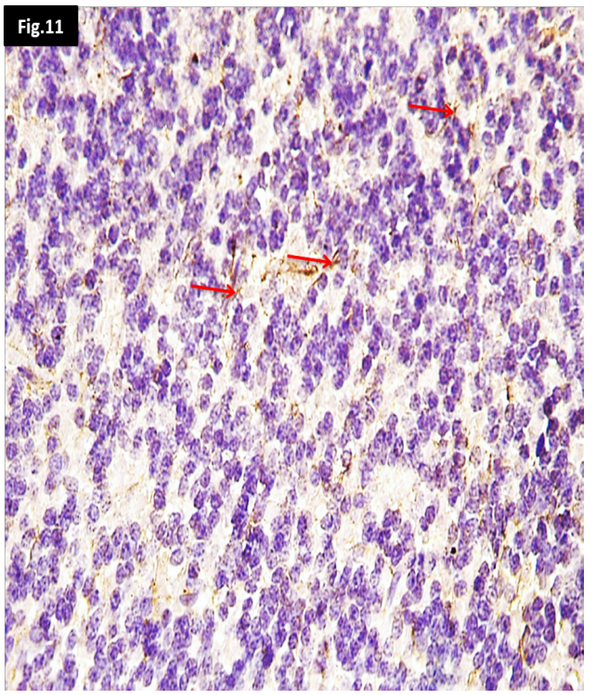

Fig.11: Photomicrograph of specimen from the cerebellar cortex of Gp. I (Control) showing star-shaped astrocytes in the granular layer (arrows) appear with mild positive immunoreaction for GFAP.

(Anti-GFAP x400)

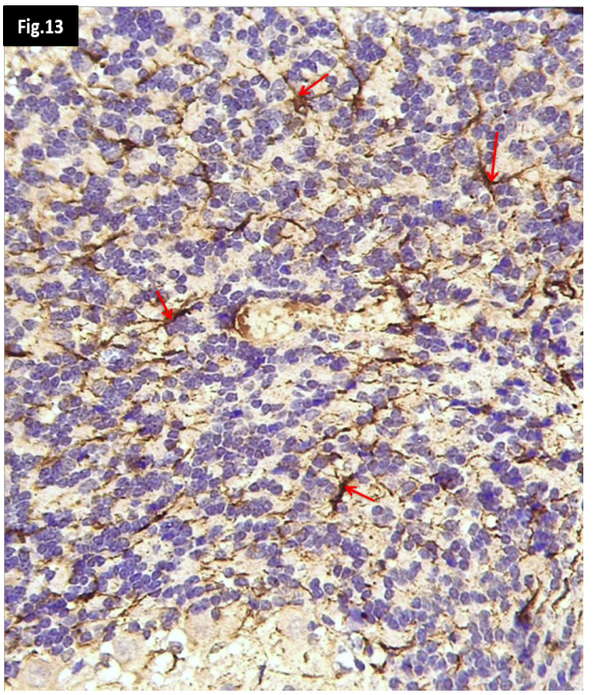

Fig.13: Photomicrograph of specimen from the cerebellar cortex of Gp. III (NaB) showing abundant astrocytes in the granular layer (arrows) appear with strong positive immunoreaction for GFAP.

(Anti-GFAP x400)

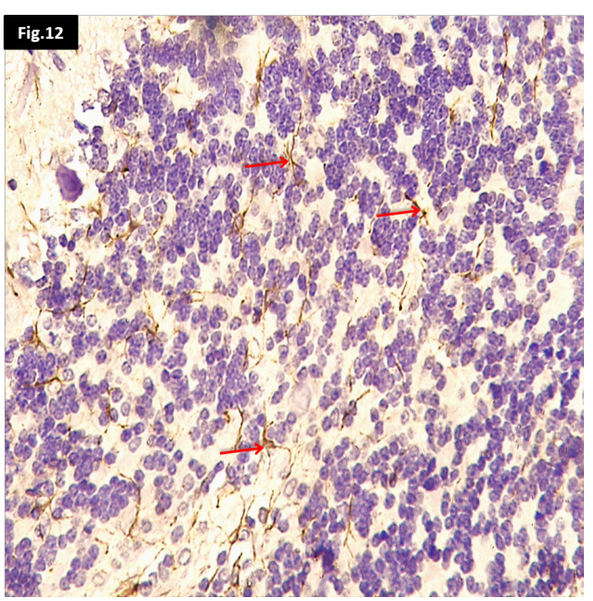

Fig.12: Photomicrograph of specimen from the cerebellar cortex of Gp. II (Zn) showing star-shaped astrocytes in the granular layer (arrows) appear with mild positive immunoreaction for GFAP.

(Anti-GFAP x400)

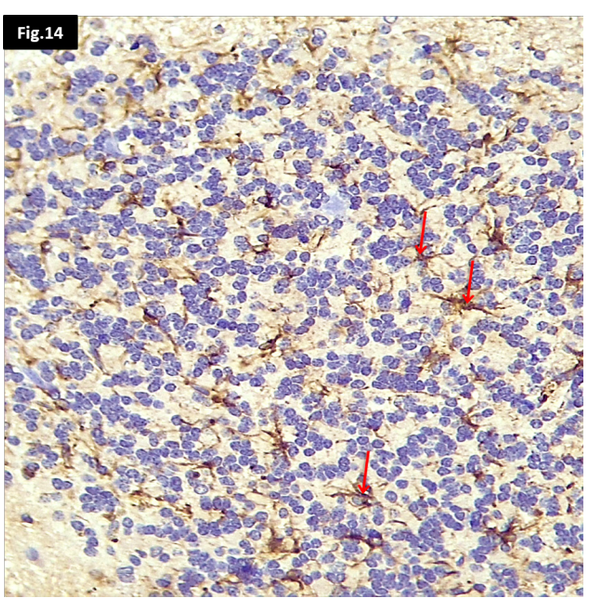

Fig.14: Photomicrograph of specimen from the cerebellar cortex of Gp. IV (NaB withdrawal) showing moderate GFAP immunoreaction (arrows).

(Anti-GFAP x400)

Fig.15

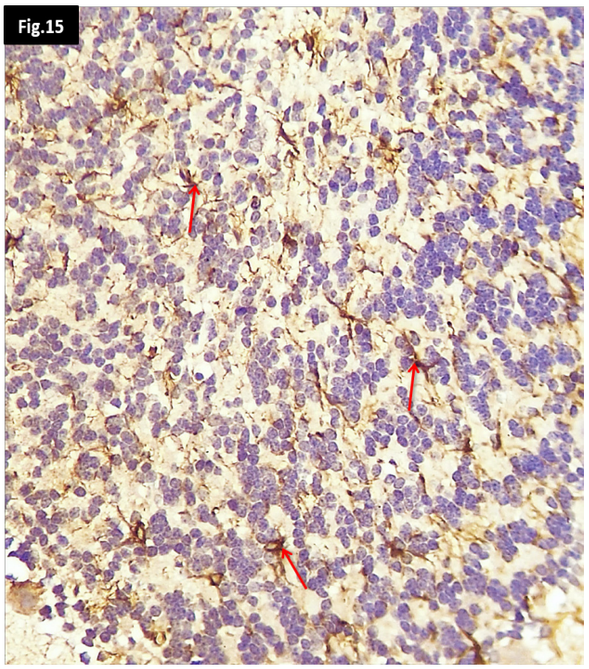

Fig.15: Photomicrograph of specimen from the cerebellar cortex of Gp. V (NaB + Zn) showing moderate GFAP immunoreaction but less intense compared with group IV (arrows).
(Anti-GFAP x400)

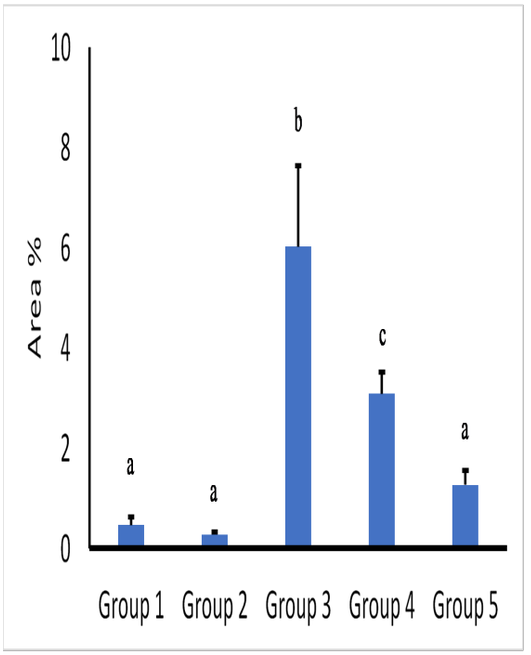

Fig. 16: Bar chart representing the area percentage of GFAP positive expression in the granular layer. Different letters indicate significant difference.

Morphometric and Statistical results:

GFAP positive expression area percentage in the granular layer was significantly higher in Gp III in comparison to both Gp I and II. Significantly higher in Gp IV in comparison to both Gp I and II. Significantly higher in Gp III in comparison to Gp IV (Table 1, Fig.16).

Table 1: Showing the mean ± SD values, regarding area percentage of GFAP positive expression in the granular layer between different groups.

	Group 1	Group 2	Group 3	Group 4	Group 5
1	0.646	0.269	4.713	2.897	1.593
2	0.4	0.236	5.516	3.58	1.055
3	0.358	0.332	7.829	2.773	1.156
Mean	0.468	0.279	6.019333	3.083333	1.268
SD	0.155576	0.048775	1.61783	0.434571	0.285953

Significantly higher in Gp III in comparison to both Gp I and II (P=0.000), (P=0.000), respectively.
Significantly higher in Gp IV in comparison to both Gp I and II (P=0.018), (P=0.011), respectively.
Significantly higher in Gp III in comparison to Gp IV (P=0.008).

Biochemical results

The biochemical results revealed the BDNF levels in different treatment groups. For the control group, range from 235.6 to 245.3 ng/ml, with a mean of 240.24 ± 3.57 ng/ml. The Zn group showed slightly higher BDNF levels than the control, ranging from 240.7 to 255.2 ng/ml, with a mean of 245.81 ± 4.85 ng/ml. The NaB group displayed significantly lower BDNF levels, ranging from 106.4 to 123.7 ng/ml, with a mean of 117.53 ± 6.11 ng/ml. The NaB withdrawal group showed partial recovery of BDNF levels, ranging from 144.5 to 157.4 ng/ml, with a mean of 151.09 ± 4.06 ng/ml. The NaB + Zn group showed BDNF levels between the control and Zn group, ranging from 215.6 to 225.7 ng/ml, with a mean of 221.44 ± 3.33 ng/ml. These results showed that NaB significantly reduced BDNF levels, while withdrawal partially restored them. Zn supplementation helped maintain or slightly increased BDNF levels (Table 2, Fig.17).

Table 2: Showing the mean ± SD values, for the comparison between different groups regarding BDNF level.

Groups	BDNF						ANOVA	
	Range			Mean	±	SD	f	*P-value*
Control	235.6	-	245.3	240.24	±	3.57		
Zinc	240.7	-	255.2	245.81	±	4.85		
Na Benzoate	106.4	-	123.7	117.53	±	6.11	1309.848	<0.001*
Na Benzoate withdrawal	144.5	-	157.4	151.09	±	4.06		
Na Benzoate + Zinc	215.6	-	225.7	221.44	±	3.33		

Tukey's test

	Control	Zinc	Na Benzoate	Na Benzoate withdrawal
Zinc	0.119			
Na Benzoate	<0.001*	<0.001*		
Na Benzoate withdrawal	<0.001*	<0.001*	<0.001*	
Na Benzoate + Zinc	<0.001*	<0.001*	<0.001*	<0.001*

Significant level:>0.05 Non-significant <0.05* significant <0.001* High

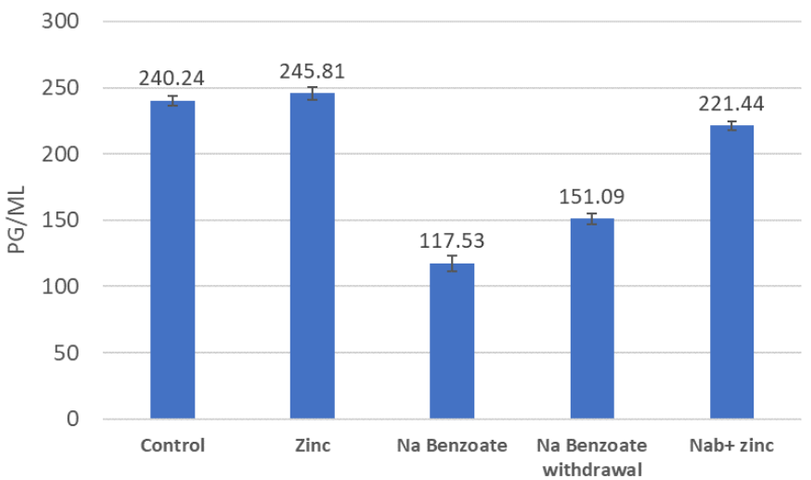

Fig. 17: Bar chart representing the BDNF levels in different treatment groups and the results of statistical analyses (ANOVA and Tukey's test).

DISCUSSION

The hazards and adverse impacts of NaB on the body were observed. The current work was done to observe the harmfulness of NaB on the cerebellum and the potential benefits of its withdrawal and zinc administration.

The present study revealed histological changes in the cerebellum after administration of NaB. The changes affect mainly the Purkinje and granular cell layers.

Our findings were consistent with earlier findings that proved a major structural alteration in the cerebellum after NaB treatment. Rats treated with NaB showed decrease in the cells, reduction in the bulk of the hemisphere and cortex[27, 28]. Similar findings indicated that the rats treated with NaB revealed changes in the cerebral cortex such as proliferation of glial cells, mild degeneration and vasogenic edema[5]. More researches which agreed this have shown that usage of NaB might change the histological architecture of body parts e.g. testes, heart and lung[29, 30].

These amendments in the tissues can be attributed to the rise in the lipid peroxidation and production of numerous quantities of free radicals and elevation of oxidative stress[15, 31, 32]. The administration of NaB was accompanied with enhancement of oxidative harm with induction of apoptosis[14, 33].

Our results also revealed that the granular layer in NaB group showed abundant astrocytes with strong positive reaction for GFAP. GFAP is a marker of astroglia injury. Astroglia cells are broadest cells in the nervous tissue.

They are supportive structurally and functionally for neurons. When brain injury occurs, astroglia cells respond by "reactive astrogliosis". The rise in GFAP is a hallmark of degenerative illnesses and brain damage[34-36].

The histological results in the current study were supported by the biochemical test which showed that the BDNF levels in different groups, these results displayed that BDNF is significantly reduced in NaB group. BDNF is a neurotropic factor uttered in the cerebrum continuously, it has a vital part in neuronal protection, survival, differentiation and in alleviating neuronal loss in neurodegeneration[37].

Previous studies reported that the hazards of NaB could lead to alterations in cellular architecture, disrupting biological and physiological status within the cells[14, 38].

Biochemical studies related reduction in serum BDNF levels with various mental illness, suggesting a common patho-physiological etiology in these disorders[39,40]. Furthermore, previous studies demonstrated the connotation relating the usage of NaB-rich drinks and signs allied with attention-deficit hyperactivity disorder (ADHD)[41, 42]. Cerebellar malfunction may have crucial role in the etiology of ADHD[43]. Furthermore, the animal model for ADHD showed reduced BDNF gene expression and studies stated a reduction in BDNF in patients with ADHD[44,45]. Similar results were observed that rats given NaB revealed diminished motor function[27, 33]. The cerebellum has a major role regarding motor and cognition functions[43].

In the present study we noticed that withdrawal from NaB led to partial histological improvement and moderate GFAP immunoreactivity. Withdrawal from NaB partially restored BDNF levels. Previous studies reported that the symptoms associated with ADHD were reduced after the withdrawal of sodium benzoate-rich beverages[41, 42].

In addition, our results revealed that co-administration of Zn mitigated the histological changes as some Purkinje cells appeared almost the same as the control. Moderate GFAP immunoreactivity was observed but less intense compared with NaB withdrawal group. Zn supplementation also helped maintain or slightly increased BDNF levels in comparison to the control.

Previous studies reported that the effect of NaB could be attributed to decrease the level of Zn in the brain and this enhances inflammatory response and the proapoptotic effects[27, 33, 46]. NaB also could induce cerebellar damage by enhancing oxidative stress[46].

Zinc level regulation has a crucial role in nervous functions, and any change in Zn can lead to neurological illnesses[3]. Zn deficiency is associated with cognitive loss, synaptic dysfunction, difficulties in learning and memory[47,48]. Zn is also vital for tissue's growth, renewal and repair. It also can protect the cell membrane[49].

In committing with our results studies which reported that Zinc treatment in rats exhibited partial enhancement in hippocampal histological architecture, with marked improvement of neurons and protection of its layers[50]. Zn also can a protect the spinal cord from injuries[51]. Nutrient rich in Zn up-regulating BDNF and prevents the cognitive loss in mouse[52-54].

Previous studies revealed that Zn therapy enhances serum BDNF concentrations[55]. Additionally, many studies proved that Zn increases the antioxidant level and can resist oxidative stress[56, 57]. It also has been reported that Zn exhibits a principal role in neuronal functioning and adaptation[22].

CONCLUSION

This study demonstrates that NaB a commonly used food preservative induces structural damage in the cerebellar cortex. Partial improvement was observed following NaB withdrawal. Co-administration of zinc mitigated the deleterious effects of NaB on the cerebellar cortex.

FINANCIAL SUPPORT

None

CONFLICT OF INTERESTS

There is no conflicts of interest.

The study was done under the guidelines of the

animal research committee, faculty of medicine, Helwan University (serial No. 15-2024)

The paper isn't published in other journals or presented at a meeting, organization, or any other place

ACKNOWLEDGMENT

All authors thank the workers and technicians who aid in performing this study.

REFERENCES

1. **Tawfeeq FK and Jirjees IH.** Effect on monosodium glutamate on pregnant female rats and embryo. Al-Anbar J Vet Sci. 2013;6(2):100-107.

2. **Arnold LE, Lofthouse N, Hurt E.** Artificial food colors and attention deficit/hyperactivity symptoms: conclusions to dye for. Neurotherapeutics. 2012;9(3):599–609.

3. **Pundir C S and Rawal R.** Determination of sulfite with emphasis on biosensing methods: a review. Analytical and bioanalytical chemistry. 2013;405(10):3049-3062.

4. **Bawazir A E.** Effects of Food Colour Allura Red (No. 129) on some neurotransmitter, Antioxidant Functions and Bioelement Contents of Kidney and Brain Tissues in male albino Rats. Life Science Journal. 2016;13(12):10-17.

5. **Davidson P M, Taylor T M, David J R D.** Antimicrobials in Food, 4th ed. CRC Press: Boca Raton, FL, USA, 2021: ISBN 978-0-367-17878-9.

6. **Shahmohammadi M, Javadi M, Nassiri-Asl M.** An overview on the effects of sodium benzoate as a preservative in food products. Biotechnology and Health Sciences. 2016;3(3):e35084. DOI:10.17795/bhs-35084.

7. **CFR-Code of Federal Regulations Title 21.** Available online: https://www.accessdata.fdaGov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=184.1733 (accessed on 14 November 2021).

8. **Al-Shinnawy M S and Elkattan N A.** Assessment of the changes in some diagnostic parameters in male albino rats fed on an Azo Dye. Int J Environ Sci Eng. 2013;4:85-90.

9. **El-banna K, Sarhan O, Khider M, Abulreesh H, Shaaban M.** Microbiological, histological, and biochemical evidence for the adverse effects of food azo dyes on rats. J. Food and Drug Analysis. 2017;25(3):667-680.

10. **Amin K A and Al-Shehri F S.** Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarkers: A review. African Journal of Biotechnology. 2018;17(6):139-149.

11. **Soltan S S and Shehata M M.** The effects of using color foods of children on immunity properties and liver, kidney on rats. Food and Nutrition Sciences. 2012;3(7): 897-905.

12. **Khan IS, Dar KB, Ganie SA, Ali MN.** Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. Drug and Chemical Toxicology. 2022; 45(3):1345-54. DOI: 10.1080/01480545.2020.1825472; PMID:33003957.

13. **Oyewole OI, Dere FA, Okoro OE.** Sodium benzoate mediated hepatorenal toxicity in wistar rat: Modulatory effects of azadirachta indica (neem) leaf. European Journal of Medicinal Plants. 2012;2(1):11-18. DOI: 10.9734/EJMP/2012/619.

14. **El-Shennawy L, Kamel MA, Khalaf AH, Yousef MI.** Dose-dependent reproductive toxicity of sodium benzoate in male rats: Inflammation, oxidative stress and apoptosis. Reproductive Toxicology. 2020; 98:92-98. DOI: 10.1016/j.reprotox.2020.08.014.

15. **Khoshnoud MJ, Siavashpour A, Bakhshizadeh M, Rashedinia M.** Effects of sodium benzoate, a commonly used food preservative on learning memory and oxidative stress in brain of mice. J Biochem Mol Toxicol. 2017; 94(01):1-7. DOI: 10.1002/jbt.22022.

16. **Yetuk G, Pandir D, Bas H.** Protective role of catechin and quercetin in sodium benzoate-induced lipid peroxidation and the antioxidant system in human erythrocytes in vitro. The Scientific World Journal. 2014;18:874824 DOI: 10.1155/2014/874824; PMID: 2469325.

17. **Oloye F F.** Spectroscopic investigation of the mixture of ascorbic acid and sodium benzoate. Sci. J. Chem. 2019;7 (3), 62–66.

18. **Prasad AS.** Impact of the discovery of human zinc deficiency on health. J Am Coll Nutr. 2009; 28:257–65. DOI: 10.1080/07315724.2009.10719780.

19. **Kambe T, Matsunaga M, Takeda T-A.** Understanding the contribution of zinc transporters in the function of the early secretory pathway. Int. J. Mol. Sci. 2017;18(10):2179. DOI: 10.3390/ijms18102179.

20. **Zhao H, Wang Y, Yang X, Fei D, Mu M, Guo M, Yu H, Xing M.** Zinc alleviates arsenism in common carp: varied change profiles of cytokines and tight junction proteins among two intestinal segments. Fish Shellfish Immunol. 2019a;94,761–768. https://doi.org/ 10.1016/j.fsi.2019.09.069.

21. **Choi S, Hong D K, Choi B Y, Suh S W.** Zinc in the brain: Friend or foe?. Int. J. Mol. Sci. 2020;21(23), 8941.

22. **Frederickson CJ, Koh JY, Bush AI.** The neurobiology of zinc in health and disease. Nat Rev Neurosci. 2005; 6:449–62. DOI:10.1038/nrn1671.

23. **O Teslariu, A S Pasca, L Mititelu-Tartau, C E schiriac, C gales, P M saftencu, *et al.*** The Protective effects of zinc in experimental gentamycin induced acute renal failure in rats. Journal OF Physiology and Pharmacology. 2016; 67(5):751-757.

24. **Bancroft JD and Layton C.** Theory and practice of histological technique, 7th ed., London: Churchill Livingstone. 2010:173-214.

25. **Buchwalow I Band Böcker W.** Immunohistochemistry: Basics and Methods, Springer-Verlag Berlin Heidelberg 2;14(3):19-29.

26. **Noorafshan A, Erfanizadeh M, Karbalay-Doust S.** 'Stereological studies of the effects of sodium benzoate or ascorbic acid on rats' cerebellum', Saudi Medical Journal. 2014;35(12):1494-1500. PMID: 25491215 PMCID: PMC4362157.

27. **Chan YH.** Biostatistics 102: quantitative data-parametric & non-parametric tests. Singapore Med J. 2003;44(8):391-6.

28. **Murray RK, Granner PA, Mayer PA, Rodwell VW.** Harper 's biochemistry. 20th ed. England: McGraw-Hill, 2000:594 –602.

29. **Nawras A Alwan1, Alia M Kudayer, Eman Aboud Al-Masoudi1.** Toxic Effect of Oral Gavaged of Sodium Benzoate (SB) on Adult Male Rabbits. Mal J Med Health Sci. 2022;18(SUPP4): 64-69.

30. **Kehinde OS, Christianah OI, Oyetunji OA.** Ascorbic acid and sodium benzoate synergistically aggravate testicular dysfunction in adult Wistar rats. Int. J. Physiol. Pathophysiol. Pharmacol. 2018;10(1):39-46.

31. **Mazin Sh, Alwan N A, Al-Masoudi E A A.** Study of toxic effect of sodium benzoate, Vit.C alone and their combination on reproductive functions of adult male rabbits. Basrah J. of Veteri. Res. 2018;17(3): 533-543.

32. **Khodaei F, Kholghipour H, Hosseinzadeh M, Rashedinia M.** Effect of sodium benzoate on liver and kidney lipid peroxidation and antioxidant enzymes in mice. J. Rep. Pharma. Sci. 2019; 8:217-223.

33. **Olofinnade A T, Onaolapo A Y, Onaolapo O J, Olowe O A.** The Potential Toxicity of Food-Added Sodium Benzoate in Mice Is Concentration-Dependent. Toxicol. Res. 2021;10,561–569.

34. **Lopategui Cabezas I, Herrera Batista A, Penton Rol G.** The role of glial cells in Alzheimer disease: potential therapeutic implications. Neurologia. 2014;29:305–9. DOI: 10.1016/j.nrl.2012.10.006.

35. **Czlonkowska A, Kurkowska-Jastrzebska I.** Inflammation and gliosis in neurological diseases–clinical implications. J Neuroimmunol. 2011;231:78–85. DOI: 10.1016/j.jneuroim.2010.09.020.

36. **Sofroniew MV.** Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 2009;32:638–47. DOI:10.1016/j.tins.2009.08. 002.

37. **Jana A, Modi K K, Roy A, Anderson JA, van Breemen R B, Pahan K.** Up-Regulation of Neurotrophic Factors by Cinnamon and Its Metabolite Sodium Benzoate: Therapeutic Implications for Neurodegenerative Disorders. J. Neuroimmune Pharmacol. 2013;8:739–755.

38. **Al-Ameen S A, Jarjees E H; Tawfeeq F Kh.** Effect of sodium benzoate on some biochemical, physiological and histopathological aspects in adult male rats1. Iraqi Journal of Veterinary Sciences. 2022;36(2):267-272.

39. **Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y *et al* (2006)** Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 30:1529–1531.

40. **Nakazato M, Hashimoto K, Shimizu E, Niitsu T, Iyo M.** Possible involvement of brain-derived neurotrophic factor in eating disorders. IUBMB Life. 2012;64:355–361.

41. **McDougal E, Gracie H, Oldridge J, Stewart T M, Booth J N, Rhodes S M.** Relationships between Cognition and Literacy in Children with Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-Analysis. Br. J. Dev. Psychol. 2022;40:130–150.

42. **Beezhold B L, Johnston C S, Nochta K A.** Sodium Benzoate-Rich Beverage Consumption Is Associated with Increased Reporting of ADHD Symptoms in College Students: A Pilot Investigation. J. Atten. Disord. 2014; 18:236–241.

43. **Stoodley C J.** Distinct Regions of the Cerebellum Show Gray Matter Decreases in Autism, ADHD, and Developmental Dyslexia. Front. Syst. Neurosci. 2014;8,92.

44. **Fumagalli F, Racagni G, Colombo E, Riva MA.** BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. Mol Psychiatry. 2003;8:898–899.

45. **Corominas-Roso M, Ramos-Quiroga JA, Ribases M, SanchezMora C, Palomar G, Valero S, Bosch R, Casas M.** Decreased serum levels of brain-derived neurotrophic factor in adults with attention-deficit hyperactivity disorder. Int J Neuro psycho pharmacol. 2013;30:1–9.

46. **El-Nouby KA, Hamouda HE, Abd El Azeem MA, El-Ebiary AA.** Food additives and Hymenolepis nana infection: an experimental study. J Egypt Soc Parasitol. 2009;39:1015-1032.

47. **Brion LP, Heyne R, Lair CS.** Role of zinc in neonatal growth and brain growth: review and scoping review. Pediatr Res. 2021;89:1627–40. DOI: 10.1038/s41390-020- 01181- z

48. **Krall RF, Tzounopoulos T, Aizenman E.** The function and regulation of zinc in the brain. Neuroscience. 2021;457:235–58. DOI: 10.1016/j. neuroscience.2021.01.010

49. **Thingholm TE, Rönnstrand L, Rosenberg PA.** Why and how to investigate the role of protein phosphorylation in ZIP and ZnT zinc transporter activity and regulation. Cell Mol Life Sci. 2020;77(16):3085-3102.

50. **Lamia M. Hafez, Hebatallah Mohammed Aboudeya, Noura A. Matar, Ashraf S. El-Sebeay, Azhar Mohamed Nomair, Shaymaa Ali El-hamshary, Hanan Mohamed Nomeir, Fawziya A. R. Ibrahim**. Ameliorative effects of zinc supplementation on cognitive function and hippocampal leptin signaling pathway in obese male and female rats. Scientific Reports. 2023;28;13(1):5072. DOI:10.1038/s41598-023-31781-8.

51. **Li X, Chen S, Mao L, *et al.*** Zinc improves functional recovery by regulating the secretion of granulocyte colony stimulating factor from microglia/macrophages after spinal cord injury. Front Mol Neurosci. 2019;12:18. DOI: 10.3389/fnmol.2019.00018.

52. **Nam S M. *et al.*** Differential effects of low- and high-dose zinc supplementation on synaptic plasticity and neurogenesis in the hippocampus of control and high-fat diet-fed mice. Neurochem. Res. 2017;42:3149–3159.

53. **Corona C. *et al.*** Dietary zinc supplementation of 3xTg-AD mice increases BDNF levels and prevents cognitive deficits as well as mitochondrial dysfunction. Cell. Death. Dis. 2010;21(10):e91. DOI: 10.1038/cddis.2010.73.

54. **Hu Y D. *et al.*** The cognitive impairment induced by zinc deficiency in rats aged 0 approximately 2 months related to BDNF DNA methylation changes in the hippocampus. Nutr. Neurosci. 2017;20(9):519–525.

55. **Solati Z, Jazayeri S, Tehrani-Doost M, Mahmoodianfard S, Gohari MR.** Zinc monotherapy increases serum brain-derived neurotrophic factor (bdnf) levels and decreases depressive symptoms in overweight or obese subjects: a double-blind, randomized, placebo-controlled trial. Nutr Neurosci. 2015;18:162–8. DOI: 10.1179/1476830513Y.0000000105.

56. **Zhao H, Wang Y, Liu J, Guo M, Fei D, Yu H, Xing M.** The cardiotoxicity of the common carp (Cyprinus carpio) exposed to environmentally relevant concentrations of arsenic and subsequently relieved by zinc supplementation. Environ. Pollut. 2019b;253:741–748. https://doi.org/10.1016/j.envpol.2019.07.065.

57. **De Grande A, Leleu S, Delezie E, Rapp C, De Smet S, Goossens E, Haesebrouck F, Van Immerseel F, Ducatelle R.** Dietary zinc source impacts intestinal morphology and oxidative stress in young broilers. Poult. Sci. 2020;99 (1):441–453. https://doi.org/10.3382/ps/pez525.

تأثير بنزوات الصوديوم على مخيخ ذكور الجرذان البيضاء البالغة والتأثير التحسيني ل الإنسحاب وإعطاء الزنك: دراسة نسيجية ومناعية كيميائية

إيمان أحمد الصواف[١] ونهى أسامة شوقي[٢]

[١]قسم التشريح والأجنة، [٢]قسم علم وظائف الأعضاء، كلية الطب، جامعة حلوان، القاهرة، مصر

المقدمة: يستخدم بنزوات الصوديوم في الصناعات الغذائية للحفاظ على الطعام ويمكن أن يؤدي استخدامه إلى العديد من الأمراض.

الهدف من الدراسة: الهدف من الدراسة هو التحقق من تأثير بنزوات الصوديوم على قشره المخيخ في ذكور الفئران وتأثير الإنسحاب وإعطاء الزنك.

المواد والطرق: تم استخدام أربعين فأرا من ذكور الفئران البيضاء و تم تقسيمهم إلى خمس مجموعات (ثمانية لكل مجموعه):

المجموعة الأولى (الضابطة): تلقت الفئران نظاما غذائيا عاديا، المجموعة الثانية (مجموعة الزينك): تم حقن الفئران بزنك كلوريد بجرعة ٥ ملجم / كجم / يوم لمدة ثمانية وعشرون يوما، المجموعة الثالثة (مجموعة بنزوات الصوديوم): تلقت الفئران بنزوات الصوديوم عن طريق الفم (٢٠٠ مجم / كجم / يوم) لمدة ثمانية وعشرون يوما، المجموعة الرابعه (مجموعة الإنسحاب من بنزوات الصوديوم): تلقت الفئران بنزوات الصوديوم بجرعة ٢٠٠ مجم / كجم / يوم لمدة ثمانية وعشرون يوما ثم إيقاف إعطاء بنزوات الصوديوم لمده ثمانية وعشرون يوما أخرين، المجموعة الخامسه (مجموعة بنزوات الصوديوم والزينك): تلقت الفئران بنزوات الصوديوم بجرعة ٢٠٠ مجم / كجم / يوم مع الإعطاء المشترك للزنك بجرعة ٥ مجم / كجم / يوم لمدة ثمانية وعشرون يوما.

في نهاية التجربة تم سحب عينات الدم للدراسات البيوكيميائية وتشريح المخيخ للدراسات النسيجية والكيميائية المناعية.

النتائج: أظهر فحص عينات المخيخ لمجموعة بنزوات الصوديوم تغيرات نسيجية في قشره المخيخ وأظهر الإنسحاب من بنزوات الصوديوم تحسنا جزئيا وقد أظهر الإعطاء المشترك للزنك مع بنزوات الصوديوم تأثيرا وقائيا ضد التغيرات النسيجية.

وقد خفضت بنزوات الصوديوم بشكل كبير من مستويات العوامل العصبية المشتقة من الدماغ، لكن الإنسحاب من بنزوات الصوديوم أعادها جزئيا. كما حافظ الزنك عليها أو زادت بشكل طفيف.

الإستنتاج: أظهرت هذه الدراسة أن ماده بنزوات الصوديوم تسبب أضرارا هيكلية في قشرة المخيخ وقد ظهر تحسن جزئي بعد الإنسحاب من بنزوات الصوديوم كما أن الإعطاء المشترك للزنك يخفف من هذا التأثير.