Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2021.100454.2376 Volume 30, Issue 1.7, Oct. 2024, Supplement Issue

Manuscript ID ZUMJ-2110-2376
DOI 10.21608/zumj.2021.100454.2376

ORIGINAL ARTICLE

Comparative Neurotoxic Effect of Aspartame and Stevia Sweeteners on Rat Cerebellar Cortex and the Possible Protective Role of Omega-3: Histological and Immunohistochemically Study

Aml E. Mohamed, Huda M. El-Tahry, Amany Shams Marwa Abd El-kader, Aml E. Mohamed*
Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

***Corresponding Author:**
Aml E. Mohamed
Email:
amlesma3eel@gmail.com

Submit Date 07-11-2021
Revise Date 02-12-2021
Accept Date 14-12-2021

ABSTRACT

Background: Over the last two decades, the market for sweeteners has greatly exploded. Aspartame is one of the most common artificial sweeteners that is considered a risky compound. On the other hand, stevioside is a sugary plant that seemed to be a hopeful natural alternative to synthetic sweeteners.

The purpose of this study was to assess the histopathological changes in the cerebellar cortex of adult rats following eight weeks of aspartame and stevioside administration, and to evaluate the protective effect of omega-3 against induced cerebellar injury.

Methods: Thirty rats were used and divided into five groups at random: Group I: was the control group, Group II: rats received aspartame (250 mg\kg\day), Group III: rats received stevia (250 mg\kg\day), Group IV: rats received omega-3 (100 mg/kg/day) followed by aspartame (250 mg\kg\day), Group V: rats received omega-3 (100 mg/kg/day) followed by stevia (250 mg\kg\day). All medications received by gavage for 8 weeks. Cresyl violet staining and immunohistochemistry staining for Cyclooxygenase-2 (COX-2) were performed on cerebellar cortex specimens.

Results: Both aspartame and stevioside-treated groups showed degenerative alterations in the cerebellar cortex but aspartame was more harmful than stevia. This is manifested by a considerable rise in COX-2 positive cells. Omega-3 supplementation with aspartame and stevia reduced degenerative and inflammatory consequences, with stevia having a stronger effect than aspartame. Such finding was corraborated by a significant decrease in COX-2 positive cells.

Conclusions: Stevioside is suggested to be less harmful on the cerebellar cortex than aspartame. Omega-3 can greatly decrease the harmful effects of stevia. However, its effects on the adverse effects of aspartame appears to be less potent.

Keywords: Aspartame; Cerebellar Cortex; Omega-3; Stevioside; COX-2.

INTRODUCTION

Aspartame is one of the most widely used synthetic sweeteners in the world today. It is artificial, white-colored, odorless, crystalline powder used chiefly in diet soft drinks and many foodstuffs like yogurts, sweets, gum, vitamins, and medications [1]. It may be ingested in very small amounts and is regarded almost non-caloric because it is 180–200 times sweeter than sucrose. [2]. Aspartame is a methyl ester of a dipeptide. It consists of phenylalanine (50%), aspartic acid (40%), and methanol (10%) [3].

Aspartame is digested in the small intestines by esterases and peptidases into its basic components, which are phenylalanine, aspartic acid, and methanol. Phenylalanine is present as D and L enantiomers. L-phenylalanine is an essential amino acid necessary for protein synthesis. Dietary consumption of L-phenylalanine and endogenous recycling of amino acid reserves are the two main sources of L-phenylalanine in the body [4]. Aspartic acid is a common amino acid in the normal diet which is an excitatory neurotransmitter.[5]Methanol is converted to formaldehyde in the liver, which is then converted to formic acid. Through the production of 10-formyl tetra hydro folate, formic acid is converted to CO2 and water [6].

On the other hand, the growing prevalence of obesity and diabetes, has heightened the interest in natural sweeteners that can replace sucrose. Stevia has received a lot of attention. *Stevia rebaudiana Bertoni* is a persistent branched bushy plant of the *compositae* family, original to the northeast of Paraguay. It also presents in the nearby parts of South America. Nowadays, its cultivation has spread to various countries around the world, including Canada, Europe, and Asia [7].

The sweet component of stevia was extracted in 1909, but it wasn't until 1931 that the extract was refined to yield stevioside which is a glycoside with three glucose molecules connected to an aglycone, the steviol moiety. The steviol glycosides are the chemicals responsible for stevia's sweet flavour, which is now well-known. Stevioside has undergone numerous safety tests, and no major hazardous effects have been documented to date. Also, several studies demonstrated multiple health benefits e.g. anti-diabetic, anti-hypertension, anti-tumor, anti-oxidant and anti-microbial effects [8].

Omega-3 long-chain polyunsaturated fatty acids (n-3 PUFA, with 20 carbon atoms) are a form of fatty acid that contains two or more double bonds, one of which is three carbon positions from the methyl terminus (“omega-3” or “n-3”). The main n-3 PUFA in the diet are eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Over the last few decades, multiple studies have found that diets high in EPA and DHA, obtained from dietary sources or pharmacological supplements have hypotriglyceridemic, anti-inflammatory, antithrombotic, vasodilator, and antiarrhythmic abilities, and that these pleiotropic effects are accompanied by a lower risk of cardiovascular diseases.[9] They are deemed essential (mammals cannot produce them on their own) and must be received from external sources such as fish oil, marine animals, and plant seeds such as flaxseed, walnut, soybean, and canola oil [10].

Long-term linolenic acid shortage has been linked to loss of learning ability, memory problems, and neurological illness (precursors of PUFAs). The consumption of OM3 by moms during pregnancy and breastfeeding may also help the mental development of their children. In nervous tissue, PUFAs are involved in synaptic transmission of catecholaminergic, cholinergic, and serotoninergic receptors, as well as cell migration and death [11].

Cyclooxygenase (COX) is a membrane-bound enzyme which is responsible for the oxidation of arachidonic acid to prostaglandin (G2) and its subsequent reduction to prostaglandin (H2) [12]. It is present in at least two forms, the expressed form, COX-1, and the inducible form, COX-2 [13] COX-1 can be expressed in a number of normal tissues and is involved in a number of homeostatic body functions such as hemostasis [14] The expression of COX-2 can be induced by various stimuli such as cytokines and growth factors [15] A strong expression of COX-2 has been associated with chronic inflammatory diseases and neurotoxic conditions such as seizures and hypoxia [16].

As a result, the goal of this study was to look at the cellular damage produced by aspartame and stevia sweeteners in the cerebellar cortex of male albino rats, as well as the probability of alleviating these alterations by use of Omega-3.

METHODS

Animals

In this study, 30 adult male albino rats weighing between 200-250 gm. were used. Animals were housed in standard clean plastic cages and were given a regular diet and water ad libitum under controlled conditions. This work was conducted in the research facility of Anatomy Department, Mansoura Faculty of Medicine. The experiments lasted for two months. All experiments followed the National Institutes of Health's (NIH) guidance for the Care and Use of Laboratory Animals. All animal experiments comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals.

The experimental design:

The animals were divided into five equal groups (6 rats each):

Group 1: It was used as a control group. Throughout the trial, the animals in this group were given a daily dose of 100ml distilled water using an intragastric tube hat was comparable to the dose provided to the other groups.

Group 2: The animals received aspartame at a dose of 250 mg/kg body weight daily [17] dissolved in 100 ml distilled water for 8 weeks using an intragastric tube. The aspartame tablets used were obtained from the Amriya group for pharmaceuticals, Egypt.

Group 3: The animals received stevia at a dose of 250 mg/kg body weight daily [18] dissolved in 100 ml distilled water for 8 weeks using an intragastric tube. Stevia was obtained in powder form from ATOS PHARMA, Egypt.

Group 4: The animals received omega-3 in a dose of 100 mg/kg/day [19] dissolved in 100 ml distilled water followed by aspartame in the same dose as the rats in group 2.

Group 5: The animals received omega-3 in a dose of 100 mg/kg/day [19] dissolved in 100 ml distilled water followed by stevia in the same dose as the rats in group 3.

After the termination of the trial, the rats were sacrificed by decapitation under halothane anesthesia. The brain was removed and the cerebellum was taken out.

Light microscopic examination:

For the light microscopic investigation, sections of rat cerebellum were obtained and fixed in 10% neutral buffered formalin. Alcohols were used to dry the samples, which were subsequently cleaned in xylene before being embedded in paraffin wax and processed for light microscopic (LM) study [20]. Cresyl violet was used to stain 5mm thick sections.

Immunohistochemical staining for localization of cyclooxygenase-2:

The expression of COX-2 was identified by using the primary antibody anti-rabbit, anti-mouse polyclonal COX-2-specific IgG (A1235, ABclonal chemicals, USA) [21].

Dewaxed sections were rehydrated in graded alcohol solutions. Then, for heat retrieval, it was placed in citrate buffer (1:10 dilution, pH 6) and heated at 120°C for 3–5 minutes. Endogenous peroxidase activity was neutralised for 5 minutes with peroxidase block. Preincubation with protein block for 5 minutes at room temperature inhibited nonspecific binding. The primary diluted antibodies rabbit polyclonal antibodies with dilution 1/200 were then treated for 1 hour at room temperature with the slides. The slides were then rinsed in PBS for 2–5 minutes before being incubated with the secondary antibody for 30 minutes. After 5 minutes of colour development in diaminobenzidine, sections were counterstained with hematoxylin, dehydrated, and mounted.

Morphometric analysis:

The morphometric study was accomplished using Image J software (Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA). The number of normal Purkinje cells was measured in Cresyl violet stained sections and the surface area of cyclooxygenase-2 immunoexpression of the three layers of the cerebellar cortex was measured. All measurements were performed at magnification X400 in 6 non-overlapping fields in 6 random sections in 6 different rats in each group.

Statistical analysis:

The statistical investigation was conducted using SPSS 22 statistical software

(IBM Corp. Armonk, NY, USA). The data's arithmetic means and standard deviation (SD) were reported. Using one-way analysis of variance (ANOVA) and the Post Hoc test, the probability (P) value was calculated. When the (P) value was less than or equal to 0.05, the statistical results were significant.

RESULTS

Light microscopic results

The control group's cerebellar sections revealed three layers of normal cortical architecture: the molecular layer, Purkinje layer, and granular layer. There were few tiny dispersed cells and many nerve fibers in the outer molecular layer. The Purkinje cell layer contained Purkinje cells arranged in one row and have a pyriform-shaped cell body with their dendrites pointing toward the molecular layer and a pale stained centrally located vesicular nuclei with prominent nucleoli and were encircled by Nissl's granules. The granular layer contained small granule cells with dense nuclei and sparse cytoplasm (Figs. 1A, 2A).

Inspection of sections of the aspartame treated group revealed disturbed cortical architecture. The Purkinje cells showed degenerative changes. They lost their normal pyriform shape and became irregular in shape with shrunken irregular outline, pale basophilic cytoplasm, pyknotic nucleus, and a few randomly scattered Nissl's granules. Some cells showed intracytoplasmic vacuoles. Many vacuolar spaces appeared between them (Figs. 1B, 2B).

Examination of sections of stevia treated group showed that many Purkinje cells seemed normal in shape. Some other Purkinje cells appeared degenerated with pale basophilic cytoplasm, Pyknotic nuclei, and a few randomly scattered Nissl's granules (Figs. 1C, 2C).

Examination of sections of rats treated with omega-3 and aspartame revealed restoration of cortical architecture except that some Purkinje cells were still degenerated and others regained their normal pyriform shape (Figs. 1D, 2D).

Examination of sections of rats treated with omega-3 and stevia revealed restoration of normal architecture of cerebellar cortex apart from few Purkinje cells that were still degenerated. Most Purkinje cells restored its normal pyriform shape (Figs. 1E, 2E).

Immunohistochemical evaluation for COX-2:

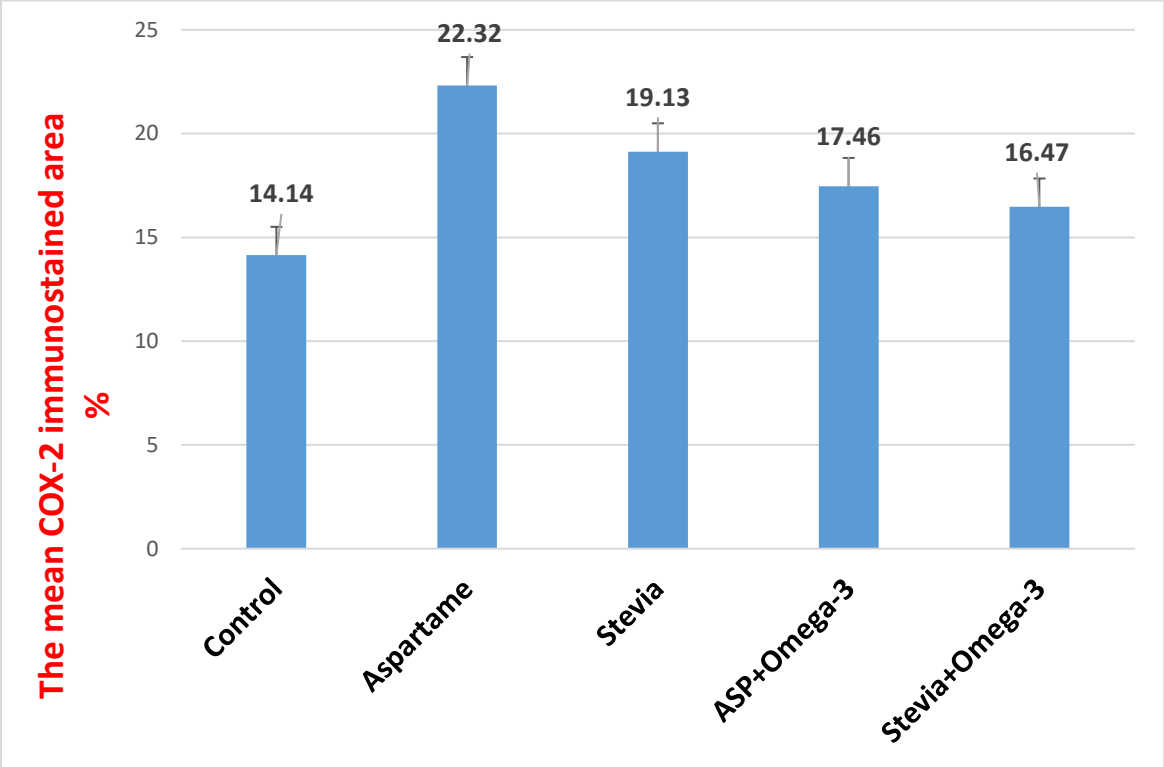
In the three layers of the cortex of the cerebellum, sections from the control group revealed a very low intensity immunological reactivity for COX-2 (Fig. 3A). A strong positive high-intensity COX-2 reaction in the form of brown colour was observed in all levels of the cerebellar cortex in the aspartame treated group (Fig. 3B). The stevia-treated group's cerebellar sections revealed a moderate positive immune response for COX-2 in the three layers of the cortex of the cerebellum. (Fig. 3C). Examination of sections of aspartame+omega-3 treated group showed a decrease in the immune reaction in the molecular and granular layers but it is still high in Purkinje cell layer (Fig. 3D). In rats treated with both stevia + omega-3, the cerebellar sections showed mild immune reaction in the three layers of the cortex of the cerebellum (Fig. 3E).

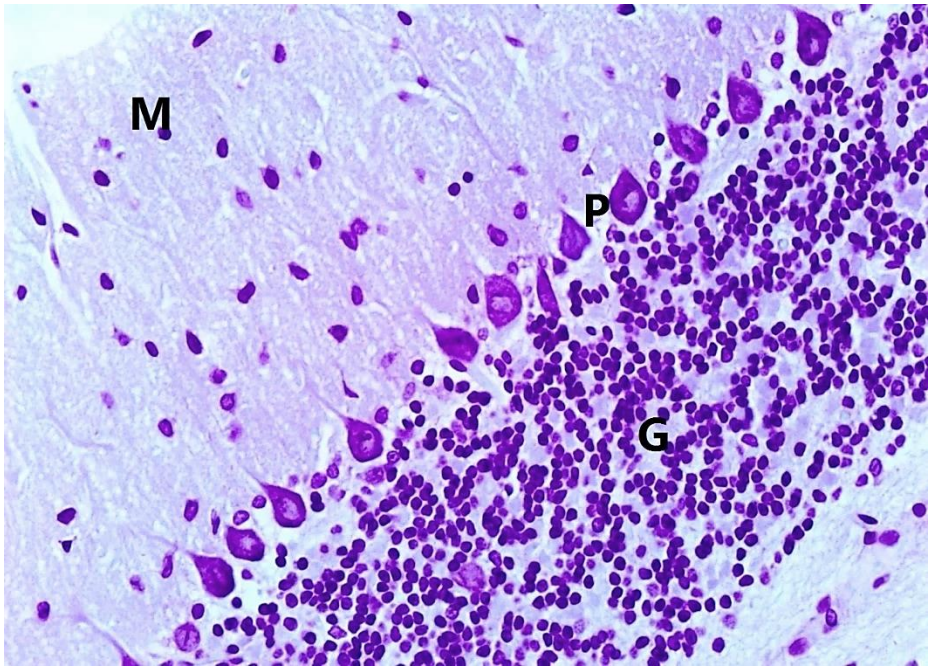
Table 1 and Histogram 1 show the mean ± SD of the positive brownish COX-2 immunostained area percentage between all studied groups:

Table. 1: Comparison of the mean COX-2 immunostained area percentage between all studied groups

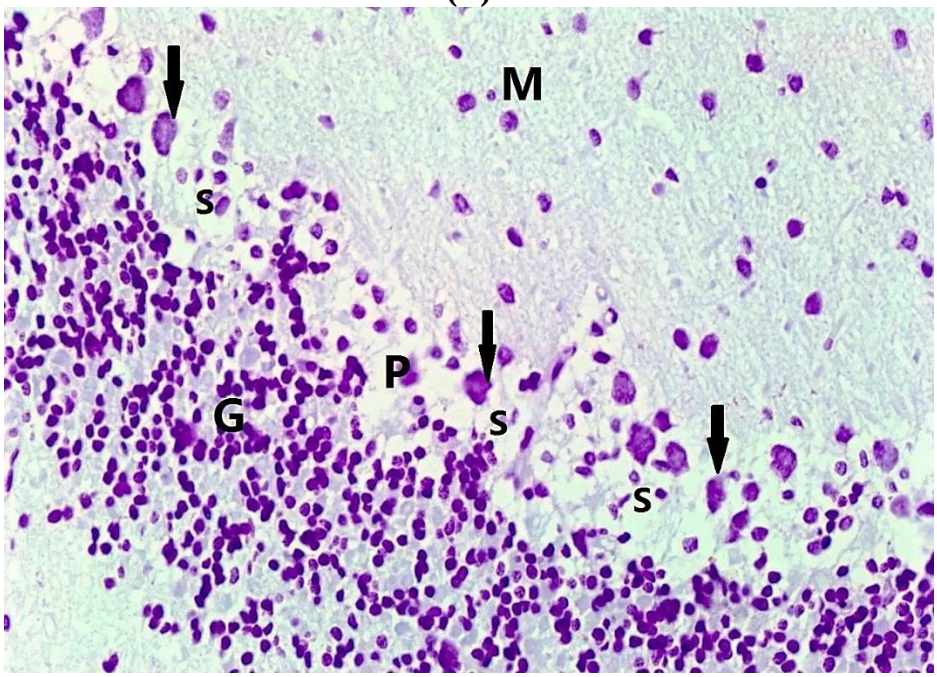
	Control	Aspartame	Stevia	ASPARTAME+Omega-3	Stevia+omega-3
Mean	14.14	22.32	19.13	17.46	**16.47**
Standard deviation	4.58	5.13	5.71	4.05	**4.81**

Histogram. 1: A Histogram showing the mean COX-2 immunostained area percentage of different groups

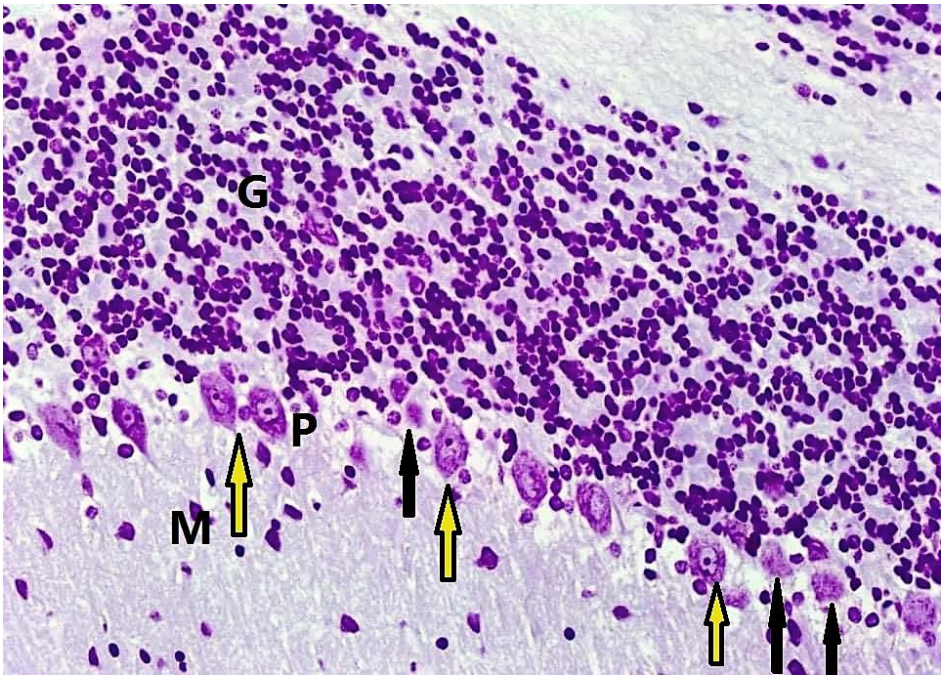




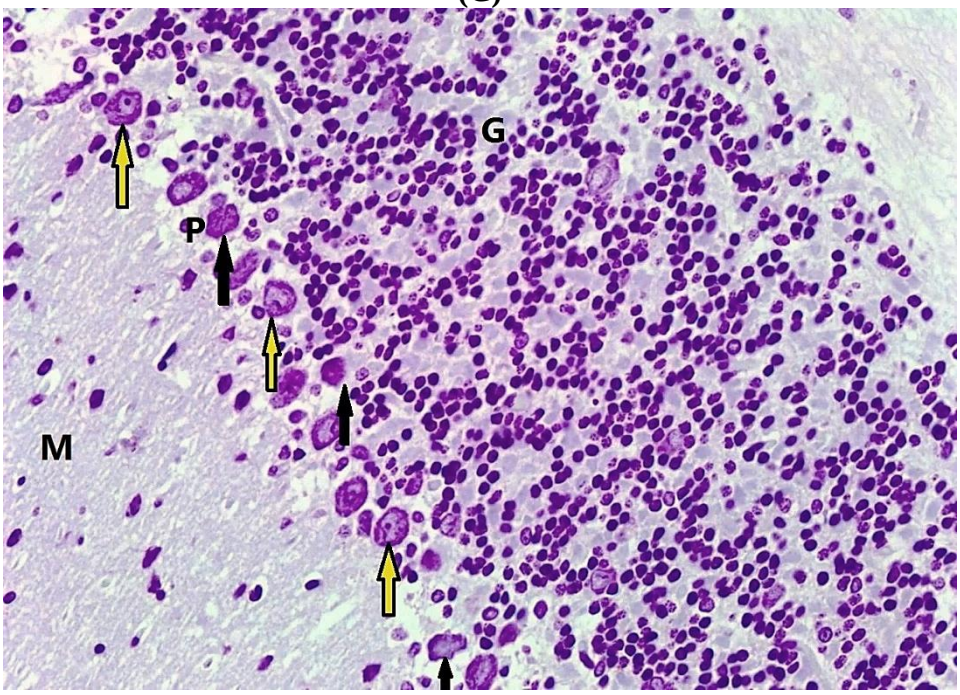
(A)



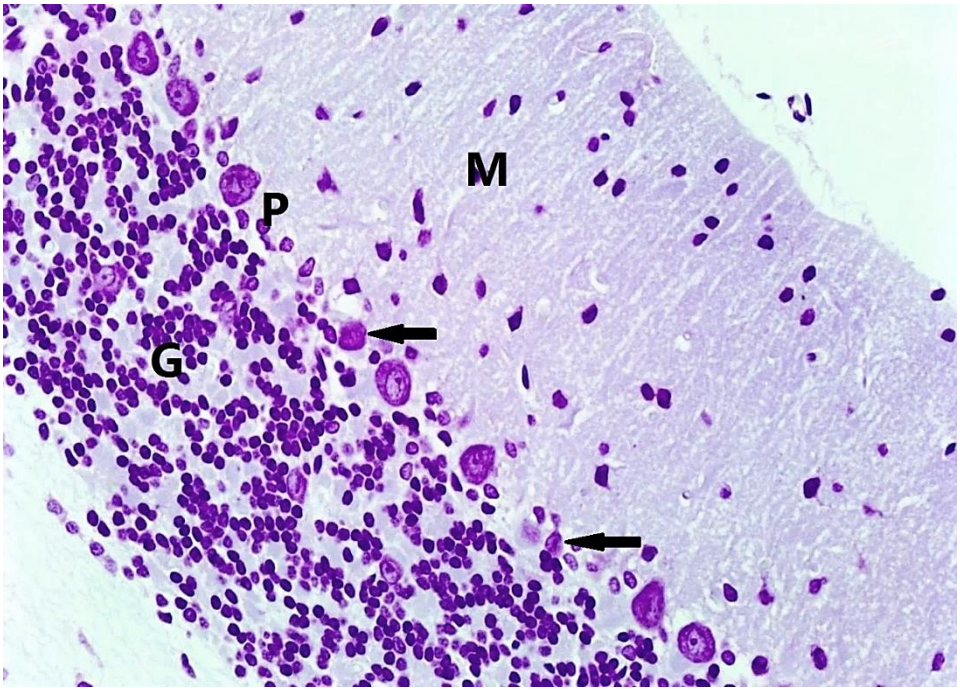
(B)



(C)

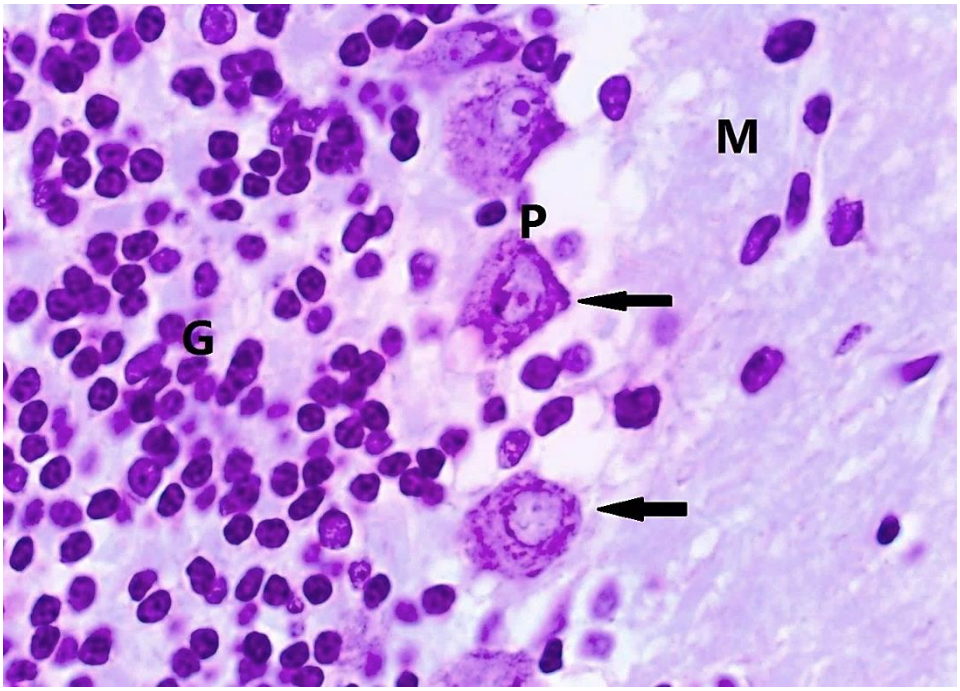


(D)

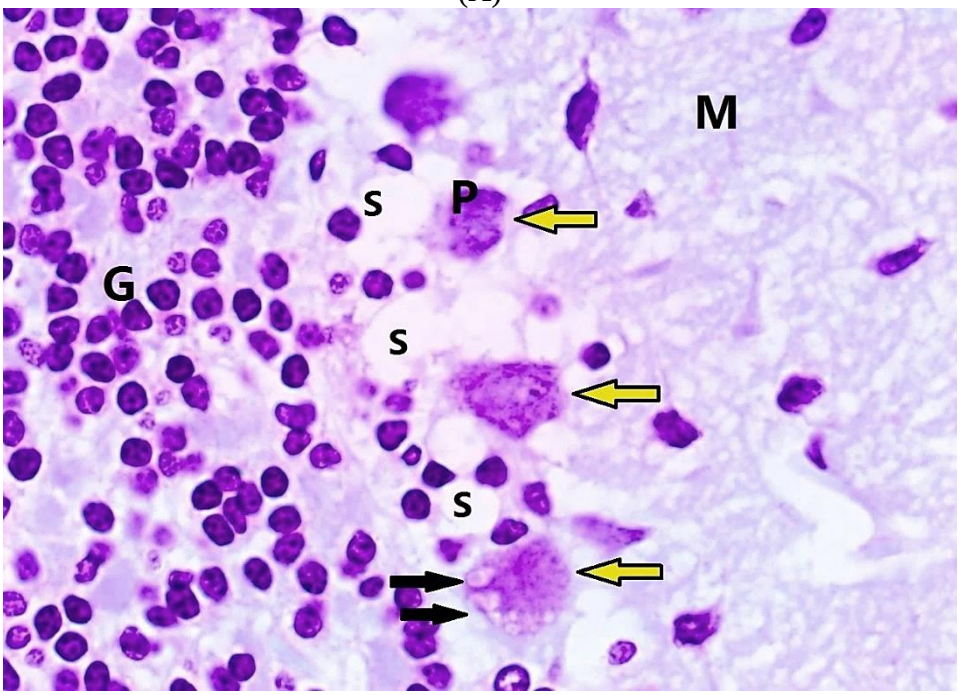


(E)

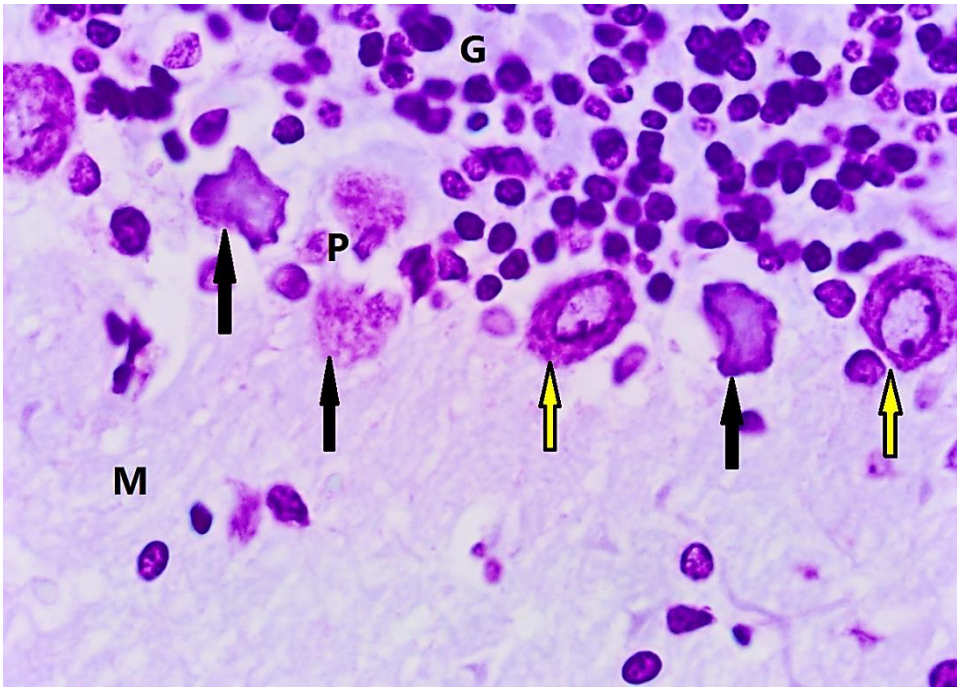
Figure 1: Representative Cresyl violet stained sections photomicrographs (magnification 400x): (A) Control group showing normal cerebellar cortex with the three layers of the cerebellar cortex: the outer molecular layer (M), which encloses a few dispersed nerve cells, the Purkinje layer, which encloses one raw of Purkinje cells with pyriform shape and centrally located vesicular nuclei (P), and the granular layer, which encloses tightly packed small granular cells (G). (B) Aspartame group showing that all Purkinje cells become degenerated with irregular outline, deep basophilic cytoplasm and faint pyknotic nuclei (arrows). Many empty spaces appear due to shrinkage and fall off many Purkinje cells (s).Multiple spaces appear between granular cells in the inner granular layer and also in the outer molecular layer. (C) Stevia group showing that many Purkinje cells have a normal shape and their nuclei appear vesicular (yellow arrows), other cells (black arrows) are degenerated with an irregular shape, pale basophilic cytoplasm, and ill-defined nuclei. (D) Aspartame + omega-3 group showing that several Purkinje cells recover their shape and their nuclei seem vesicular (yellow arrows) and other cells still degenerated with deep basophilic cytoplasm and ill-defined nuclei (black arrows). (E) Stevia + omega-3 group showing that the cerebellar cortex is very similar to normal except very few degenerated Purkinje cells (arrows).The spaces between cells in the Molecular layer (M), Purkinje cell layer (P) and granular layer (G) have decreased to a great extent.



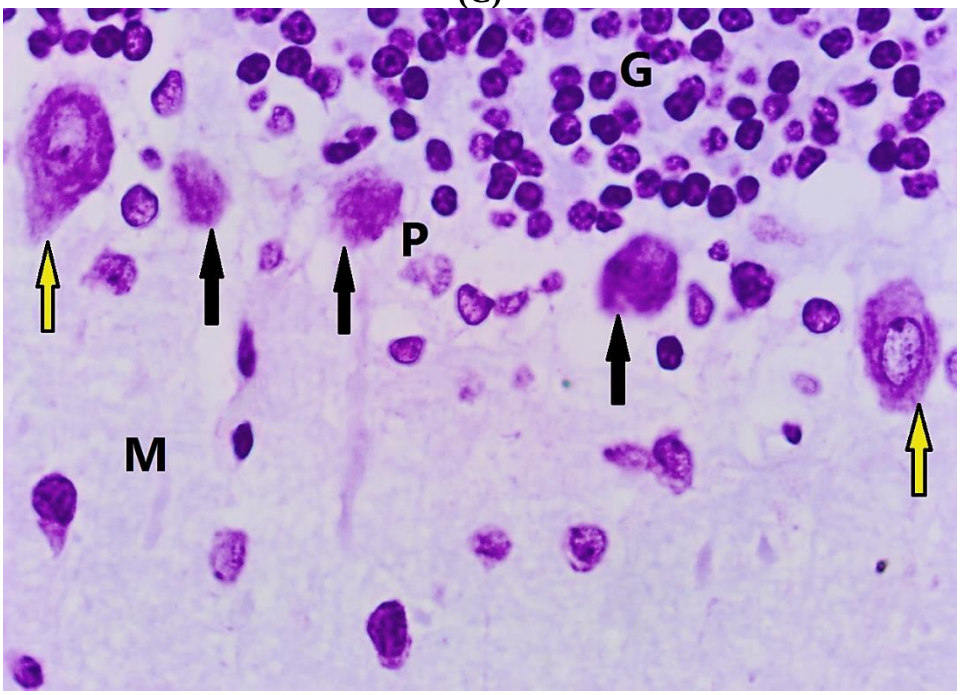
(A)



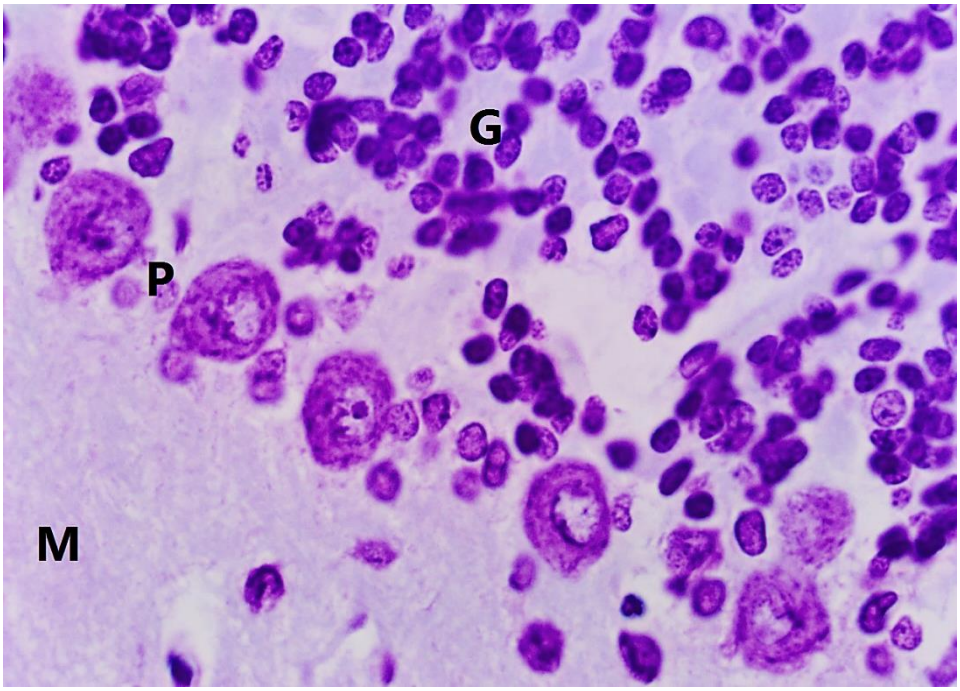
(B)



(C)

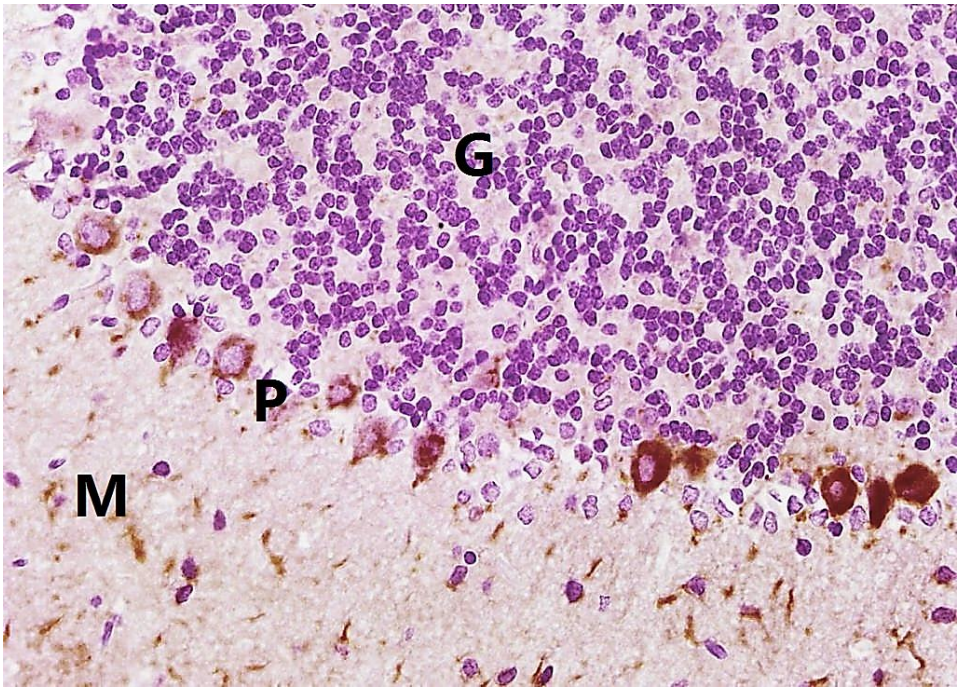


(D)

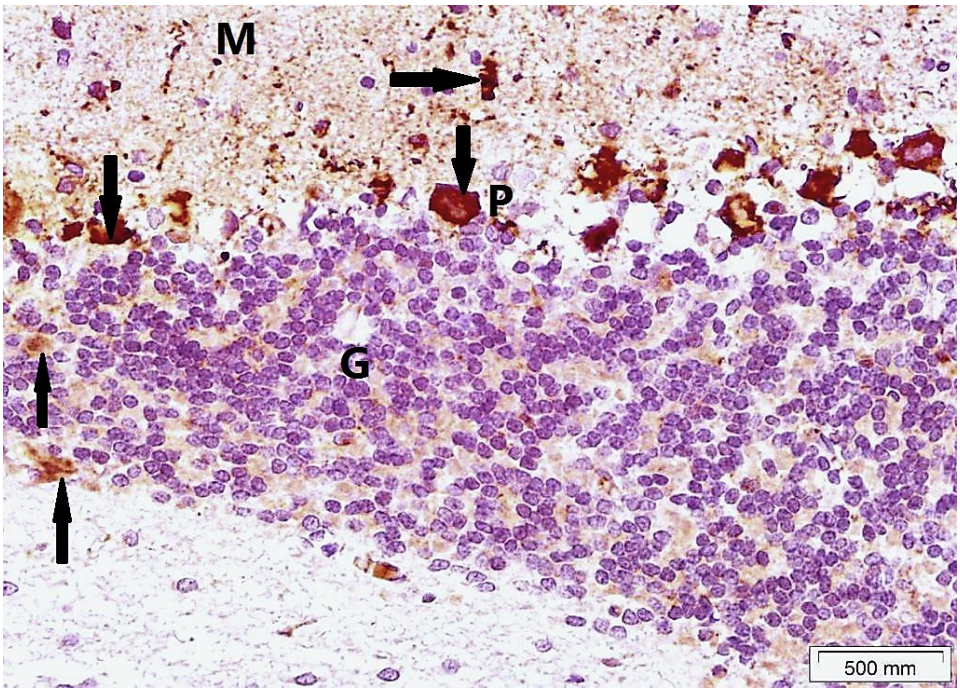


(e)

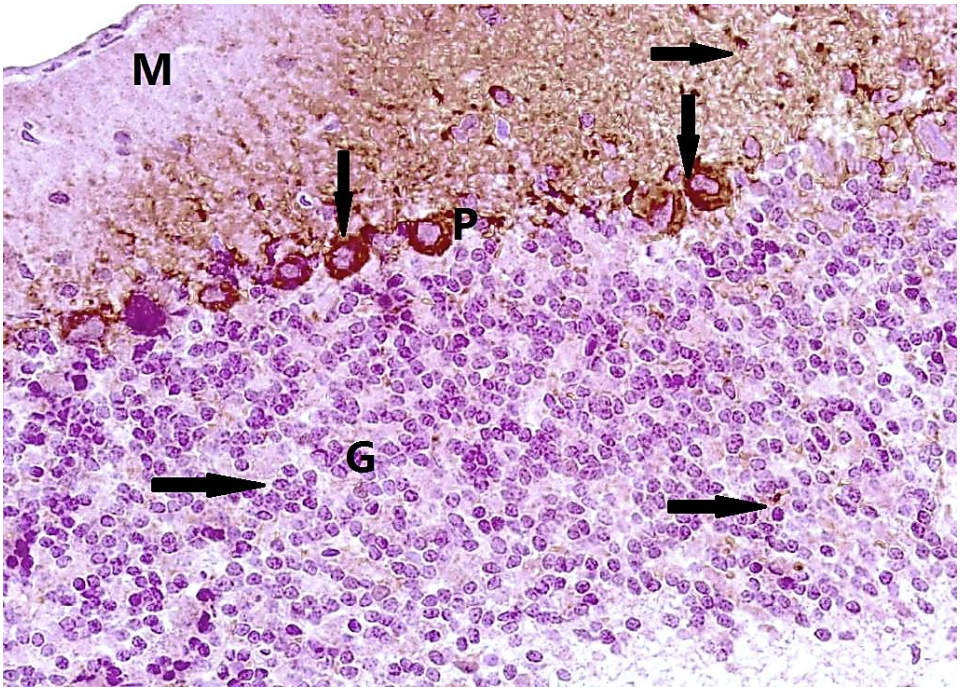
Figure 2: Representative Cresyl violet stained sections photomicrographs (magnification 1000x): (A) Control group showing Purkinje cells with normal pyriform shape, centrally located vesicular nuclei surrounded by Nissl's granules (arrows), the outer molecular layer (M), which encloses a few dispersed nerve cells and the granular layer, which encloses tightly packed small granular cells (G). (B) Aspartame group showing that Purkinje cells are degenerated with an irregular shape, pale basophilic cytoplasm, ill-defined pyknotic nucleus and a few randomly scattered Nissl's granules (yellow arrows). Some cells show intracytoplasmic vacuoles (black arrows). There are many empty spaces due to shrinkage and the death of many cells leaving empty spaces (s). (C) Stevia group showing that some Purkinje cells are normal in shape (yellow arrows) and other cells with irregular shrunken outline (black arrows), deeply basophilic cytoplasm, ill-defined nucleus, and a few randomly scattered Nissl's granules. (D) Aspartame + omega-3 group showing that many cells retain their normal pyriform shape (yellow arrows) while other cells still degenerated with deep basophilic cytoplasm and ill-defined nucleus and a few randomly scattered Nissl's granules (black arrows). (E) Stevia + omega-3 group showing that nearly all Purkinje cells regain their normal pyriform shape with central vesicular nucleus and Nissl's granules arranged around it.



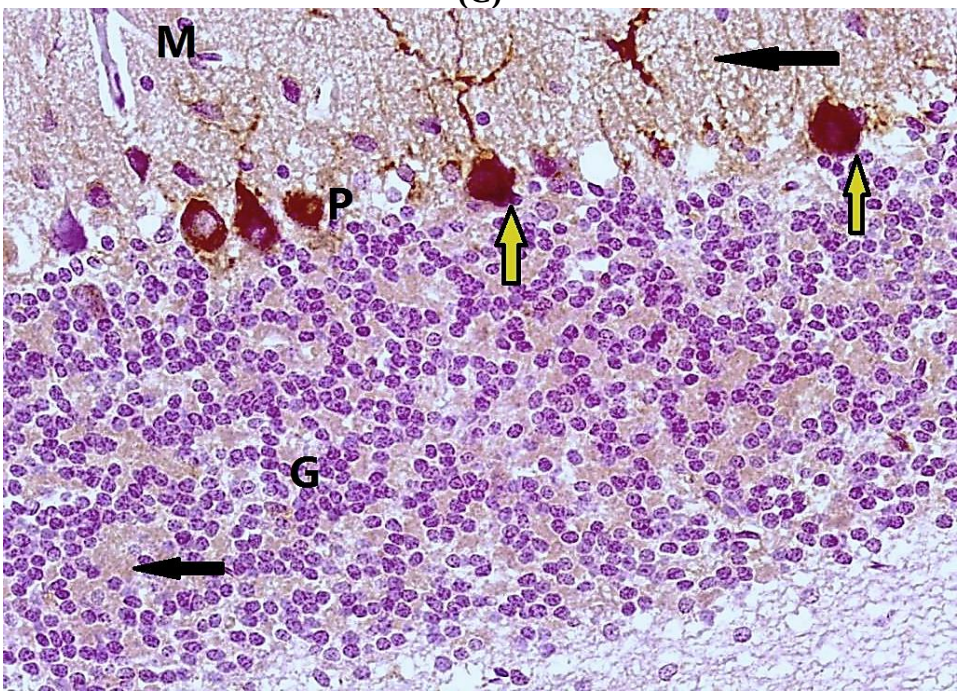
(A)



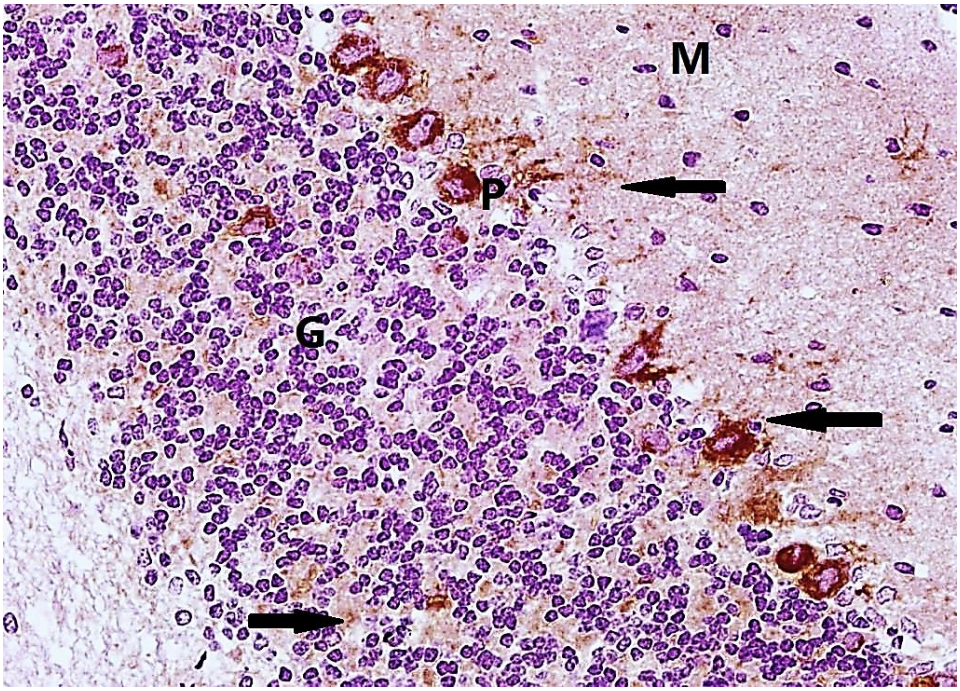
(B)



(C)



(D)



(E)

Figure 3: Representative immunohistochemical stained sections photomicrographs (magnification 400x): **(A)** Control group showing very low-intensity cytoplasmic reaction to cyclooxygenase-2 in the molecular layer (M), Purkinje cells (P), and the granular layer (G). **(B)** Aspartame group showing a very strong-intensity positive reaction to cyclooxygenase-2 (arrows) in the molecular layer (M), Purkinje cells (P), and the granular layer (G). **(C)** Stevia group showing moderate-intensity reaction to cyclooxygenase-2 (arrows) in the molecular layer (M), Purkinje cells (P), and the granular layer (G). . **(D)** Aspartame + omega-3 group showing low-intensity reaction to cyclooxygenase-2 in the molecular layer and the granular layer (black arrows) and high intensity reaction in Purkinje layer (yellow arrows). **(E)** Stevia + omega-3 group showing low-intensity reaction to cyclooxygenase-2 in the three layers of the cortex of the cerebellum (arrows).

Discussion

Sweeteners are frequently employed to a wide range of food and pharmaceutical items. As well, they're utilized by diabetic individuals and for diet control. The artificial sweetener aspartame is the most extensively used one. Stevioside is a promising natural herb that has just been introduced on the market in a number of forms [22].

The present study was established to compare the histopathological changes and the immunoreactivity of inflammatory (COX-2) marker in the rat cerebellar cortex after administration of aspartame and stevia sweeteners for 8 weeks. It aimed also to study the possible protective effect of omega-3 against these menacing effects. We selected the cerebellum to elucidate the changes as the brain is biologically the most sensitive organ to oxidative stress due to its high content of unsaturated lipids; and could reflect minor changes [23,24].

The albino rat was selected as an animal model because aspartame data from rats is quite similar to human data [22]. Furthermore, human stevioside absorption and oxidative metabolism are similar to that of rats [8]. Also, we selected male animals to avoid any possible effects for the hormones of estrus cycles in female animals [25].

To simulate the real practical use of sweeteners, the experimental model of long-term dosing for 8 weeks was used. Each rat was given aspartame dissolved in distilled water (250 mg/kg/day) and this dose was adjusted to correspond to the acceptable intake of aspartame per day in humans as determined by the world health organization (40-50 mg/kg/day), then this dose was augmented up to 5 times as the rats metabolize aspartame

quicker than human [26]. Also, an equivalent dose of stevia (250 mg/kg/day) dissolved in distilled water was used. Agreeing with the WHO food additive series for stevioside, this dose was delivered within the permissible daily intake range. Omega-3 was administered at a dose of (100mg/kg/day) [19].

In the current study, the cortex of the cerebellum of aspartame-treated rats demonstrated apparent structural abnormalities in the Purkinje cell layer. Cresyl violet stained sections exhibited irregular, shrunken outlines with darkly stained cytoplasm and faint pyknotic nuclei and loss of the characteristic pyriform shape of the Purkinje cell body. The cytoplasm of Purkinje cells in cresyl violet stained sections of the aspartame group exhibited that some Purkinje cells were darkly stained as compared to the control group with an apparent reduction in their Nissl's granules content. This process is called chromatolysis that was explained by Hanz and Fainzilber as the disintegration of the basophilic Nissl bodies. The reaction occurs in the neuronal cytoplasm following traumatic or metabolic injuries [27].

Measurement of the thickness of the granular layer in the aspartame-treated group revealed a highly significant decrease as compared to the control group. This result agreed with Mohamed who noticed decreased number of granular cells in rats receiving a dose of aspartame (20 mg/kg /day) for 6 months [22].

In the present study, the aspartame group showed a robust positive COX-2 immunohistochemical reactivity in the molecular layer, Purkinje cells, and granular layer, whereas the control group had a low level. This finding coincided with Abdel Baky who discovered a robust positive high-intensity COX-2 reaction in the molecular layer, Purkinje cells, and the granular layer in the rats received a dose of aspartame (75 mg/kg/day) for three months [21]. Also, El Desoky and Mohamed found that aspartame-treated rats (250 mg/kg/day) had a substantial increase in COX-2 reaction intensity after six weeks [28].

COX-2 is highly elevated in the brain in diseases linked with inflammation, resulting in cytotoxicity in vitro and in vivo [29]. Proinflammatory stimuli such as IL-1, TNF, and trauma can activate COX-2 [30]. Abdul-Hamid and Gallaly mentioned that increased liability to excitotoxicity in COX-2 overexpressing neurons and neuroprotection by COX-2 inhibition has been exposed in several experimental models [31]. So, increased COX-2 expression might be an adaptive response to pathological conditions like cerebrovascular dysfunction, early inflammatory processes, or oxidative stress as a way to repair lost physiological functions [16].

Aspartame is metabolized in the intestine into triple components that are phenylalanine, aspartate that also called aspartic acid and methanol [32]. Salmann et al explained increased COX-2 enzyme activity to the increased level of Phenylalanine and aspartic acid which modify the brain's activity and change its enzymes [33] However, Humphries et al correlated these changes to methanol, which is a component formed from aspartame hydrolysis and known to be neurotoxic and carcinogenic[34].

Aspartic acid (40% of aspartame) is an excitatory neurotransmitter in the central nervous system. It works by causing the postsynaptic membrane to become more depolarized which causes the neurons to fire more quickly [34]. Increased quantities of these chemicals have the potential to influence brain activity and function [35] Also, aspartame is similar to glutamate in its structure, aspartame and glutamate are normally found neurotransmitters in the central nervous system and become neurotoxic when their levels increased more than the critical values [26].

Methanol is the third component of aspartame (10% of aspartame). It is converted to formaldehyde in the liver [34]. Formaldehyde is considered as a neurotoxin because it could attach to proteins and DNA of different cells and might lead to the breaking of DNA [36].

In the current study, the stevia-treated group revealed similar changes in the cortex of the cerebellum to those of the aspartame group but their levels were less than the changes induced by aspartame as verified by statistical analysis. These findings came in agreement with other studies like Mohamed who noticed that stevia has less neurotoxic effects than aspartame on the cerebellar cortex [22]. Also, Abdelhaiem and Mohamed mentioned that stevia has minimal effects on Adrenal-Pituitary Axis when compared to aspartame [37] This might be related to the rapid elimination of stevia from the body.

Immunohistochemical staining revealed increased COX-2 immunoreactions in the three layers of the cortex of the cerebellum indicating increased inflammation of cortical cells. The previous changes could be attributed to steviol and isosteviol [8,38]. It had an inhibiting influence on the efficiency of mitochondrial phosphorylation and oxidative metabolism [39].

Different studies reported that stevia has multiple beneficial effects on human health including anti-hypertensive, anti-hyperglycemic, anti-oxidant, and anti-carcinogenic activities [40,41] However, other studies proved the harmful effects of stevia related to stevioside, which is a steviol derivative. Steviol causes toxicity in mammalian cells by creating lesions in their chromosomal DNA [42] In mammalian cells, Chromosome breakage and gene mutation have also been linked to steviol.[37]

Multiple experimental studies have shown that omega-3 has neuroprotective properties against a variety of harmful substances. In a culture model of hypoxic circumstances, researchers discovered that omega-3 fatty acids obtain a neuroprotective impact via increasing neurogenesis [43]. Patten et al validated the protective role of omega-3 fatty acids in inhibiting oxidative stress in the brains of rats that were exposed to ethanol during intra-uterine life [44].

Several neurodegenerative disorders have early pathogenic processes that can be ascribed to oxidative stress [45]. Inflammation and the formation of reactive oxygen species are induced by oxidants such as free radicals and hydrogen peroxide, leading to cell injury [46]. Recent research suggests that Omega-3 fatty acids have neuroprotective effects and may protect oligodendroglia cells [47], retinal ganglion cells, and eliminate intracellular free compounds caused by superoxide anion, hydrogen peroxide, and hydroxyl radicals [48] and can exert beneficial effects on cerebral ischemia and other brain disorders [49].

In the present study, omega-3 was administered with aspartame and with stevia to evaluate its protective effects on the construction of the cerebellar cortex. These two groups showed improvement in the cells of the cerebellar cortex. This was in agreement with Ali and Sonpol who reported that omega-3 has a neuroprotective and ameliorating effect against aspartame-induced neuronal and astrocytic degeneration [11]. This improvement might be explained as follows: omega-3 fatty acids have essential roles in normal cellular metabolism [50], They are required for membrane fluidity, the activity of membrane-bound enzymes, the quantity and affinity of receptors, the performance of ion channels, the synthesis and activities of neurotransmitters, and signal transduction [19].

Simões et al conveyed that omega-3 fatty acid supplementation considerably reduced acute inflammatory markers levels [51]. Ali and Sonpol considered omega-3 as an anti-inflammatory element that has inhibitory effects on a variety of inflammatory illnesses, and DHA can be converted to neuroprotectin D-1(NPD1), which is an anti-inflammatory oxygenated complex [11]. This could explain why the COX-2 immune reactions were lower in the omega-3 groups in the current investigation.

CONCLUSIONS

Both aspartame and stevioside are suggested to have a negative impact on the cortex of the albino rat's cerebellum. Stevioside might be less hazardous than aspartame. Omega-3 could have an ameliorating effect on the adverse effects of both stevia and

aspartame. It is suggested to decrease the harmful effects of stevia to a great extent so that the cerebellar cortex returned near to normal in structure. However, its effects against the adverse effects of aspartame are less potent.

RECOMMENDATION

It's better to use stevia than aspartame as a non-caloric sugar and also recommended to use omega-3 with them to ameliorate their hazardous effects after confirming the results through future clinical trials.

REFERENCES

1. Bawazir AE and Bokhary LE. Study the chronic effect of aspartame on some neurotransmitters content and histological structure of cerebellar cortex in male albino rats. Adv Environ Biol 2015; 9(11): 19-26.
2. Czarnecka K, Pilarz A, Rogut A, Maj P, Szymanska J, Olejnik Ł, et al. Aspartame—True or False? Narrative Review of Safety Analysis of General Use in Products. Nutrients 2021; 13: 1957-74.
3. Adaramoye OA and Akanni OO. Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats. J Basic Clin Physiol Pharmacol 2016; 27(1):29–37.
4. Salamanca N, Giráldez I, Morales E, deLaRosa I, Herrera M. Phenylalanine and Tyrosine as Feed Additives for Reducing Stress and Enhancing Welfare in Gilthead Seabream and Meagre. Animals 2021, 11, 45.
5. Rycerz K and Jaworska-Adamu JE. Effects of aspartame metabolites on astrocytes and neurons. Folia Neuropathol 2013; 1: 10-17.
6. Samy AH, Yakout AA, Mahamoud MA, Hamam AE. Attenuating effect of spirulina platensis and alpha-lipoic acid against aspartame induced oxidative stress and brain damage in rabbits Benha Vet Med J 2019; 37 (1): 229-36.
7. Bender C. Stevia Rebaudiana's Antioxidant Properties. Sweeteners Pharmacology, Biotechnology, and Application, Springer 2018; 350-71.
8. Momtazi AA, Esmaeili SA, Abdollahi E, Sahebkar A. A Review on the Pharmacology and Toxicology of Steviol Glycosides Extracted from Stevia rebaudiana. Curr Pharma Design 2017; 23 (11): 1616-22.
9. Von Schacky C. Omega-3 fatty acids in cardiovascular disease— an uphill battle. Prostaglandins, Leukot and Essent Fatty Acids (PLEFA) 2015; 92(1):41–47.
10. Punia S, Sandhub KS, Sirohaa AK, Dhulla SB. Omega 3-metabolism, absorption, bioavailability and health benefits–A review. PharmaNutr 2019; 10: 100162.
11. Ali EM and Sonpol HM. Neuroprotective and Ameliorating Impacts of Omega-3 Against Aspartame-induced Neuronal and Astrocytic Degeneration. Anat Rec 2017; 300: 1290–8.
12. Marnett LJ. Cyclooxygenase mechanisms. Curr Opin Chem Biol 2000; 4:545–2.
13. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular and molecular biology. Annu Rev Biochem 2000; 69:145–182.
14. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996; 271:33157–60.
15. Singer II, Kawka DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. Gastroenterology 1998; 115:297–6.
16. Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J Neuropathol Exp Neurol 2004; 63:901–10.
17. Chatsudthipong V and Muanprasat C. Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacol Ther 2009; 121 (1): 41–54.
18. Akbarzadeh S, Eskandari F, Tangestani H, Bagherinejad ST, Bargahi A, Bazzi P, et al .The effect of Stevia rebaudiana on serum omentin and visfatin level in STZ-induced diabetic rats. J Diet Suppl 2015; 12(1):11-22.
19. Bi J, Chen C, Sun P, Tan H, Feng F, Shen J. Neuroprotective effect of omega-3 fatty acids on spinal cord injury induced rats. Brain and Behavior 2019; e01339.
20. Hegazy R, Hegazy A. Hegazy' simplified method of tissue processing (consuming less time and chemicals). Ann. Int. Med. Dent. Res. 2015; 1(2):57-61.
21. Abdel Baky F. (2016). Immunohistological study of the effect of extravirgin olive oil on aspartame-treated cerebellum of male albino rat. Menoufia Med J 2016; 29 (3): 728–5.
22. Mohamed NA. Chronic effect of aspartame versus stevioside on the cerebellar cortex of the adult albino rat: a histological and immunohistochemical study. Egypt J Histol 2013; 36 (1): 213-32.
23. Cobley JN, Fiorello ML, Bailey DM. 13 reasons why the brain is susceptible to oxidative stress. Redox Biol. 2018 May; 15:490-503.
24. Mowafy SM, Hegazy AA, Mandour D, Abd El-Fatah S. Impact of copper oxide nanoparticles on the cerebral cortex of adult male albino rats and the potential protective role of crocin. Ultrastruct Pathol. 2021 Aug 29:1-2.
25. Hegazy AA, Abd Al Hameed EA, El-Wafaey DI, Khorshed OA. Potential role of Moringa Oleifera in alleviating paracetamol-induced nephrotoxicity in rat. Eur. J. Anat. 2020; 24(3):179-91.
26. Okasha EF. Effect of long term-administration of aspartame on the ultrastructure of sciatic nerve .J Microsc Ultrastruct 2016; 4: 175-83.

27. Hanz S and Fainzilber M. Retrograde signaling in injured nerve – the axon reaction revisited. J Neurochem 2006; 99 (1): 13–19.
28. El Desoky RE and Mohamed OM. Mesenchymal Stem Cells Derived Extracellular Vesicles Ameliorate Cortical Cerebellar Changes-induced by Aspartame in Rats, Histological and Immunohistochemical Study. Egypt J Histol 2019; 43 (2): 380-9.
29. Boje KM, Jaworicz DJ, Raybon JJ. Neuro inflammatory role of prostaglandins thru experimental meningeitis: evidence suggestife of an in vivo relationship between nitric oxide and prosataglandins. J Pharmacol Exp Ther 2003; 304 (1): 319–25.
30. Bedoui Y, Septembre-Malaterre A, Giry C, Jaffar-Bandjee MC, Selambarom J, Guiraud P, et al. Robust COX-2-mediated prostaglandin response may drive arthralgia and bone destruction in patients with chronic inflammation post-chikungunya. PLoS Neglected Tropic Dis 2021; 15(2): e0009115.
31. Abdul-Hamid M and Gallaly SR. (2014). Ameliorative Effect of Pimpinella anisum Oil on Immunohistochemical and Ultrastuctural Changes of Cerebellum of Albino Rats Induced by Aspartame. Ultrastruct Pathol 2014; 38(3): 224–6.
32. Hassen EZ, Mahmoud AA, Ibrahim NE, El-Shal AS. The Effect of Long Term Administration of Aspartame on the Sciatic nerve of adult male albino rats and the Possible Therapeutic Role of Ozone (Histological and Biochemical Study). Egypt J Histol 2019; 42(1): 191-01.
33. Salmann NA, Abdiel-Fattah NA, El-Kholy WB, El-Shereef NM. Histological and genetic studies on the effect of mon0sodium glutamete on the cerebellar cortex and the conceivable protective role of ascorbic acid in adult and neonate albino rat. J Am Sci 2012; 43:179–6.
34. Humphries P, Pretorius E, Naude H. Direct and indirect cellular effects of aspartame on the brain. Eur J Clin Nut 2008; 62: 451–62.
35. Eluwa MA, Inyangmme II, Akpantah AO, Ekanem TB, Ekong MB, Asuquo OR, et al. A comparative study of the effect of diet and soda carbonated drinks on the histology of the cerebellum of adult female albino Wistar rats. Afr Health Sci 2013; 13(3): 541 –5.
36. Abd El-Samad AA. Light and Electron Microscopic Study on the Effect of Aspartame on the Cerebellar Cortex of Male Albino Rat. Egypt J Histol 2010; 33(3): 419 – 30.
37. Abdelhaliem NG and Mohamed DS. Comparative Study on the Safety of Aspartame and Stevia on the Adrenal-Pituitary Axis of Adult Male Albino Rats: Histological and Immunohistochemical Study. Egypt J Histol 2017; 40 (2): 216- 5.
38. Ghosh S, Subudhi E, Nayak S. Antimicrobial assay of Stevia rebaudiana Bertoni leaf extracts against 10 pathogens. Int J of Integr Biol 2008; 2: 27- 31.
39. Braguini WL, Gomes MA, de Oliveira BH, Carnieri EG, Rocha ME, deOliveira MB. Activity of isosteviol lactone on mitochondrial metabolism. Toxicol Lett 2003; 143: 83– 92.
40. Chen J, Xia Y, Sui X, Peng Q, Zhang T, Li J, et al. Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. Oncotarget 2018; 9(41): 26299–8.
41. Ahmad J, Khana I, Blundellb R, Azzopardib J, Mahomoodally MF. Stevia rebaudiana Bertoni. : An updated review of its health benefits, industrial applications and safety. Trends Food Sci Technol 2020; 100: 177–89.
42. Nunes AP, Ferreira-Machado SC, Nunes RM, Dantas FJ, De Mattos JC, Caldeira-de Araújo A. Analysis of genotoxic potentiality of steviosidebycomet assay. Food Chem Toxicol 2007; 45 (4): 662–6.
43. Lo Van A, Sakayori N, Hachem M, Belkouch M, Picq M, Fourmaux B et al. Targeting the brain with a neuroprotective omega-3 fatty acid to enhance neurogenesis in hypoxic condition in culture. Molec Neurobiol 2019; 56(2): 986–99.
44. Patten AR, Brocardo PS, Christie BR. Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure, J Nutr Biochem 2013; 24 (5): 760–9.
45. Ullah H, Di Minno A, Santarcangelo C, Khan H, Daglia M. (2021). Improvement of Oxidative Stress and Mitochondrial Dysfunction by β-Caryophyllene: A Focus on the Nervous System. Antioxidants 2021; 10 (4): 546.
46. Finamor IA, Ourique GM, Pes TS, Saccol EM, Bressan CA, Scheid T, et al. The protective effect of N-acetylcysteine on oxidative stress in the brain caused by the long-term intake of aspartame by rats. Neurochem Res 2014; 39 (9): 1681–90.
47. Brand A, Schonfeld E, Isharel I, Yavin E. Docosahexaenoic acid-dependent iron accumulation in oligodendroglia cells protects from hydrogen peroxide-induced damage. J Neurochem 2008; 105 (4): 1325– 5.
48. Shimazawa M, Nakajima Y, Mashima Y, Hara H. Docosahexaenoic acid (DHA) has neuroprotective effects against oxidative stress in retinal ganglion cells. Brain Res 2009; 1251: 269–5.
49. Nobre ME, Correia AO, Mendonça FN, Uchoa LR, Vasconcelos JT, de Araújo CN, et al. Omega-3 fatty acids: Possible neuroprotective mechanisms in the model of global ischemia in rats. J Nutr Metab 2016; 12: 1-13.
50. Scorletti E, & Byrne CD. Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. Annu Rev Nutr 2013; 33: 231–48.
51. Simões M, Coqueiro D, Bueno P. (2011). Acute effect of omega-3 polyunsaturated fatty acids on muscle lesion and inflammation markers in rats subjected to resistive exercises. Gazzetta Med Ital Arch Sci Med 2011; 170: 397–3.

Citation

Mohamed, A., ELtahry, H., Shams, A., Abd El-kader, M. Comparative Neurotoxic Effect of Aspartame and Stevia Sweeteners on Rat Cerebellar Cortex and the Possible Protective Role of Omega-3: A Histological and Immunohistochemical Study. *Zagazig University Medical Journal*, 2024; (4280-4298): -. doi: 10.21608/zumj.2021.100454.2376