BioBacta

Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

Opuntia ficus indica fruit alleviates the cerebellar neurotoxicity induced by monosodium glutamate and aspartame in female rats and their pups

Abd El-Fattah B. M. El-Beltagy[1], Heba Rashed[1], Karoline K. Abdelaziz[1], and Rania F, Abou-El-magd[2]

[1]Zoology Department, Faculty of Science, Damanhur University, Egypt

[2]Biology Department, College of Science, Jazan University, Kingdom Saudi Arabia

DOI: 10.21608/jmals.2023.320163

ABSTRACT

Monosodium glutamate (MSG) has a long history of use enhancer to flavor food but modern scientific research has shown that it has very serious damage to the different organs of the body, especially the nervous system. Aspartame (ASP) is one of the most widely used artificial sweeteners in the world. ASP was assured to induce many hazardous effects on the structure and functions of the brain. *Opuntia ficus indica* fruit as a source of natural liquid sweetener cactus fruit juice laden with total phenolic, total flavonoids, vitamin C, vitamin E, and β-carotene can safeguard the body against oxidative stress and minimize cataract risk in diabetes patients. This work aims to evaluate the possible ameliorative role of fruit juice of *O. ficus indica* against MSG and ASP-induced cerebellar toxicity in female rats and their fetuses. For this study, 36 pregnant females were used in this study and randomly divided into six groups on the fourth day of gestation: control, *Opuntia* group, MSG group, ASP group, MSG plus *O. ficus indica* group, and ASP plus *O. ficus indica* group. The experiment extended from the fourth day of pregnancy till the end of weaning. At the end of the experiment, the mother's rats and their pups were dissected for collection of blood and removal of the cerebellum for estimation of biochemical and histopathological changes. The obtained results revealed pronounced histopathological signs in the cerebellar cortex of MSG, and ASP-treated dams and their pups. These signs included disorganized cerebellar layers, pyknotic Purkinje and granular cells, and scattered vacuoles. The immunohistochemical results revealed strong expression for GFAP and P53 protein while weak expression for Bcl-2 in the cerebellar cortex of MSG and ASP groups compared with control. Additionally, the obtained biochemical analysis shows a significant increase in the levels of caspase-3 and IL-1 in the cerebellar tissue while a significant decrease in the levels of serum insulin and IGF-1 compared with control. Co-supplementation of *O. ficus indica* fruit juice with MSG or ASP successfully alleviated the deleterious histopathological, immunohistochemical, and biochemical changes induced by MSG and ASP.

Keywords: MSG, aspartame, gestation, cerebellum, offspring, histopathology, caspase-3

Receive Date: 10 July 2023, Accept Date: 24 September 2023, Published: 5 October 2023

INTRODUCTION

One of the most important problems in the human health nutrition field is the use of food additives. Food additives are usually used in all types of food, propagated from the minimally processed sorts to the highly processed and modified ones (**Carocho et al., 2015**). Food additives are widely used by the food industry to increase the product shelf life and/or attribute as well as enhance certain characteristics of particular foods, which are often lost during processing. Wide use of food additives, capable of triggering adverse reactions, just as any other drug does, including allergic reactions, behavioral changes, and carcinogenicity (**Aun et al., 2011; Cardoso et al., 2017**).

Monosodium glutamate (MSG) and aspartame (ASP) are famous substances used as food additives. MSG is a natural constituent of many protein-rich food items such as meat, cheese, and some vegetables and is used worldwide as a flavor enhancer increasing the palatability of food and thus food intake. In the body, MSG dissociates into sodium and glutamate ions. Glutamate is a naturally occurring amino acid and one of the most abundant amino acids in the central nervous system (CNS). It is found in high concentration in regions of the brain that are important in mediating cognition such as the cerebral cortex, dentate gyrus of the hippocampus, and striatum (**Park et al .,2000**). Regardless of dietary source, all glutamate molecules entering the circulation from the gastrointestinal tract are structurally identical (**Geha et al., 2000**). The average daily intake of MSG is estimated to be 0.3–1.0 g in industrialized countries but can be higher occasionally, depending on the MSG content of individual food items (**Zai et al., 2009**). Furthermore, MSG is one of the most popular flavoring agents of modern times and is widely used in many commercially packed food and restaurant and household cooking.

It is reported that neonatal exposure to MSG (4 mg/g body weight) in rats and mice causes learning difficulty **(Abu-Taweel et al., 2014**). Acute ingestion of MSG has been associated with adverse symptoms that include general weakness, muscle tightness or tenderness, flushing or sweating, headache, parenthesis, arrhythmias, and tachycardia in healthy individuals (**Shimada et al., 2015**). MSG was suggested to trigger symptoms, which were referred to collectively as ‘‘Chinese restaurant syndrome" consisting of numbness at the back of the neck and arms, weakness, and palpitations (**Geha et al., 2000).** Moreover, MSG can induce the production of free radicals, activation of proteases, phospholipases, and endonucleases, transcriptional activation of apoptotic programs, and genotoxicity in mice and rats **(Goldsmith, 2000; Farombi & Onyema, 2006).** Additionally, MSG has a neurotoxic effect leading to degenerative changes in neurons and astrocytes in the cerebellar cortex of albino rats **(Hashem et al., 2012).** It had been documented that acute administration of low-dose MSG was associated with changes consistent with hepatic and renal injury in mice (**Onaolapo et al., 2013**).

The neurotoxic effect of MSG could be mediated by an oxidative stress process where high levels of extracellular glutamate result in the depletion of glutathione and acute concentration-dependent efflux of ascorbate from the cells leading to a form of cell injury called oxidative glutamate toxicity (**Loo et al., 2003; Shih et al., 2006).** The young and elderly are most at risk from MSG. In the young, the blood-brain barrier is not fully developed. The elderly are at increased risk because the blood-brain barrier can be damaged by aging diseases (**Taylor, 1993**).

Aspartame (ASP) is a dipeptide (L-aspartyl-L-phenylalanine methyl ester) and is used as an artificial sweetener that is now in wide and frequent use. ASP is used in a variety of food products; however, ASP-related neurological disturbances such as dizziness,

headaches, gastrointestinal symptoms, mood alterations, allergic-type reactions, and alterations in menstrual patterns have also been reported (**Abu–Taweel et al., 2014**). The effect of aspartame consumption on behavior is the most important aspect to be considered because controversial reports do exist for aspartame (**Ashok et al., 2014**).

Following oral administration to humans and experimental animals, ASP is rapidly and completely metabolized by intestinal esterases and dipeptidases to aspartic acid, phenylalanine, and methanol **(Mourad & Noor, 2011**). The aspartic acid in ASP is a well-documented excitotoxin **(Prakash et al., 2014)**. Excitotoxins are usually amino acids, such as glutamate and aspartate. Phenylalanine is a precursor of catecholamines in the brain, increased levels of these molecules could change the basic activity level of the brain to an unhealthy state (**Nosti-Palacios et al., 2014).** The methanol metabolized from ASP is converted to formaldehyde and then formic acid **(Choudhary & Devi, 2015).** Methanol and its metabolites are responsible for the generation of oxidative stress in the brain region (**Iyyaswamy & Rathinasamy, 2012**). Furthermore, Chronic formaldehyde exposure at very low doses has been shown to cause the immune system and nervous system changes and damage as well as headaches, general poor health, irreversible genetic damage, memory loss, and several other serious health problems (**Sun-Edelstein & Mauskop, 2009; Abdel-Salam et al., 2012**).

The newborn rats prenatally treated with ASP showed a remarkable increase in the lactoperoxidase (LPO) rate and depletion of SH groups in the brain homogenate. Further, ASP is a potential angiogenic agent that can induce reactive oxygen species (ROS) production that stimulates the induction of cytokines and growth factors as it enhances IL-6, vascular endothelial growth factor, and their soluble receptors release from the endothelial cells (**Alleva et al., 2011**) as well as brain oxidative stress increased by repeated ASP administration (**Abdel-Salam et al., 2012**).

Natural foods have recently received immense attention from health professionals as well as consumers in the wake of the discovery of their health-promoting potential. Cactus *(Opuntia*) pears have emerged as promising candidates. *Opuntia ficus indica* is the most common among *Opuntia* species and is regarded to be the most delicious **(DeWit et al., 2010). El Kossori et al. (1998)** previously investigated the composition of *Opuntia ficus-indica* pears and reported the ethanol-soluble carbohydrates to be the most abundant components in the skin and pulp. The pulp contained glucose and fructose while the skin essentially contained glucose. Protein content was found to be highest in the seeds. The pulp fibers were rich in pectin while the skin and seeds were rich in cellulose. Moreover, the skin had a remarkable content of calcium (2.09 %) and potassium (3.4 %). **Galati et al. (2003)** found that the juice of *Opuntia ficus-indica* contains ascorbic acid, total polyphenols, and flavonoids. Ferulic acid emerged as the chief component of total phenolic compounds. The flavonoid fraction consisted of rutin and isorhamnetin derivatives, responsible for antioxidant activity. Phenolic compounds like anthocyanins, phenolic acids, stilbenes, and tannins were reported. **Ramadan & Morsel, 2003** compared the seeds and pulp of *O. ficus-indica* pears in terms of fatty acids, lipids, sterols, fat-soluble vitamins, and β-carotene. High amounts of neutral lipids were found in seed oil, while glycolipids and phospholipids occurred at high levels in pulp oil. **Galati et al. (2003)** added that in both oils, linoleic acid was the major fatty acid, followed by palmitic and oleic acids. Vitamin E level was higher in the pulp oil than in the seed oil, whereas ɤ-tocopherol was the predominant component in seed oil.β -carotene was also higher in the pulp oil compared to seed oil.

Opuntia ficus indica juice has been reported to be efficient against hyperlipidemia (**Wolfram et al., 2002**), immunological disorders (**Aires et al., 2004)**, chronic hyperglycemia (**Ennouri et al., 2006),** tumor growth **(Garcia-Solis et al., 2009)**, and cataract risk in diabetic patients (**Liu et al., 2010; Abd El Razek et al., 2012).** This work is mainly designed to evaluate the possible protective and ameliorative effects of *Opuntia ficus indica* fruits against the cerebellar neurotoxicity induced by ASP and MSG in pregnant rats and their offspring.

MATERIALS and METHODS

1. **Chemicals**: Monosodium glutamate (MSG) and aspartame in the form of white crystals were purchased from Sigma Chemical Company, Cairo, Egypt. Aspartame was purchased from Amyria Pharmaceutical Company, Cairo, Egypt. It was available in the form of tablets, each tablet contained 20mg.

2. **Preparation of *Opuntia ficus indica* fruit juice**

Mature prickly pears of *O. ficus indica* (purple-skinned) were purchased from the local market of Damanhur city. The whole unpeeled fruit (10 Kg) was washed, ground by a Musermax double-bladed mill, and filtered through a colander (0.5 mm mesh size) to discard seeds. The resulting juice was centrifuged at 3000 × g for 10 min to remove hard fibers. The clarified juice (10.5 L) was then collected and stored at −21°C until use.

3. **Experimental design**

For this study, 36 females and 12 males Wistar albino rats weighing 180-200 g were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). The animals were kept in wire-bottomed cages in a room under standard conditions of illumination with a 12-hour light-dark cycle at 25 ± 1°C and 50% relative humidity. They were provided with tap water and a balanced diet of libitum. After an acclimatization period of two weeks; the animals were mated in the special matting cages (1 male: 3 females) overnight. After 3-4 days and ensuring pregnancy via observation of vaginal plug and using vaginal smear method, pregnant females were separated from males and randomly divided into six groups as follows, six for each group (n=6): Control group: they have received a daily oral dose of 0.5ml saline solution, *Opuntia* juice group*:* they were given a daily oral dose of *Opuntia* juice (4ml /100g b.wt) **(Alimi et al., 2013),** MSG treated group: they were given a daily oral dose of MSG (400mg/Kg. body weight) **(Diniz et al., 2004; Waer & Edress, 2006),** ASP group: they were given a daily oral dose of 0.5ml of aspartame (40mg/Kg. body weight)**(Abd Elfatah et al., 2012),** MSG & *Opuntia* group: the pregnant rats were given a daily oral dose of MSG 400mg /kg b. wt. simultaneously with *Opuntia* juice in a dose of 4ml /100g b.wt, and ASP and *Opuntia*: the pregnant rats were given a daily oral dose of aspartame (40mg /kg b. wt.) simultaneously with *Opuntia* juice in a dose of 4ml /100g b.wt. All groups were exposed to the appropriate dose of treatment from the fourth day of gestation till the end of weaning. The mother rats and their offspring of all groups were weighed and examined on post-natal day 21.

4. **Sample collection and tissue preparation**

At the end of the experimental period (21th days postnatal), the fasted rats were weighed and sacrificed under diethyl ether anesthesia. The animals were dissected and the whole brain of mothers and their offspring were removed immediately, and washed in normal saline. The cerebellum was separated from each brain and then, cut longitudinally into two halves; one half was kept frozen for estimation of biochemical parameters while the other was fixed in fixed in 10% neutral buffered formalin for histological and immunohistochemical studies.

5. Investigated parameters

5.1 Histological technique for hematoxylin and eosin stain.

Formalin-fixed cerebella were dehydrated with an ascending ethanol series, cleared with xylene, and embedded in paraffin. The 5-6 µm thick longitudinal sections of the cerebellum were obtained, and stained with hematoxylin and eosin **(Bancroft & Gamble, 2008).** The obtained sections were investigated under a bright field light microscope and photographed.

5.2 Immunohistochemical labeling of B-cell lymphoma 2 (BCl-2), Glial Fibrillary Acidic Protein (GFAP) and P53

The cerebellar-embedded paraffin sections were blocked by goat serum for 20 minutes at 37 °C to reduce non-specific antibody binding and then incubated separately with primary antibodies (mouse-anti-human BCl-2) at 4 °C overnight. After being washed three times (three minutes each time) in PBS, the sections were incubated with the biotin-labeled goat anti-mouse IgG at 37 °C for 30 minutes, washed again with PBS, followed by incubation with streptavidin peroxidase complex for 30 minutes at 37 °C. Staining was visualized with 3, 3′-diaminobenzidine (DAB) for 10 minutes at room temperature. Finally, the sections were counterstained by hematoxylin solution.

The deparaffinized 5 µm paraffin sections of the cerebellum on charged slides were used for labeling GFAP using avidin–biotin-complex (ABC) immunoperoxidase technique. The cerebellar sections were incubated in hydrogen peroxide for 10 min to block the endogenous peroxidase and then incubated with the primary anti-GFAP antibody at 1:100 dilutions for 20 min at room temperature. The primary antibody used was a mouse monoclonal antibody (Glial Fibrillary Acidic Protein) Ab-1 (Clone GA-5), specific to astrocytes obtained from Lab Vision Corporation, Medico Co., Egypt (Cat. #MS-280-B0). The slides were washed with phosphate buffer and then incubated with the secondary anti-mouse antibodies universal kits obtained from Zymed Corporation. Staining was completed by incubation with substrate chromogen DAB (3,30Diaminobenzidine) for 5–10 min which resulted in a brown-colored precipitate at the antigen sites and Mayer's Hematoxylin was used as a counter stain. Incidences of cellular accumulations of GFAP were determined for each group.

For immunohistochemical detection of P53 protein, the paraffin-embedded tissue sections of cerebellum were incubated with primary antibodies (monoclonal antibody RM-2103-R7, 7.0 ml of antibody pre-diluted in 0.05 mol/l tris–HCl, pH 7.6 containing stabilizing protein and 0.015 mol/l sodium azide, Produced by Epitomics, Inc. Using Technology Licensed Under Patent no. 5,675,063, Thermo Fisher Scientific, Anatomical Pathology, 46360 Fremont Blvd., Fremont, CA 94538, USA) for 16 min. at 37◦C. Sections were stained with p53-immunoperoxidase stain (Cuello, 1993). The intensity of nuclear staining for P53 protein was recorded as a weak, moderate, or strong reaction.

5.3 Measurment of of caspase3 and Interleukin-1 (IL-1).activity in cerebellar tissues

For measurement of caspase3 activity, caspase3 colorimetric substrate (Ac-DEVD-pNA; Cal-biochem) was used. The plate was incubated at 37 °C for 1h. Cleavage of the chromophore pNA from the substrate molecule was monitored at 405nm. Caspase3 activity was expressed as picomoles of pNA released per microgram of protein per minute **(Zhang et al., 2012).**

Interleukin 1 activity of cerebellar tissue homogenate was determined by its induction to

interleukin 2 production by murine EL-4 cells as described previously **(Simon et al., 1985).** Briefly, 0.25 ml cultures of 2 x 105 EL-4 cells in a 96-well flat bottom plate are cocultured with the sample and 2x 10^{-7} M calcium ionophore A23187 for 24 hours. The culture fluids are then tested for interleukin 2 activity using the CTLL-20 interleukin 2 dependent cell line. The interleukin 2 activity is directly proportional to the input of interleukin 1. Units of interleukin 1 activity were calculated relative to a standard of pure recombinant rat interleukin 1 beta by a computer program.

5.4 Determination of insulin and insulin growth Factor-I (IGF-I) in serum

For the mother's rats and their offspring, the serum insulin was measured by an enzyme-linked immunosorbent assay (ELISA) Kit purchased from Boehringer Mannheim, Germany, using Boehringer analyzer ES300 **(Flier et al., 1976)**.

The IGF-I levels were measured from serum samples of mothers and their offspring of all experimental groups using an automated competitive immunoassay that was calibrated to the new World Health Organization standard. This assay does not cross-react with insulin, proinsulin, or IGF-II, and shows no interference with the high-affinity insulin-like growth factor binding proteins (**Bougoussa et al., 2010).**

Statistical analysis: Data are expressed as mean± standard error {n=5 per group} statistical analysis one way ANOVA followed by post hoc test means in the same row with different superscript (*) are significantly different when p<0.05 * significant at value <0.05, **significant at p- value<0.01 and ***significant p-value < 0.001 in comparis+on with control.

RESULTS

1. Body weight

The results of the present work revealed that the mean body weights of MSG and ASP treated mother's rats and their offspring were significantly higher (P<0.001) if compared with control. In the two protective groups of mothers and their offspring (MSG & ASP simultaneously supplemented with *Opuntia* fruit extract), the mean body weights showed non-significant (P>0.05) changes with control **(**Table 1 and Figures 1&2).

2. Histological observations

In control and *Opuntia* supplemented mother's rats (Fig.3A&B) and their offspring (Fig.4 A&B), the sections of the cerebellum showed the normal characteristic appearance of the cerebellum and its layers; the outer cerebellar cortex (gray matter) and inner medulla (white matter). The cerebellar cortex presented three layers; the outer molecular, intermediate Purkinje, and inner granular layers. The molecular layer showed sparsely distributed stellate and basket cells in addition to axons and dendrites as well as capillaries that penetrate deep into this layer. The Purkinje cell layer is represented by one cell thick of a monolayer of flask-shaped uniformly arranged cells between the molecular and granular layers with deeply stained basophilic nuclei. The granular layer is well-defined and darkly stained due to the presence of densely packed rounded and oval cells of variable sizes.

In MSG (Fig.3 C) and aspartame (Fig.3 D) exposed mother's rats, the cerebellar cortex presented the three layers with apparently partial loss and degenerated Purkinje cells, cellular hypertrophy in the molecular and granular layers and deep-staining pyknotic granule cells. Also, little vacuoles were noticed in some areas of granular and Purkinje cell layers. On the other hand, the cerebellar cortex of 21th days old rats induced with MSG showed multiple pyknosis of Purkinje cells and granular cells with scattered vacuoles, especially in the granular layer (Fig.4C). Moreover, the aspartame-treated offspring showed remnants of

the external granular layer; the Purkinje cells appeared atrophied with remarkable vacuolation and condensed fragmented fibers. Also, few stellate cells were noticed among the granular cells (Fig.4 D).

For mothers (Fig.3E&F) and their offspring (Fig4.E&F), the two ameliorative groups; MSG or ASP post supplemented with *Opuntia* fruit extract the cerebellar cortex showed obvious recovery in their histological pattern despite little pyknotic Purkinje cells still found in some area of the sections.

3. Immunohistochemical Observation:

3.1 Immunohistochemical localization of GFAP

In the present work, the sections of the cerebellar cortex of control mother's rats (Fig.5A) and their offspring (Fig.6A) displayed weak to moderate immunoreactivity for glial fibrillary acidic protein (GFAP). In MSG (Fig.5B) induced mothers rats, a strong positive immune expression of GFAP appeared while the aspartame-induced mothers showed less intense immune reactivity if compared with MSG-treated mothers(Fig.5C). For the two ameliorative groups; MSG or ASP post supplemented with *Opuntia* fruit extract, the cerebellar sections were revealed moderate immune expression for GFAP respectively (Fig.5D&E).

In 21^{-day}-old rats, the cerebellar GFAP immune-reactivity of MSG and aspartame was stronger than that of control (Fig.6B&C). On the other hand, the cerebellar cortex of the two ameliorated groups of 21-day-old rats (Fig.6D&E) revealed a weak to moderate GFAP immune expression if compared with MSG or ASP groups alone.

As a general aspect, the GFAP reactivity was more localized in the intermediate cells of the molecular layer and granular layer however for both mother and their offspring.

3.2 Immunohistochemical localization of BCl-2

The cerebellar cortex of the control mother's rats (Fig.7A) and their offspring (Fig.8A) revealed a moderate to strong BCL-2 immune expression. Such expression was more confined to the granular cells, Purkinje cells, and dispersed basket cells of the molecular layer. However, the cerebellar sections from MSG (Fig7B) and ASP (Fig 7C) treated mother's rats; the BCL-2 immunoreactivity was negative in the granular and molecular layer however the Purkinje cells were positively stained. Moreover, the cerebellar sections from MSG and ASP maternal treated offspring showed negative BCL-2 reaction in the molecular and granular cell layers but very weak immune expression was recorded in the Purkinje cells (Fig.8B&C).

In MSG or ASP /*Opuntia* treated mother rats (Fig.7 D&E) and their offspring (Fig.8D&E), the BCL-2 immunoreactivity appeared moderately expressed that appeared similar more or less to control.

3.3 Immunohistochemical localization of P53

The cerebellar sections from control mother rats (Fig.9A) and their offspring (Fig.10A) showed negative or very weak expression for P53 protein. In MSG or ASP-treated mother rats (Fig.9B&C) and their offspring (Fig.10B&C), the cerebellar cortex exhibited moderate to strong positive P53 immune expression. In comparison with other induced groups, the P53 immuno-reactivity was intensive in the cerebellar cortex of ASP-induced offspring. The P53 activity appeared more prominent in the Purkinje cells and granular cells, but less prominent in the molecular cells. Moreover, weak immune reaction for P53 protein was recorded in the cerebellar cortex of the two groups of mothers rats treated with MSG or ASP plus *Opuntia* juice (Fig.9D&E) while their offspring exhibited negative immunoreactivity for P53(Fig.10D&E).

Table 1: **Illustrating the body weight (g) of mother rats and their offspring in different studied groups.**

gps(n=6) / B.Wt	Control	OP	MSG	ASP	MSG+OP	ASP+ OP
Mothers	181±0.8	190±0.7	202+0.8***	194±8.2***	185±1.1	185±0.7
Offspring 21 day	20.45±0.52	20.31±0.43	29.11±1.41*	26.03±1.53*	24±0.76	25.2±1.5

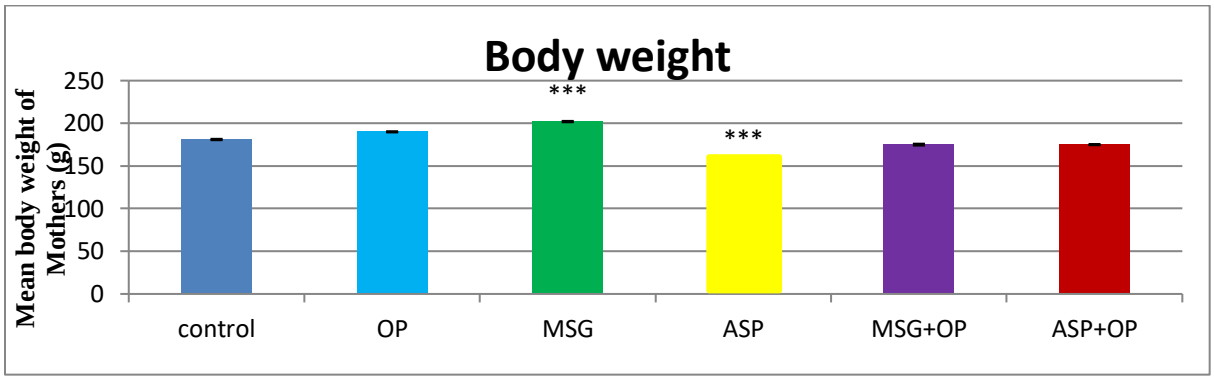

Fig 1: The body weight (g) changes among the different studied groups of mother rats.

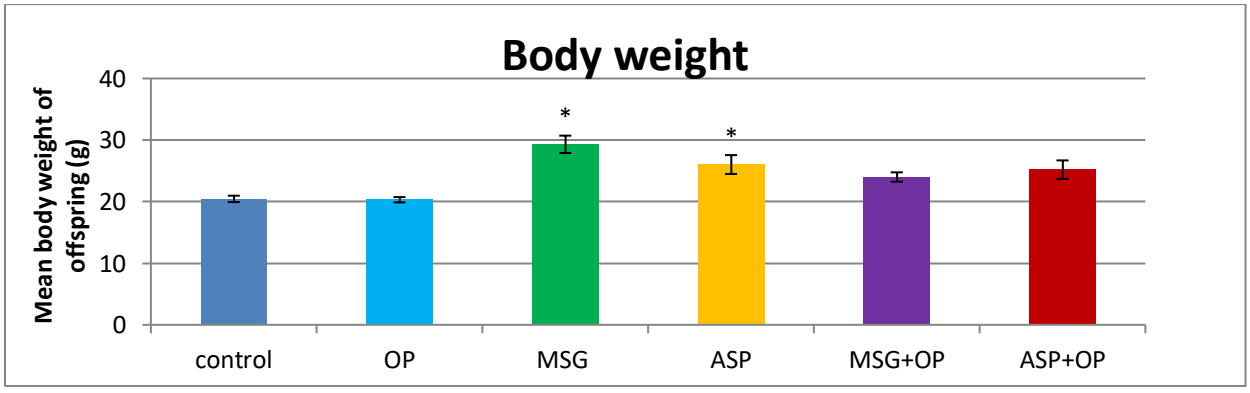

Fig 2: The body weight (g) among the different studied groups of offspring at post-natal day 21

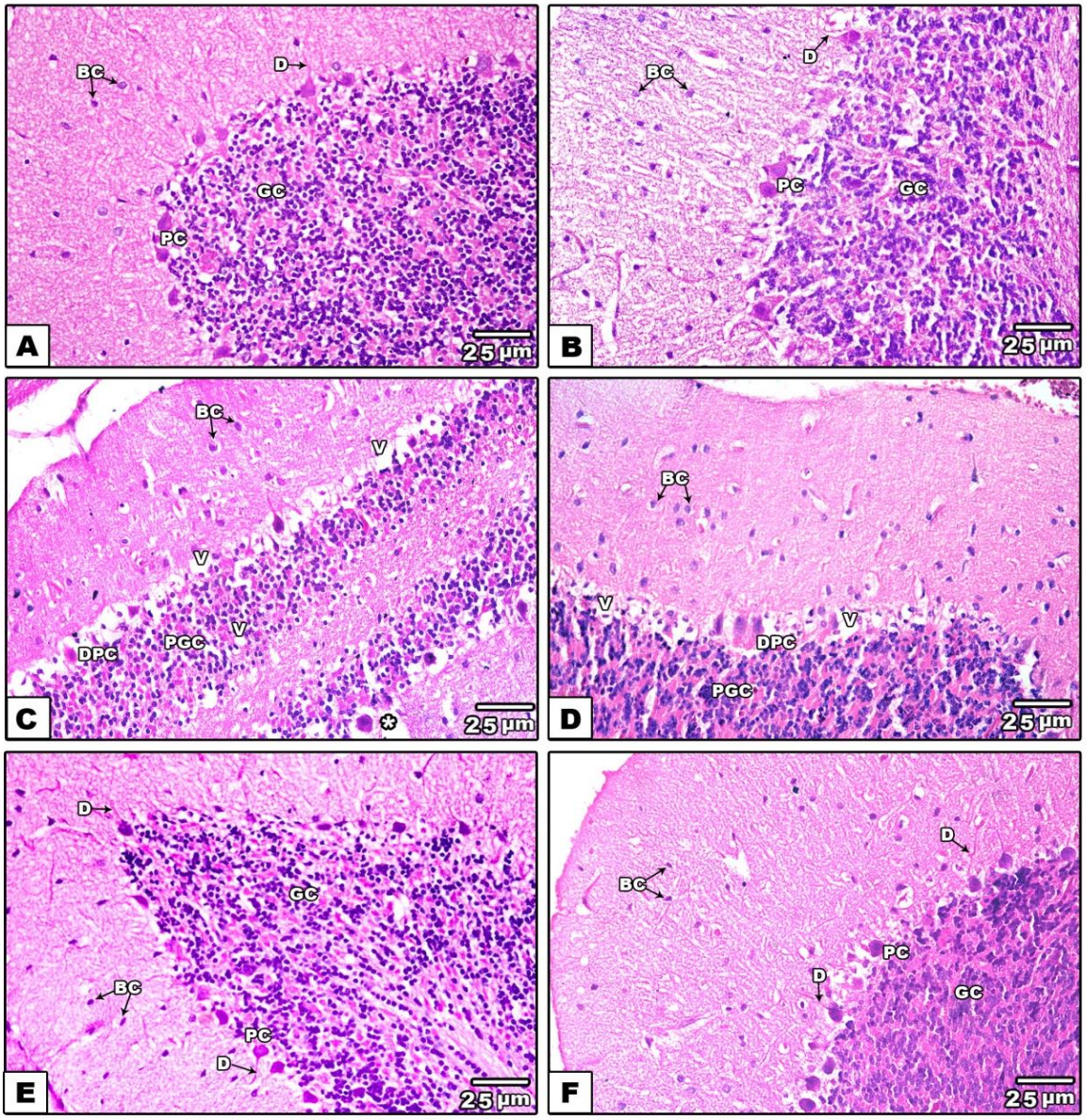

Fig.3: Photomicrograph of histological sections through the cerebellar cortex of mother rats for different studied groups. Panel A: control, panel B: *Opuntia ficus* (OP), panel C: MSG treated group, panel D: ASP treated group, panel E: MSG&OP, and panel F: ASP&OP. In panels A&B, the cerebellar sections appeared with normal histological architecture. Panels C&D show obvious degenerated Purkinje cell (PC), scattered vacuoles, less organized and dislocated PC, and pyknotic granular cells. In panels E&F, the cerebellar sections show remarkable amelioration that tends to be more or less as control. (H&E stain , *Scale bar* =25). **Abbreviations**: D: dendrite cell, BC: basket cell, PGC: pyknotic granular cells, GC: Granular cells, DPC: degenerated Purkinje cell. V: vacuoles

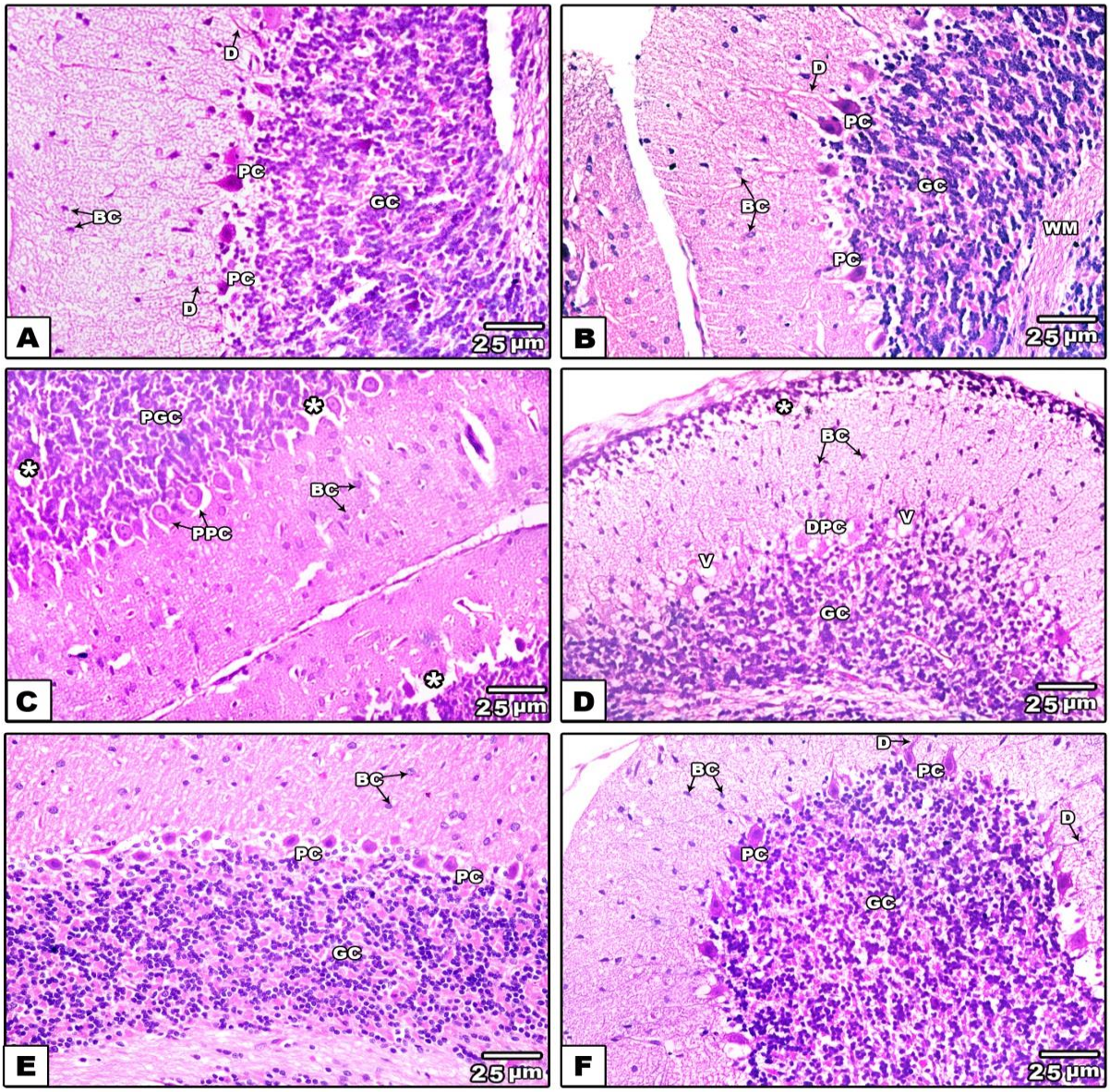

Fig 4: Photomicrograph of histological sections through the cerebellar cortex of 21th days old rats for different studied groups. Panel A: control, panel B: *Opuntia ficus* (OP), panel C: MSG treated group, panel D: ASP treated group, panel E: MSG&OP, and panel F: ASP&OP. Panel A&B show a normal histological architecture of the cerebellum. Panels C&D show obvious degeneration with remarkable separation between cerebellar layers (star), pyknotic granular cells, and pyknotic and vacuolated Purkinje cells. In panels E&F, the cerebellar sections illustrate remarkable amelioration in the histological architecture of cerebellar layers and cells. (H&E stain , Scale bar =25). **Abbreviations:** PM: pia matter, ML molecular layer, PL: Purkinje cells layer, GL: Granular layer, WM: white matter. PGC: pyknotic granular cells, GC: Granular cells, PPC: pyknotic Purkinje cell. V: vacuoles

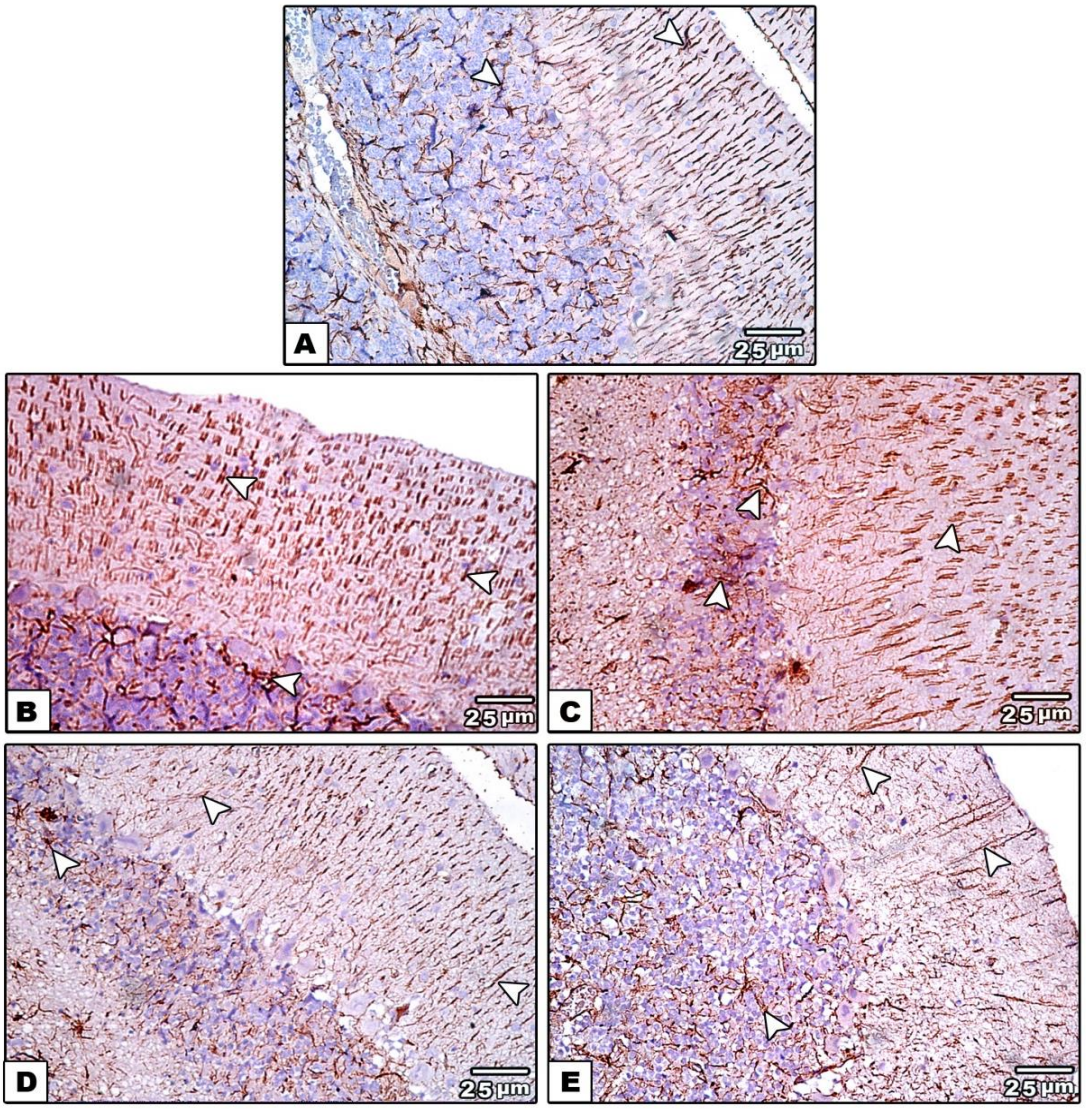

Fig.5: Photomicrograph of embedded formalin-fixed paraffin sections of cerebellar cortex of mother Albino rats stained with GFAP antibody. Panel A:control, panel B: MSG, panel C: ASP, panel D: MSG&OP and panel E:ASP&OP. **Note**: strong positive immune expression in the cerebellar cortex of MSG &ASP treated groups if compared with other studied groups. (GFAP antibody stain, Scale bar =25). The arrowheads point to the localization of GFAP reactivity

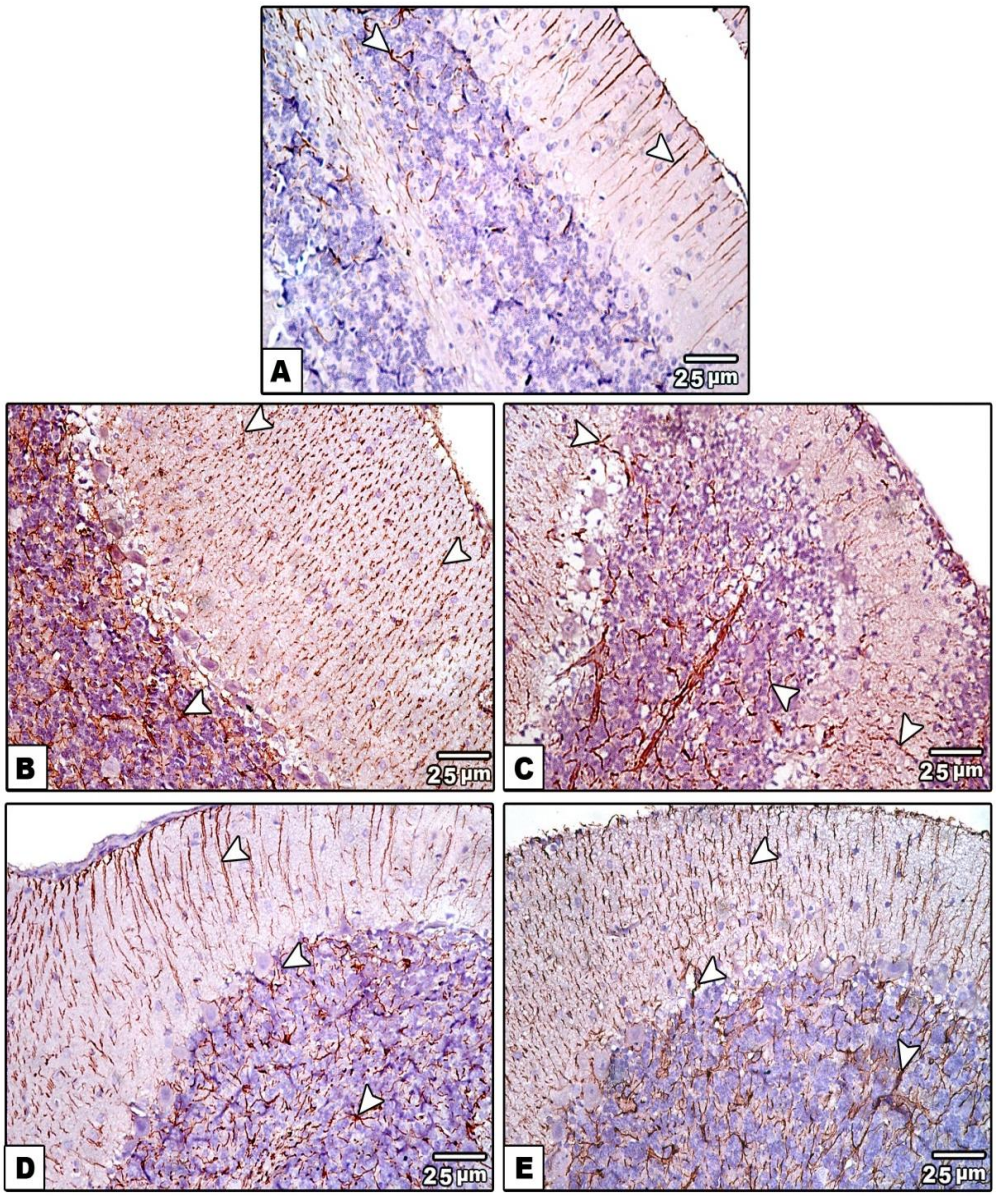

Fig. 6: Photomicrograph of embedded formalin-fixed paraffin sections of the cerebellar cortex of offspring 21-day rats stained with GFAP antibody. Panel A:control, panel B: MSG, panel C: ASP, panel D: MSG&OP and panel E:ASP&OP. Note: an intensive immune expression in the cerebellar cortex of MSG&ASP (B&C) groups stained with GFAP reaction, weak immune expression in the control group (A), moderate GFAP reaction in (D&E). (GFAP antibody stain, Scale bar =25). The arrowheads point to the localization of GFAP reactivity

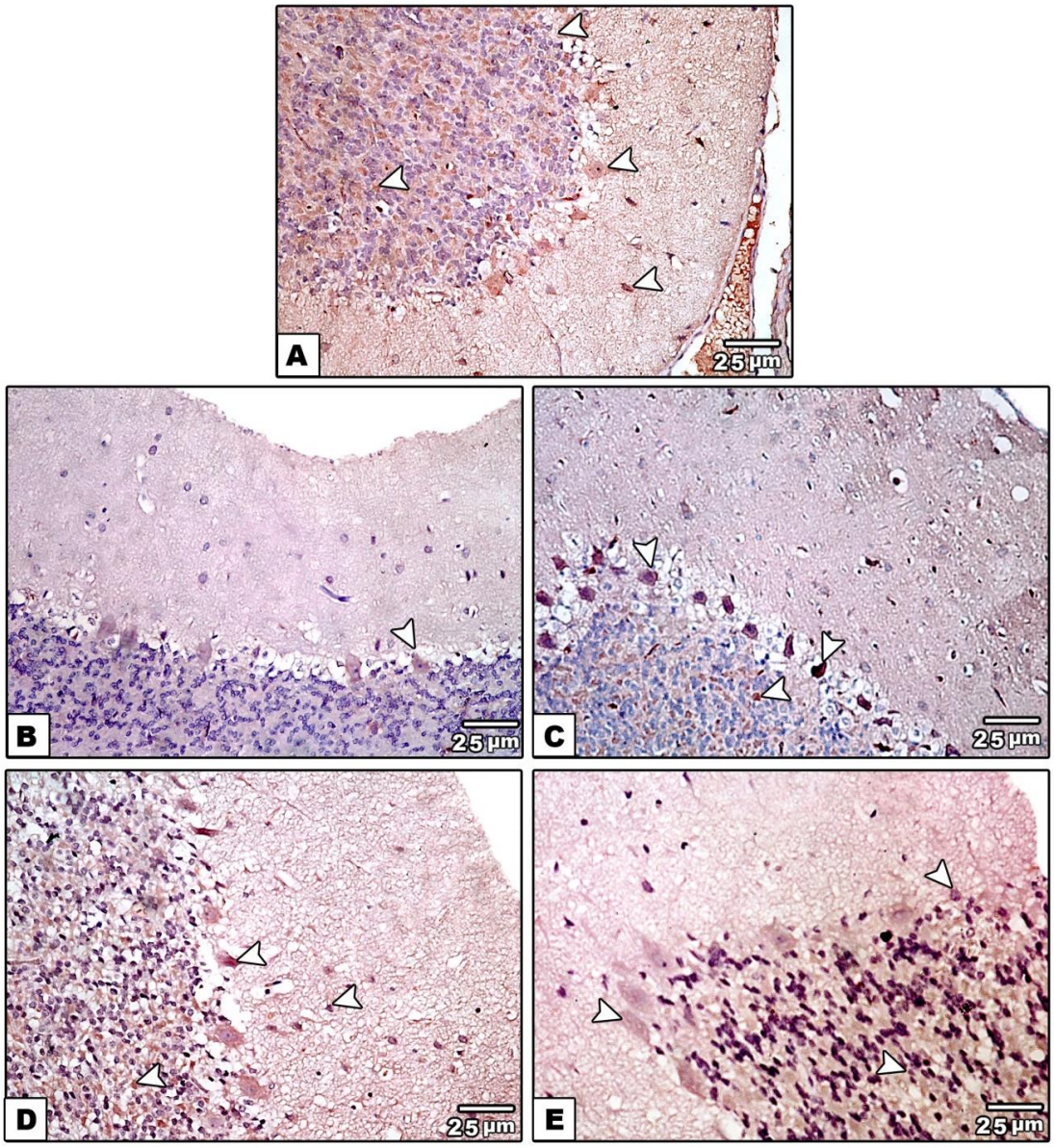

Fig. 7: Photomicrograph of paraffin-embedded sections of cerebellum cortex of mother rats stained with BCL-2 antibody. Panel A:control, panel B: MSG, panel C: ASP, panel D: MSG&OP and panel E:ASP&OP.Note: a moderate positive immune expression of BCL-2 in the control group, weak expression in MSG and ASP groups, and moderate to strong immune reaction in MSG and ASP /Op groups.(BCL-2antibody stain , Scale bar =25). The arrowheads point to the localization of BCL-2reactivity.

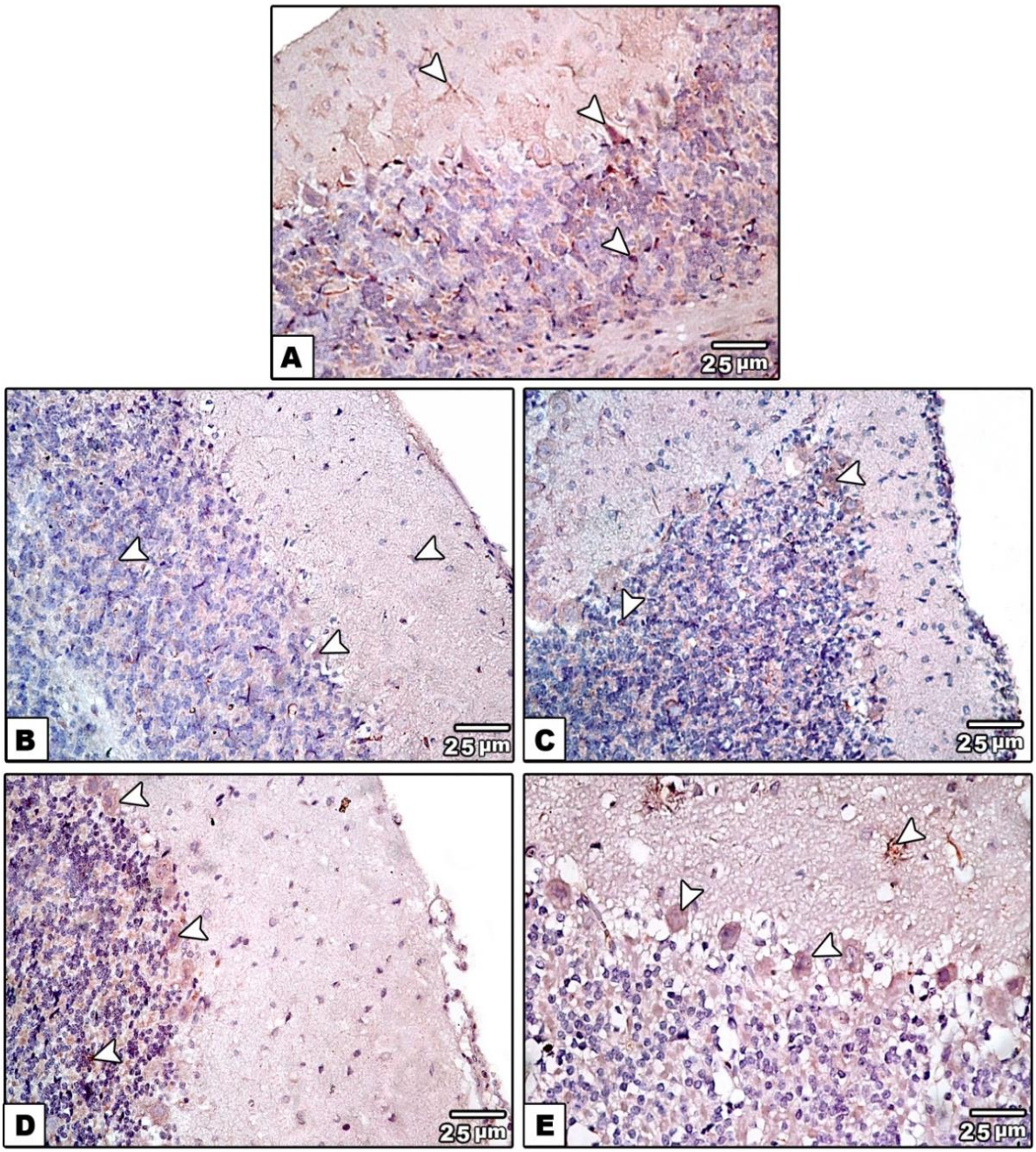

Fig 8: Photomicrograph of paraffin-embedded sections of cerebellum cortex of 21-day-old rats stained with BCL-2 antibody. Panel A:control, panel B: MSG, panel C: ASP, panel D: MSG&OP and panel E:ASP&OP. **Note:** A moderate positive immune expression of BCL-2 in panel A, weak expression in panels B&C, and moderate to strong immune reaction in panels D&E. (BCL-2antibody stain, Scale bar =25). The arrowheads point to the localization of BCL-2reactivity.

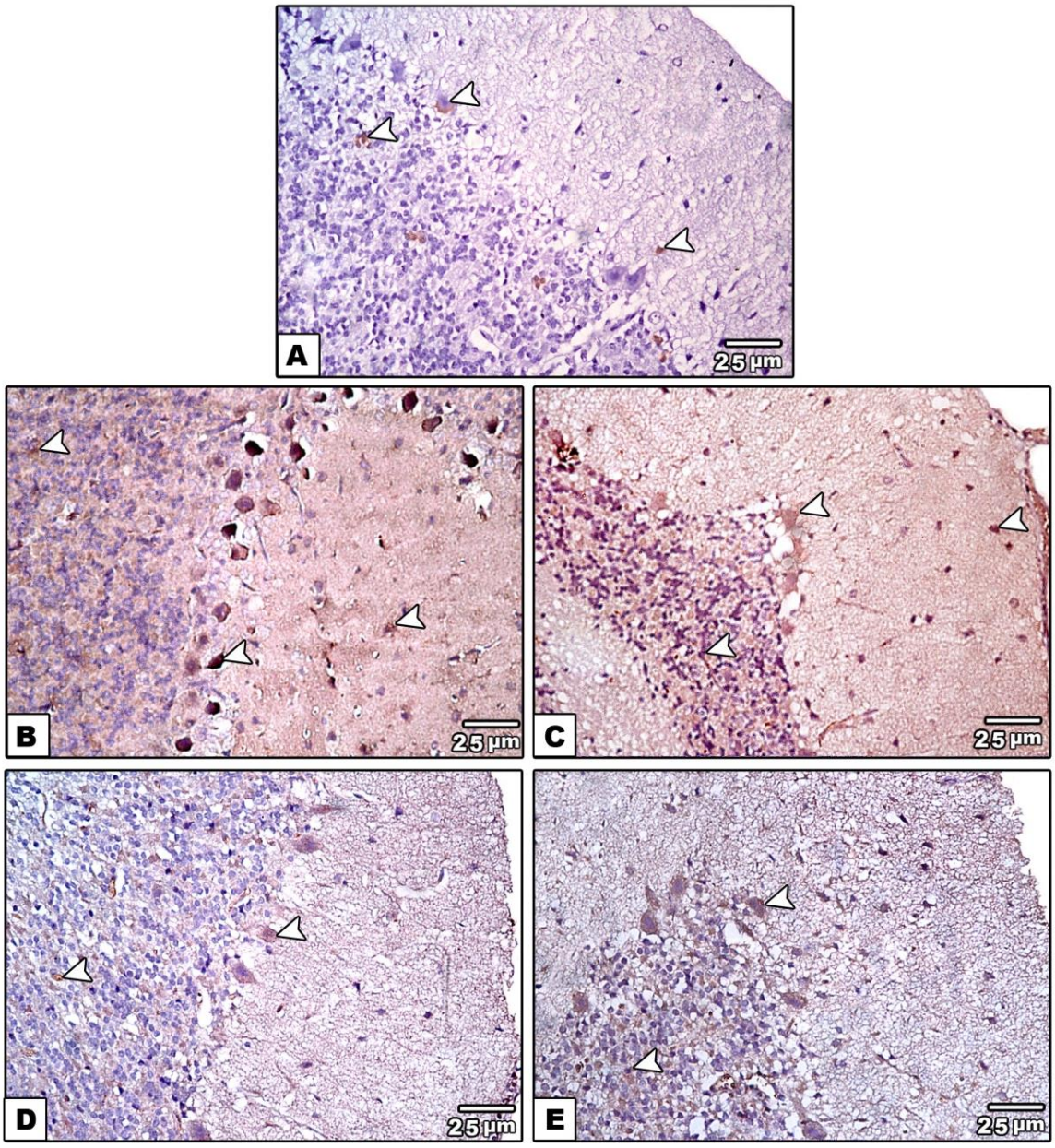

Fig.9: photomicrograph of embedded formalin-fixed paraffin sections of cerebellum cortex of mother Albino rats stained with the P53 antibody. Panel A:control, panel B: MSG, panel C: ASP, panel D: MSG&OP and panel E:ASP&OP. Note: weak immune expression in panel A group, strong positive immune reaction in B&C groups, and moderate to strong expression of P53 in D&E groups. (P53 antibody stain, Scale bar =25). The arrowheads point to the localization of P53 reactivity.

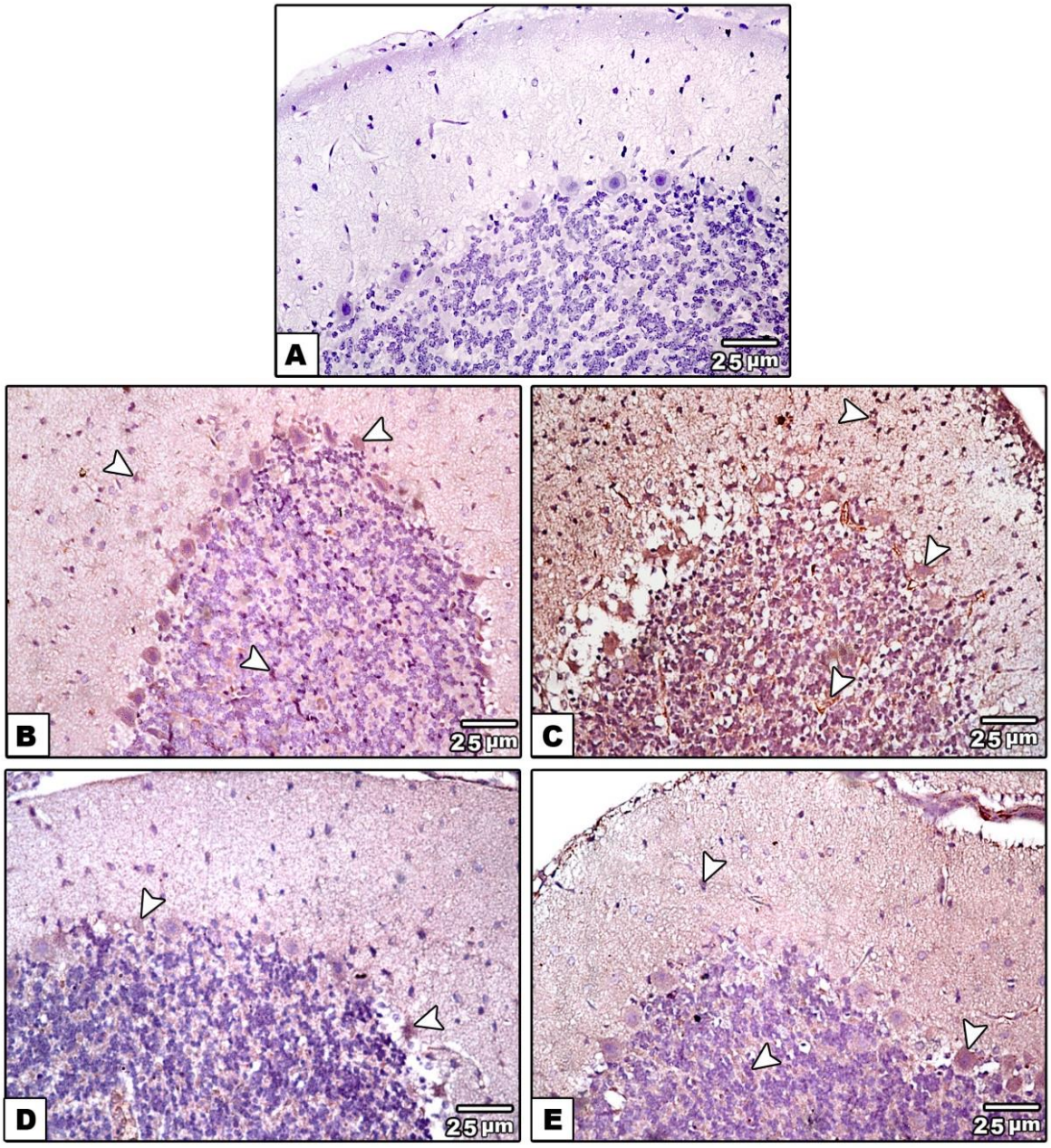

Fig.10: photomicrograph of embedded formalin-fixed paraffin sections of cerebellum cortex of 21-day-old rats stained with the P53 antibody. **A** Panel A: control, panel B: MSG, panel C: ASP, panel D: MSG&OP, and Panel E: ASP&OP **A**. **Note**: weak immune expression in panel A group, a strong positive immune reaction in panels B&C groups and moderate expression of P53 in panels D&E groups. (P53 antibody stain, Scale bar =25). The arrowheads point to the localization of P53 reactivity.

4. Biochemical results in cerebellar tissue

4.1 Caspase-3

As shown in Table (2) and Figures (11&12), the level of caspase-3 in the cerebellar cortex of *Opuntia*-supplemented mothers and their offspring appeared with non-significant variation compared with control. On the other hand, a highly significant increase (P<0.001) in caspase-3 activity was noticed in MSG and ASP-treated mothers while their offspring showed a low significant increase (P<0.05) if compared with control. In the two protective groups of mothers, the cerebellar level of caspase-3 was lowered but showed a low significant increase (P<0.05) with control whereas the level of caspase-3 activity in two ameliorative groups of offspring showed remarkable non-significant variation with their control.

4.2 Interleukin-1 (IL-1)

The obtained result of the present work revealed that the level of cerebellar IL-1 was significantly higher (P<0.001) in MSG-treated mothers and their offspring if compared with the control. On the other hand, the level of IL-1 in ASP-supplemented mothers and their offspring showed a low significant increase (P<0.05) in comparison with control and *Opuntia*-supplemented mother rats. In MSG-induced rats that supplemented with *Opuntia* fruit extract the level of IL-1 showed a remarkably low significant increase (P<0.05) compared with control while ASP-induced rats that co-supplemented with *Opuntia* showed obvious improvement in the level of cerebellar IL-1 (Table 3, Figures 13&14).

5. Serum Analysis

5.1 Serum insulin

In the present work, the serum insulin level in MSG and ASP groups of mother's rats showed a high significant decrease (P<0.001) however, their offspring revealed a low significant decrease (P<0.05) if compared with control. On the other hand, the two ameliorated groups of mother's rats (MSG-ASP plus Op) showed a significant increase in serum insulin level if compared with MSG and aspartame groups but still significantly lower than control (P<0.05). In the two ameliorated groups of offspring, a remarkable significant improvement in the levels of serum insulin was recorded (Table 4, Figures 15&16).

5.2 Insulin-like growth Factor-I (IGF-I).

In MSG and ASP-treated mothers and their pups, the levels of serum IGF-1 showed a low significant decrease (P<0.05) if compared with control. On the other hand, a remarkable amelioration was noticed in the level of IGF-1 for the two ameliorative groups of mothers and their offspring. Such a result confirms that *Opuntia* fruit extract has a powerful role in ameliorating the serum level of IGF-1 rather than insulin level (Table 4, Figures 17&18).

Table 2: illustrates the levels of caspase-3 in the cerebellar tissues of mother's rats and their offspring (Ug/ml) for the different experimental groups.

Groups N=5 / Age	Control	MSG	ASP	MSG+OP	ASP+OP
Mother	150.1±10.4	197±5.98***	180.1±16.6***	145.2±2.57*	148.5±2.57*
Offspring 21 day	186.66±1.89	197.8±1.81*	216.5±0.76*	188.5±0.42	190.3±2.12

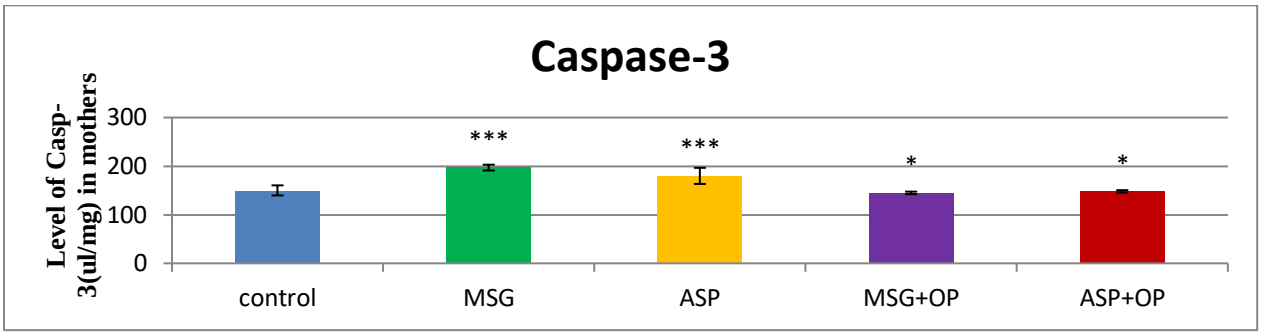

Fig 11: Showing the levels of caspase-3 in the cerebellar tissues of mother rats among different studied groups

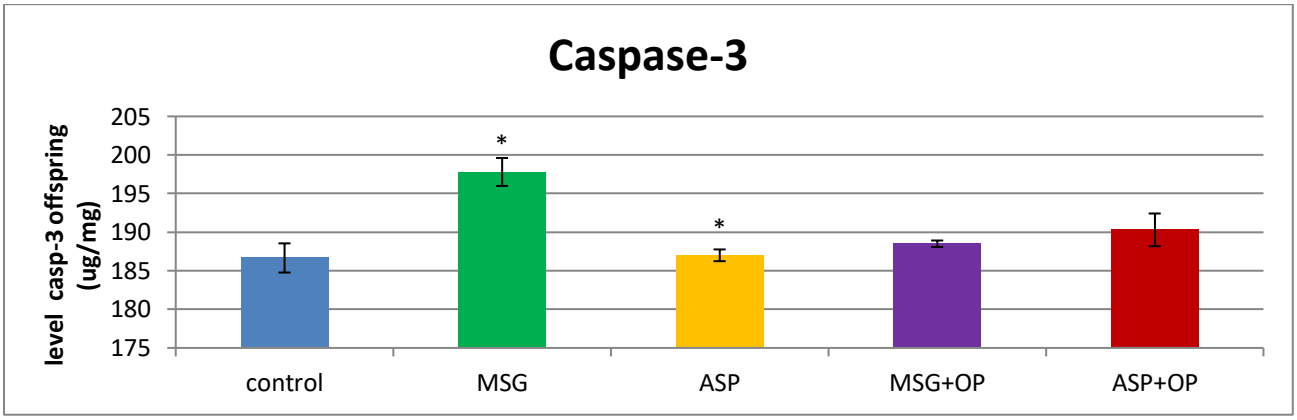

Fig 12: Showing the levels of caspase-3 in the cerebellar tissues of 21day rats among different studied groups

Table 3: illustrates the level of Interleukin-1 (IL-1) in the cerebellar tissues of mother rats and their offspring (ug/mg) in the different experimental groups.

Group n=5 / Age	Control	MSG	ASP	MSG+OP	ASP+OP
Mother	88.5±8.13	115.5±12.18***	116.7±9.61*	88.3±3.14*	98.3±3.33
Offspring 21 days	82.4±0.77	106.5±1.38***	94.8±1.13*	89.33±0.49*	85.6±0.16

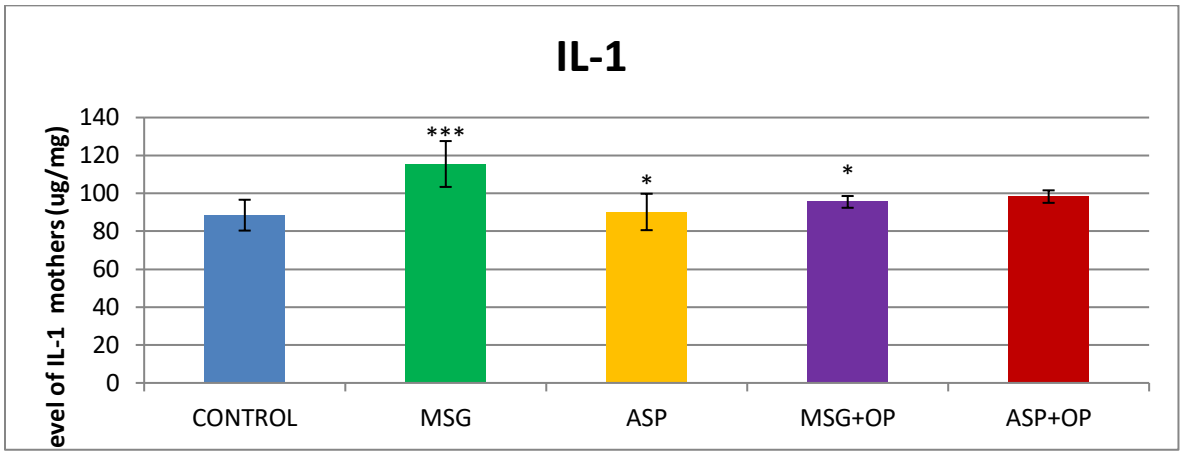

Fig 13: Showing the level of IL-1 in the cerebellar tissues among different studies of mothers groups

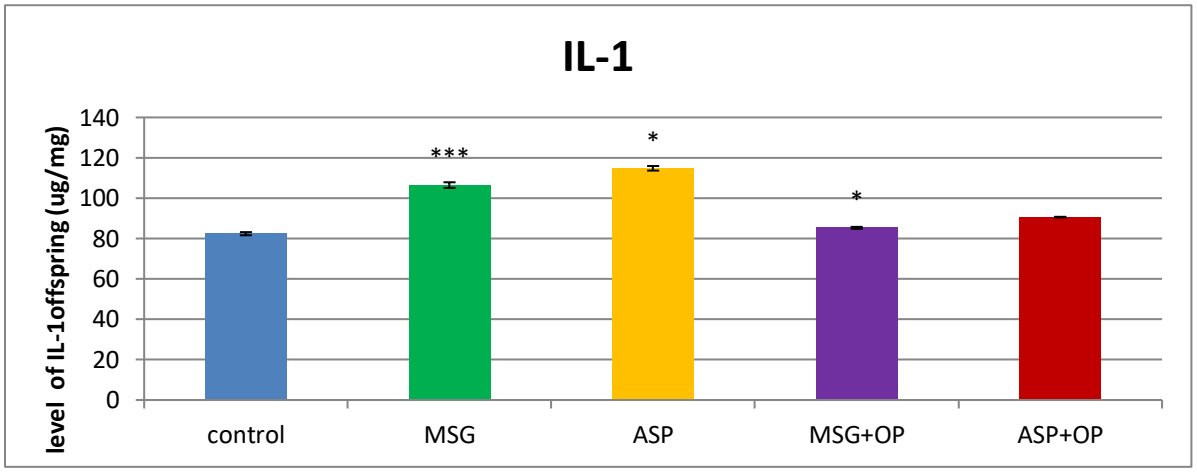

Fig 14: Showing the level of IL-1 in the cerebellar tissues among the different studied groups of offspring.

Table 4: Illustrates the levels of serum Insulin (IN) and Insulin-like growth factor-1 (IGF-1) in mothers and their offspring in different experimental groups.

Groups (N=5)		**Control**	**MSG**	**ASP**	**MSG+OP**	**ASP+OP**
Mother	IN	6.9±0.206	5.7±0.32***	5.9±0.43***	6.1±0.16*	6.4±0.22*
Offspring 21 day		7.56±0.61	10.6±1.5*	12±2.4*	8.4±2.4	9.1±1.11
Mother	IGF-1	500±16.5	200.1±16.7*	314.1±16.2*	354.1±16.3	307.7±19.8
Offspring 21 day		503±1.2	204±1.2*	350±3.3*	400±4.7	480±4.2

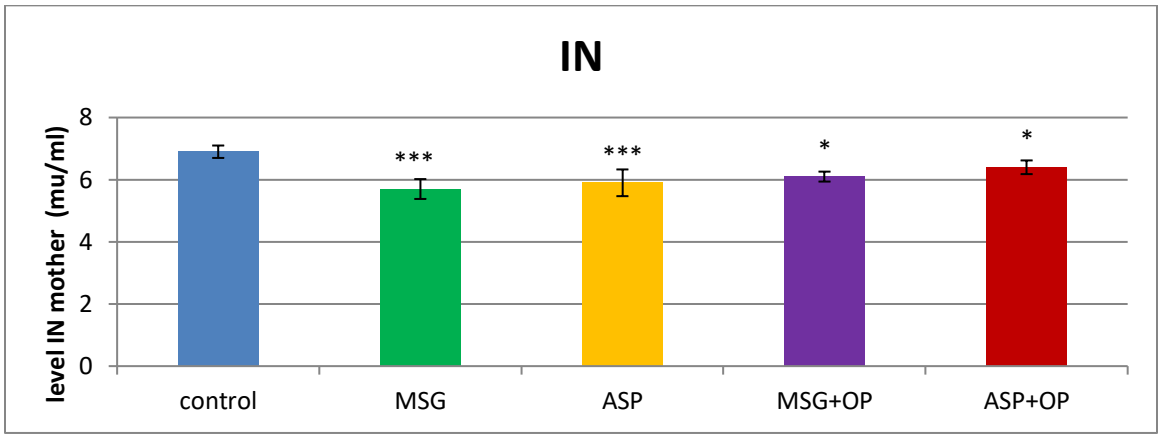

Fig.15: Showing the serum level of insulin (IN) among the different studied groups of mother rats

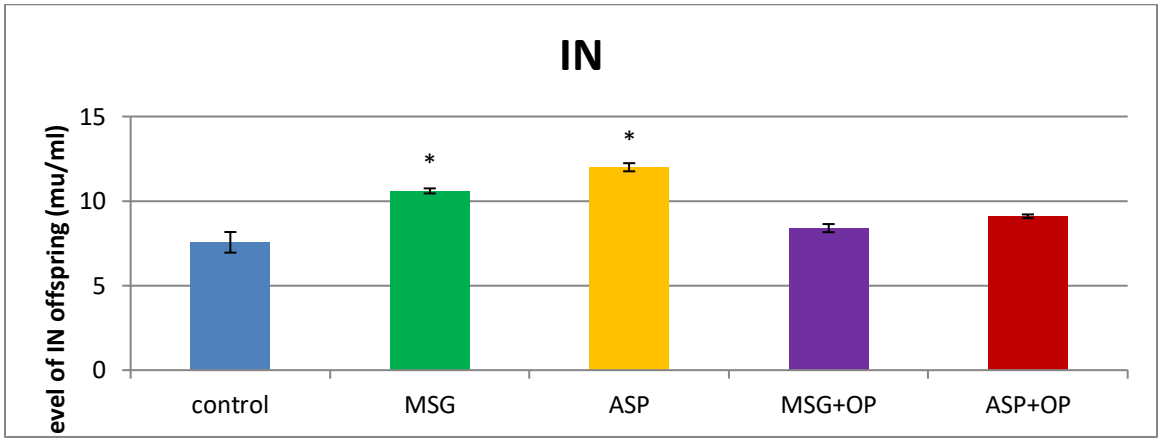

Fig.16: showing the levels of serum insulin (IN) among the different studied groups of offspring.

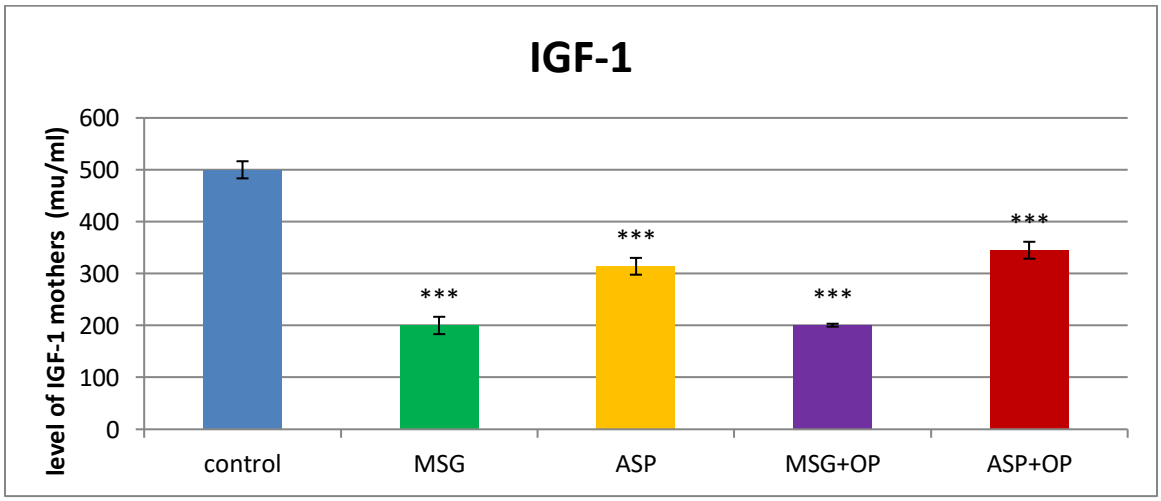

Fig.17: Showing the levels of serum IGF-1 among the different studied groups of mothers rats

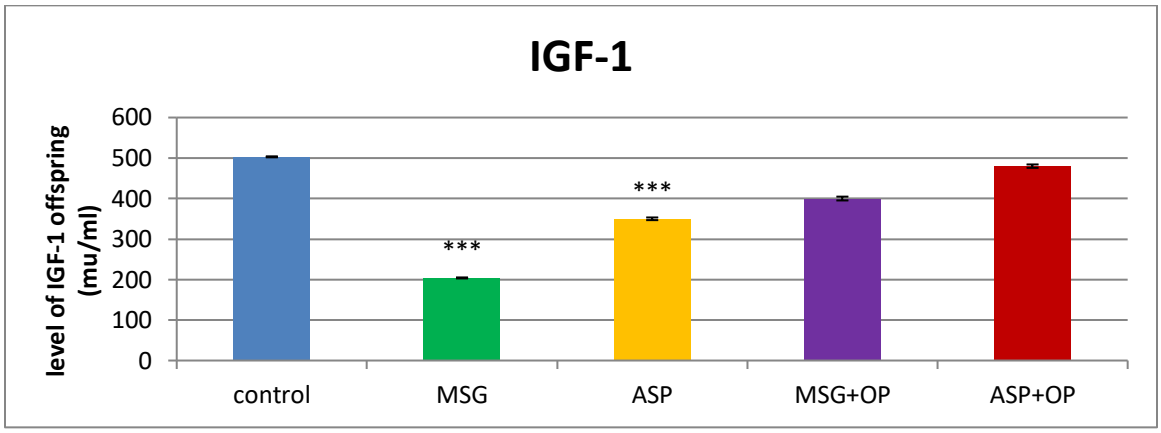

Fig.18: Showing the serum level IGF-1 among the different studied groups of offspring

DISCUSSION

Most food additives act either as preservatives or enhancers of palatability. Food additives have been implicated in causing harmful effects **(Moore, 2003; El-Beltagy, 2016).** One such food additive is monosodium glutamate (MSG) and aspartame (ASP). It has been reported that MSG has neurotoxic effects resulting in brain cell damage, retinal degeneration, and some pathological conditions like neuropathic pain and depression (**Salman et al., 2012**). ASP contains a large number of compounds, including phenylalanine, tyrosine, and aspartic acid that compete with each other for a binding site on the neutral amino acid transporter (NAAT), because it is the only manner in which they can cross the blood-brain barrier (BBB).

Previous reports revealed that food additives utilization during gestation and breastfeeding is not to be considered completely safe whereas these substances induce alterations in the differentiation of several cell types, resulting in the development of disease during the adult age (**El-Beltagy, 2016).**

It is generally accepted that the beneficial effects of medicinal plants can be obtained from active constituents present in the whole plant, parts of the plant, or plant materials or combinations, whether in the crude or processed state (**De Smet, 2002).** One medicinal plant that has been proposed as having interesting antioxidant activity and protective capacities due to the presence of components such as vitamins C and E, phenolics, and other non-nutrient substances is *Opuntia ficus indica* (L). *Opuntia* fruit contains a rich variety of natural antioxidants, many phenol compounds, ascorbic acid, betalains, betacyanins, and a flavonoid fraction that consists mainly of rutin and isorhamnetin derivatives (**Kuti, 2004; Fernández-López et al., 2010**). Accordingly, the present work was mainly designed to evaluate the ameliorative role of *Opuntia ficus indica* fruit against MSG and ASP-induced neurotoxicity in the cerebellum of mother rats and their offspring.

The obtained data revealed that the body weight of MSG and ASP induced mother's rats were significantly higher while that of offspring showed low significant increase in their body weights. Previous reports on experimental animals found a possible correlation between MSG intake and obesity, this might be attributed to an increase in appetite or even to consuming large amounts of food (**Iwase et al., 2000),** and by stimulation of the appetite center

(**Hermanussen et al., 2006**). Another study explained that the increased body weight under the influence of MSG consumption is mainly attributed to a reduction in the secretion of growth hormone that subsequently results in increased obesity **(Corder et al., 1990). Hassan (2016)** reported that a low intake of aspartame can promote body weight gain in weanling Syrian hamsters. Further study revealed that aspartame affected appetite, followed by a sustained increase in hunger ratings **(Tordoff & Alleva, 1990**). Also, it had been found that the increase in the consumption of the foods sweetened by ASP was parallel with an increase in the consumption of the foods sweetened by caloric sweeteners (**Duffey et al., 2008**). Additionally, aspartame might lead to increased body weight and obesity by interfering with the fundamental equilibrium of physiological processes mediated by taste receptors (**MacKinnon et al., 1999**). Another mechanism was revealed increment palatability of the food items using aspartame (**Mattes & Popkin, 2009)**

In the current work marked harmful toxic degenerative effects in the form of partial loss and degenerated Purkinje cells, cellular hypertrophy, and scattered vacuoles in the granular layer were noticed in the cerebellar cortex of MSG and ASP-treated mothers and their offspring. The obtained results go parallel with the findings of the previous studies (**Eweka & Om`Iniabohs**, **2007; Salman et al., 2012) González-Burgos et al.(2000)** found that MSG can induce neurodegenerative process in neonatal rats in the form of apoptotic cell death and they attributed these findings to the incidence of several neurochemical alterations of surviving neurons in the brain. Another study confirmed that MSG produces neuro-degeneration with severe damage to the cells in different brain regions when it was administered to neonatal rats, from an early embryonic age to adulthood (**Falalieieva et al., 2010)**. **Pavlovic et al. (2011)** explained that administration of high concentrations of MSG can induce oxidative stress in different body organs. **Salman et al. (2012)** reported that MSG can delay the mechanism of cerebellar cortex development during postnatal life**.**

Nweze et al. (2015) reported that some people suffer neurological or behavioral reactions in association with aspartame consumption through the induction of oxidative stress. **Ashok et al. (2015**) revealed that frequent ASP administration can alter the functional activity in all brain regions by elevating the free radical levels. Moreover, the daily acceptable intake of aspartame can disrupt cerebellar function, apoptosis, and neural cell damage as a result of Tau aggregation **(Su et al., 2016**). Also, it has been confirmed that consumption of ASP leads to neurological and behavioral disturbances in sensitive individuals and these may be attributed to changes in regional brain concentrations of catecholamines **(Humphries et al., 2008).** Aspartame metabolites like aspartic acid, phenylalanine, and methanol are toxic at high levels. These products were proven to be responsible for the disruption of the blood-brain barrier and induction of neuronal damage (**Trocho et al., 1998**). Methanol and aspartic acid could emit free radicals which produce cytoplasmic vacuoles by reacting with the proteins and lipids of different organelles as well as alteration of nuclear chromatin (**Butchko et al., 2002).** Furthermore, methanol is oxidized to formaldehyde and formic acid, these metabolites cause neural damage and prevent DNA replication **(Ashok et al., 2015; Carol-Chibuzo et al., 2015**). The authors added that excess aspartic acid from aspartame can kill certain neurons in the brain through stimulation of excess calcium influx into the cells. This influx triggers the liberation of excess free radicals, which kill the cells, thus, giving this amino acid the name "excitotoxin" because it excites or stimulates the neural cells to death (**Hassan, 2016).** Phenylalanine, the third metabolite of aspartame, has been associated with neurotoxicity and also affects the synthesis of inhibitory monotransmitters, and has been

shown to mediate neurological disorders (**Nweze et al., 2015**).

In the current work, *Opuntia* fruit extract successfully restored the cerebellar histopathological alterations induced by MSG&ASP. Previous reports revealed that the beneficial effect of *Opuntia* on the brain is mainly attributed to their polyphenol constituents that play a major role as powerful antioxidants and in free radical scavenging activities. For instance, gallic acid exhibits high antioxidant activity that is responsible for its ability to reduce brain DNA damage (**Khan et al., 2000**). Nicotiflorin polyphenol is also thought to be an anti-inflammatory and neuroprotective component, whereas it can reduce brain cell damage and attenuate neurological deficits induced by ischemia (**Li et al., 2006**). Also, nicotiflorin is neuroprotective against hypoxia-glutamate- or oxidative stress-induced retinal ganglion cell death at nanomolar concentrations (**Nakayama et al., 2011**). Other studies, explained that pre- and post-natal exposure of rats to MSG and ASP has been shown to have deleterious effects on the developing cerebellum. The exogenous antioxidant vitamin E in *opuntia* can promote neuronal survival (**Heaton et al., 2004).**

Immunohistochemical markers have emerged as one of the tools available for studying neuronal morphology and morphometry. The immunohistochemical results of the present work revealed a strong positive expression for GFAP and P53 markers but weak to moderate expression for BCL-2 marker in the cerebellar cortex for both MSG and ASP-treated groups of mother's rats and their pups.

Glial fibrillary acidic protein (GFAP) is an intermediate filament (IF) protein that is expressed by numerous cell types of the central nervous system (CNS) including astrocytes and ependymal cells. Moreover, GFAP forms a network to provide support and strength to neural cells. It is thought to control their shape, movement, and function. A strong GFAP reactivity was recorded in astrocytes of all brain regions post-administration of ASP at 40, 80, and 160 mg/kg (**Omar, 2009). Ahmed &Abd El-Samad (2010)** revealed a strong immune expression of GFAP in the cerebellar cortex of ASP-treated rats could be considered a marker for neural cell damage **(Omar, 2009).** Other reports found obvious production of GFAP under the conditions of brain injury (trauma or disease) (**Onaolapo et al., 2017).**

Currently, the intense immune expression of GFAP in the cerebellar cortex of MSG treated mother's rats and their offspring may be attributed to the excitotoxicity effect of glutamate which may lead to severe neuronal damage and other complications (**Lau &Tymianski, 2010**). **Beas-Zarate et al. (2002)** revealed that L-glutamate during neonatal periods of Wistar rats' can result in neuronal degeneration and cytoarchitectural changes in the brain which subsequently leads to proliferation of GFAP.

In the current work, there was apoptotic cell death in all cerebellar cortical layers especially Purkinje one, due to MSG and aspartame usage that appeared in histological and confirmed immunohistochemically using P53 and BCL-2 as an apoptotic marker. This result goes parallel with the finding of **Eweka & Om``Iniabohs (2007**) who reported neuronal cell death post-MSG supplementation in rats. They also explained that cell death in response to neurotoxins might trigger an apoptotic death pathway in cerebellar cells. Other studies reported that MSG administration to animals significantly increased the apoptotic rate and oxidative stress of the mocytes **(Pavlovic, et al., 2011). Narayaman et al. (2010)** explained that the excitotoxic effect of MSG was mediated by an interaction of NMDA (N- malondialdehyde) receptors that led to an elevation of intracellular calcium concentration which activates various apoptotic enzymes. In contrast to the obtained results, **González-Burgos et al. (2000)** reported that the

prefrontal cerebral cortex did not show any apoptotic signs when MSG was administered to male neonate rats. Another similar observation on the cerebellar cortex of aspartame-exposed rats elucidated strong expression for pro-apoptotic markers Bax and caspases-3 with a marked decrease in the Bcl-2 expression **(Iyaswamy et al., 2014).** They also explained that aspartame metabolites can induce oxidative stress which is a main cause for neuronal cell apoptosis.

In the present work, the cerebellar sections of the two ameliorated groups with *Opuntia* fruit extract exhibited a pronounced decrease in the pro-apoptotic markers P53 and an increase in anti-apoptotic markers BCl-2. Previous studies showed the antioxidant of *Opuntia* fruit extract would support biological resistance to free radicals, suggesting the capacity of this extract to play a role in antigenotoxicity and anti-apoptotic effects (**Brahmi et al., 2011& 2012**). The protective effects of *Opuntia* extract against oxidative damage and apoptosis are mainly attributed to the presence of several antioxidants such as ascorbic acid, vitamin E, carotenoids, reduced GSH, flavonoids, and phenolic acids detected in this fruit (**Lee et al., 2012).**

Interleukin-1 is a complex network of proinflammatory cytokines that plays a central role in the regulation of immune and inflammatory responses to infections or sterile insults (**Dinarello, 2011).** Moreover, IL-1 has a major role in neuro inflammation **(Moynagh, 2005**), whereas, the increased levels of TNF and IL-1 in the brain, may cause the breakdown of the blood-brain barrier (**Hofman et al., 1986)**.

In the current work, the cerebellar cortex of MSG MSG-induced group showed a remarkable and significant increase in IL-1 level rather than ASP exposed group and control respectively. A previous study has shown links between elevated levels of inflammatory cytokines including elevated levels of IL-1β and TNF-α and mood disorder (**Kamel, 2015).** The obtained result in this study goes parallel with the finding of **Ye et al. (2013)** who declared that interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), two pro-inflammatory cytokines are elevated in response to neurodegenerative state (neuronal death and apoptosis) induced by excess glutamate. Moreover, excessive levels of glutamate are well known to induce neurotoxicity **(Erdmann et al., 2006**) which is a potential mediator of IL-1β mediated neurotoxicity.

In the present study, ASP administration caused a slight elevation in the levels of the pro-inflammatory cytokines IL-1 in cerebellar tissue homogenate. The obtained result follows the results of **Kamel (2015**) who reported that ASP administration resulted in a significant increase in IL-1 levels in the rat brain tissues. These results indicate that ASP metabolites have a powerful role in the induction of inflammatory processes (**Catena-Dell'Osso et al., 2013**). Furthermore, ASP metabolites like methanol or formaldehyde may be the causative factors behind the significant increase in interleukins (**Choudhary and Sheela-Devi, 2015).** Moreover, the increase in proinflammatory cytokines would lead to increased oxidative damage with a production of tryptophane catabolites and consequently reduced availability of tryptophane and serotonin (**Dantzer and Kelley, 2007).**

In the current work, the anti-inflammatory effect of *Opuntia* fruit extract against MSG and ASP-induced neurotoxicity may be attributed to its vital component, indicaxanthin which prevents activations of NF-kB and attenuates the rise in inducible nitric oxide synthase leading to a decreased level of IL-1 **(Panico et al., 2007; Tesoriere et al., 2014)**

The obtained data of the present study revealed that the levels of serum insulin and insulin-like growth factor 1 (IGF-1) were significantly decreased in MSG and aspartame exposed groups, especially in mothers rather than their offspring. Previously it has been

reported that the decreased level of NGF induces apoptosis in pancreatic beta cells **(Pierucci et al., 2001)**. **Palmnas et al. (2014)** revealed that individuals consuming aspartame presented elevated fasting glucose and impaired insulin sensitivity**.** Also, an experimental study on ASP-exposed male and female mice revealed a remarkable weight gain, elevated fasting glucose levels, and decreased insulin sensitivity in males while females were less affected, but had significantly raised fasting glucose levels **(Collison et al., 2012).** Another study performed on rats found that MSG is a causative potent chemical for obesity and consequently induction of diabetes **(Pinterova et al., 2001)**. **Iwase et al. (2000**) added that intraperitoneal injection of 4 mg/kg of MSG to newborn rats resulted in more advanced obesity, higher triglyceride, and low serum insulin.

Opuntia ficus indica is known for its high content of polyphenols exhibiting antioxidant and anti-inflammatory properties (**Butera et al., 2002; Kuti, 2004**). The ameliorative role of *Opuntia* fruit extract against MSG and ASP-induced reduction of insulin and IGF-1 is mainly attributed to alkaloids, indicaxanthin, neobetanin, and various flavonoids along with polysaccharides which have a powerful antidiabetic and antiglycation effect (**Lee et al., 2012).**

In conclusion, *Opuntia* fruit extract has a powerful ameliorative role against MSG and ASP-induced cerebellar neurotoxicity in mother rats and their offspring. The beneficial effects of *Opuntia* fruit extracts are mainly attributed to their high content of natural antioxidants, like phenol compounds, ascorbic acid, betalains, betacyanins, and various types of flavonoids.

Conflict of interest

All authors declared that there were no conflicts of interest.

Fund:

No funds, grants, or other support were received.

REFERENCES

Abd Elfatah A A., Ghaly I S., Hanafy S M (2012): Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. Pak J Biol Sci 15(19): 904- 18.

Abd El-Razek FH, El-Metwally M, Shehab GMG, Hassan AA,Gomaa AM.(2012): Effects of cactus pear (Opuntia ficus indica) juice on oxidative stress in diabetic cataract rats.Exp Stud 1:23–29.

Abdel-Salam O M E, Salem N A., El-Shamarka M. E S., Hussein J. S., Ahmed N A S., El-Nagar M E S, (2012): Studies on the effects of aspartame on memory and oxidative stress in brain of mice. Eur. Rev. Med. Pharmacol. Sci. 16 (15), 2092–2101.

Abu-Taweel G, Ajarem J, Zyadah M, Ahmad M (2014): Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. Neurotoxicol. Teratol. 42:60-67.

Ahmed A and Abd El-Samad M (2010): Light and electron microscopic study on the effect of aspartame on the cerebellar cortex of male albino rat Egyptian Journal of Histology; 33(3). 419 – 430.

Aires V, Adote S, Hichami A, Moutairou K, Boustani ES, Khan NA (2004): Modulation of intracellular calcium concentrations and T cell activation by prickly pear polyphenols.Mol Cell Biochem 260:103–110.

Alimi H, Hfaeidh N, Bouoni Z, Mbarki S, Sakly M and Rhouma K B (2013): Cactus (Opuntia ficus indica f. inermis) fruit juice protects against ethanol-induced hematological and biochemical damages in rats. Afric. J. of Biotech., 12(51), 7099-7105.

Alleva R, Borghi B, Santarelli L, Strafella E, Carbonari D, Bracci M, Marco T B, (2011): In

effect of aspartame in angiogenesis induction. Toxicology in Vitro 25, 286–293.

Ashok I, Sheeladevi R, Wankhar D (2015): Acute effect of aspartame-induced oxidative stress in Wistar albino rat brain. The Journal of Biomedical Research, 29(5):390-396

Ashok R, Sheeladevi R, Wankhar D (2014): Effect of long-term aspartame (artificial sweetener) on anxiety, locomotor activity and emotionality behavior in Wistar Albino rats.Biomed. Prev. Nut.4:39-43.

Aun MV, Mafra C, Phillipi JC, Kalil J, Agondi RC, Motta AA (2011): Food Additives. Rev. Bras. Alerg. Imunopatol. 34(5):177-186.

Bancroft J D, Gamble M (2008): Theory and practice of histological techniques. Elsevier Health Sciences.

Beas-Zarate, C, Perez-Vega M I, Gonzalez-Burgos I. (2002): Neonatal Exposure to Monosodium L-Glutamate Induces Loss of Neurons and Cytoarchitectural Alterations in Hippocampal CA1 Pyramidal Neurons of Adult Rats. Brain Research 952(2), 275–281.

Bougoussa M, Manolopoulou J, Barnes AK, Lecane S, Garrity ML, Bidlingmaier M.(2010): Development of a new Insulin Like Growth Factor-I Immunoassay on the IDS-iSYS Automated Analyser. Clin Chem 56: A64-5.

Brahmi D, Ayed Y, Hfaiedh M, Bouaziz C, Ben Mansour H, et al. (2012): Protective effect of cactus cladode extract against cisplatin induced oxidative stress, genotoxicity and apoptosis in balb/c mice: combination with phytochemical composition. BMC Complementary and Alternative Medicine 12: 1-14.

Brahmi D, Bouaziz C, Ayed Y, Ben Mansour H, Zourgui L, et al. (2011): Chemopreventive effect of cactus Opuntia ficus indica on oxidative stress and genotoxicity of aflatoxin B1. Nutr Metab (Lond) 8: 73.

Butchko H H, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL, et al (2002): Aspartame: review of safety. Regul Toxicol Pharmacol; 35 (Pt 2): S1–S93.

Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea MA (2002): Antioxidant activities of Sicilian prickly pear (Opuntia ficus indica) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J Agric Food Chem 50:6895–6901.

Cardoso LAC, Karp SG, Vendruscolo F, Kanno KYF, Zoz LIC, Carvalho JC(2017)

Carocho M, Morales P, Ferreira ICFR (2015): Natural food additives:Quo vadis? Trends Food Sci. Technol. 45(2):284-295.

Carol-Chibuzo N., Mustapha A.A. and Olose M. Aspartame Food Additive and its Biochemical Implication: A Review, (2015): Food Science and Quality Management, 36.

Catena-Dell'Osso M, Rotella F, Dell'Osso A, Fagiolini A and Marazziti D(2013): Inflammation, serotonin and major depression. Curr Drug Targets.; 14(5):571-7.

Choudhary A K, Sheela Devi R.2015 Longer period of oral administration of aspartame on cytokine response in Wistar albino rats. Endocrinol Nutr. 62(3):114-22.

Collison K S Makhoul N J, Zaidi M Z, Maqbool Z, Saleh S M, Bakheet R, Mondreal R, Al-Rabiah R, Shoukri M, Milgram N W, Al-Mohanna F A (2012): Gender dimorphism in aspartame-induced impairment of spatial cognition and insulin sensitivity. PLoS One, 7(4): 1-13.

Corder R, Saudan P, Mazlan M, McLean C, Gaillard RC (1990): Neuroendocrinology. 51(1):85-92.

Dantzer R, Kelley KW. (2007): Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun. 21:153–160.

De Smet PA (2002:) Herbal remedies. N Engl J Med 347: 2046-2056.

Dinarello CA (2011). "Interleukin-1 in the pathogenesis and treatment of inflammatory diseases". Blood. 117 (14): 3720–32.

Diniz Y S, Fernandes A A, Campo K E, Mani F Ribas B O, Novelli E L (2004): Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. Food Chem. Toxicol. 42(2):319-325.

Duffey K J, & Popkin B M. High-fructose corn syrup (2008): is this what's for dinner The American Journal of Clinical Nutrition, 88(6), 1722S-1732S, December.

El Kossori RL, Villaume C, El Boustani E, Sauvaire Y, Me jeanL (1998): Composition of pulp, skin and seeds of prickly pears fruit (Opuntia ficus indica sp.). Plant Foods Hum Nutr 52:263–270.

El-Beltagy ABM, (2016): Ameliorative effects of melatonin on the liver of pregnant albino rats and their offspring induced by monosodium glutamate.Res. Opin. Anim. Vet. Sci., 6(2): 59-68.

Ennouri M, Fetoui H, Bourret E, Zeghal N, Attia H (2006): Evaluation of some biological parameters of Opuntia ficusindica. 1. Influence of a seed oil supplemented diet on rats.Bioresour Technol 97:1382–1386.

Erdmann NB, Whitney NP, Zheng J. (2006): Potentiation of excitotoxicity in HIV-1 associated dementia and the significance of glutaminase. Clin Neurosci Res. 6:315–328.

Eweka A O and Om`'Iniabohs F A E (2007): Histological Studies of the Effects of Monosodium Glutamate on the Cerebellum of Adult Wistar Rats. The Internet Journal of Neurology. 8: (2).

Falalieieva T M, Kukhars'ky ı V M, Berehova T V, Taylor, (2010) Effect of long-term Monosodium Glutamate is not associated with obesity or a greater prevalence Of weight gain over 5 years: findings from the Jiangsu Nutrition Study, Br. J. Nutr.104(3) 457–463.

Farombi EO, Onyema O O (2006): Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol25:251–259.

Fernández-López JA, Almela L, Obón JM, Castellar R (2010) Determination of antioxidant constituents in cactus pear fruits. Plant Foods Hum Nutr 65: 253-259.

Flier J, Kahn C, Jarrett D, Roth J(1976). Characterization of antibodies to the insulin receptor –a cause of insulin-resistant Diabetes in man. J of clin Inves, 58(6):1442-1449.

Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, Taviano MF (2003): Chemical characterization and biological effects of Sicilian Opuntia ficus indica (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. J Agric Food Chem 51:4903–4908.

Garcia-Solis P, Yahia EM, Morales-Tlalpan V, Diaz-Munoz M (2009): Screening of antiproliferative effect of aqueous extracts of plant foods consumed in Mexico on the breast cancer cell line MCF-7. Int J Food Sci Nutr 26:1–15.

Geha R S, Beiser A, Ren C, Patterson R, Paul A, Greenberger PA, Leslie C, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA Yarnold PR, Corren J, Saxon A (2000): Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo-controlled study. J Nutr 130:1058S–1062S

Goldsmith PC (2000): Neuroglia responses to elevated glutamate in the medial basal hypothalamus of the infant mouse. J Nutr 130:1032S–1038S

González-Burgos M I, Pérez-Vega, and Beas-Zárate, C. (2000): Neonatal exposure to monosodium glutamate induces cell death and dendritic hypotrophy in rat prefrontocortical pyramidal neurons Seguro Social, 7(70). 58261.

Hashem HE, El-Din Safwat MD, Algaidi S. (2012): The effect of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immune histochemical study). J Mol Histol. ;43(2):179-86. doi: 10.1007/s10735-011-9380-0.

Hassan M I (2016) Low Intake of Aspartame Induced Weight Gain and Damage of Brain & Liver Cells in Weanling Syrian Hamsters. Journal of Food and Nutrition Research, 2016, Vol. 4, No. 3, 152-156

Heaton H B, Madorsky I, Paiva M & Siler-Marsiglio K I. (2004): Vitamin E amelioration of ethanol neurotoxicity involves modulation of apoptosis-related protein levels in neonatal rat cerebellar granule cells. Brain Res. Dev. Brain Res., 150(2):117-24.

Hermanussen M, Garcia A P, Sunder M, Voigt M, Salazar V and Tresguerres J A. (2006). Obesity, voracity, and short stature: the impact of glutamate on the regulation of appetite. Eur J Clin Nutr. 60: 25-31.

Hofman FM, von Hanwehr RI, Dinarello CA, Mizel SB, Hinton D, Merrill JE.(1986): Immunoregulatory molecules and IL 2 receptors identified in multiple sclerosis brain. J Immunol. 1986 May 1;136(9):3239-45.

Humphries P, Pretorius E, Naude H. Direct and indirect cellular effects of aspartame on the brain. Eur J Clin Nutr 2008; 62:451–462.

Iwase M, Ichikawa K, Tashiro K, Iino K, Shinohara N, Ibayashi S, Yoshinari M, Fujishima M (2000). Effects of monosodium glutamate induced obesity in spontaneously hypertensive rat's vs Wistar Kyoto rats: serum leptin and blood flow to brown adipose tissue. Hypertens Res. 23: 503 -510.

Iyaswamy A, Sheeladevi R, Wankhar D (2014): Acute effect of aspartame induced oxidative stress in Wistar albino rat brain The Journal of Biomedical Research, 28(0):000-000.

Iyyaswamy A, Rathinasamy S, (2012). Effect of chronic exposure to aspartame on oxidative stress in brain discrete regions of albino rats. J. Biosci. 37, 1–10

Kamel E A. (2015). Inflammation and neurochemical alterations induced by oral aspartame administration in experimental rats. Indian. J.App.Res. 5(10):750-753.

Khan N S, Ahmad A, Hadi S M. (2000): Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. Chem. Biol. Interact. 125, 177–189.

Kuti JO (2004). Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food Chem 85:527–533.

Lau A, Tymianski M. (2010): Glutamate Receptors, Neurotoxicity and Neurodegeneration. European Journal of Physiology 2010**,** 460(2), 525–542.

Lee JA, Jung BG, Lee BJ (2012) Inhibitory effects of Opuntia humifusa on 7, 12-dimethyl-benz[a]anthracene and 12-O-tetradecanoylphorbol-13- acetate induced two-stage skin carcinogenesis. Asian Pac J Cancer Prev 13: 4655- 4660.

Li R, Guo M, Zhang G, Xu X, Li Q. (2006). Nicotiflorin reduces cerebral ischemic damage and upregulates endothelial nitric oxide synthase in primarily cultured rat cerebral blood vessel endothelial cells. J. Ethnopharmacol., 107, 143–150.

Liu HG, Liang QY, Meng HL, Huang HX (2010). Hypoglycemic effect of extracts of cactus pear fruit polysaccharide in rats. Zhong Yao Cai 33:240–242.

Loo BV, Bachschmid M, Spitzer V, Brey L, Ullrich V, Luscher TF (2003) Decreased plasma and tissue levels of vitamin C in a rat model of aging: implications for antioxidative defense. Biochem Biophys Res Commun 303:483–487.

MacKinnon B I, Frank M E, Hettinger T P, & Rehnberg B G (1999). Taste qualities of solutions preferred by hamsters. Chemical senses, 24(1), 23-35

Mattes, R. D., & Popkin, B. M. (2009). Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition, 89(1), 1-14.

Moore, K.L. (2003): Congenital malformation due to environmental factors. Developing humans. W.B. Saunders. Ltd.Philadelphia. 2:173- 183.

Mourad I M, Noor N A (2011). Aspartame (a widely used artificial sweetener) and oxidative stress in the rat cerebral cortex. Int. J.Pharm. Biomed. Sci. 2(1), 4–10.

Nakayama M, Aihara M, Chen Y-N, Araie M, Tomita-Yokotani K, Iwashina T 2011 Neuroprotective effects of flavonoids on hypoxia-, glutamate-, and oxidativestress–induced retinal ganglion cell death. Mol. Vis., 17, 1784.

Narayaman S N, Kumar R S, Paval J, Nayak S. (2010): Effect of ascorbic acid on the monosodium glutamate induced neurobehavioral changes in preadolescent rats. India. 111(5): 247-252.

Nosti-Palacios R, Gomez-Garduno J, Molina-Ortiz, D, Calzada-Leon, R Dorado-Gonzalez, Vences-MejiaV M A (2014). Aspartame Administration and Insulin Treatment Altered Bra Levels of CYP2E1 and CYP3A2 in Streptozotocin-Induced Diabetic Rats. Int. J. Toxicol. 33 (4), 325–331.

Nweze C C ,Mustapha A A ,and Olose, M (2015). Aspartame food additive and its biochemical implication: a review. Food Science and Quality Management, 36,16-22.

Omar S M M. (2009): Effect of aspartame on the frontal cortex of adult male albino rats. A light and electron microscopic study. Egyptian Journal of Histology, 32(2). 346 – 357.

Onaolapo A Y, Onaolapo O J, Nwoha PU (2017). Methyl aspartyl phenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. J. Chem. Neuroanat.86:67-77.

Onaolapo AY, Onaolapo O J, Mosaku T J, Onigbinde O A, Oyeleke A A, (2013) Histological study of the hepatic and renal effects of subchronic low Dose oral monosodium glutamate in Swiss albino mice, BJMMR 3 (2) 294–306.

Palmnas MS, Cowan TE, Bomhof MR, Su J, Reimer RA, Vogel HJ, et al. (2014): Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. PLoS One. 9: e109841.

Panico AM, Cardile V, Garufi F, PugliaC, Bonina F, Ronsisvalle, S (2007). Effect of hyaluronic acid and polysaccharides from Opuntia ficus indica (L.) cladodes on the metabolism of human chondrocyte cultures. J. Ethnopharmacol., 111, 315–321.

Park C. H., Choi S. H., Piaoa Y, Kim S., Lee Y, Kim J. S., Rah J, Seo J, Lee J, Chang K, Jung Y, Suh, (2000) Glutamate and aspartate impair memory retention and Damage hypothalamic neurons in adult mice, Toxicol. Lett. 115(2) 117–125.

Pavlovic V, Pavlovic D, Kocic G, SokolovicD, Stoimenov T J Cekic, S. and Velickovic D., (2011): Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus.

Molecular and Cellular Biochemistry Volume 303, Numbers 1-2, P. 161-166.

Pierucci D, Cicconi S, Bonini P, Ferrelli F, Pastore D, Matteucci C, Marselli L, Marchetti P, Ris F, Halban P, Oberholzer J, Federici M, Cozzolino F, Lauro R, Borboni P, Marlier LN (2001). "NGF-withdrawal induces apoptosis in pancreatic beta cells in vitro". Diabetologia. 44 (10): 1281–95.

Pinterova, L.; Zelezna, B.; Fickova, M.; Macho, L.; Krizanova, O.; Jezova, D.; Zorad, S (2001). Elevated AT1 Receptor Protein but Lower Angiotensin II-Binding in Adipose Tissue of Rats with Monosodium Glutamate-Induced Obesity. Hormone and Metabolic Research, 33(12), 708–712.

Prakash A, Jaspreet Kaur Kalra J K, Kumar A (2014). Neuroprotective effect of N-acetyl cysteine against streptozo- tocin-induced memory dysfunction and oxidative damage in rats.J. Basic Clin. Physiol. Pharmacol. 26 (1), 0792–6855.

Ramadan MF, Morsel J-T (2003) Oil cactus pear (Opuntia ficus- indica L.). Food Chem 82:339–345.

Salman N A, Abdel-Fattah N A, El-Kholy W B, El-Shereef N V.(2012). Histological and genetic studies on the effect of monosodium glutamate on the cerebellar cortex and the possible protective role of ascorbic acid in adult and neonate albino rat. Journal of American Science;8(5)

Shih AY, Erb H, Sun X, Toda S, Kalivas PW, Murphy TH (2006) Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation Neurosci 26:10514–10523.

Shimada ADDS, Baad-Hansen L, Castrillon E, Ghafouri B, Stensson N, Gerdle B, Ernberg M, Cairns B, Odont PS (2015). Differential effects of repetitive oral administration of monosodium glutamate on interstitial glutamate concentration and muscle pain sensitivity. Nutrition 31:315-323.

Simon P L, Laydon J T, Lee J C. A (1985) modified assay for interleukin-1 (IL-i). ImmunolMeth; 84: 85-94.

Su T, C Monte W, Hu X, He, Y, & He R (2016). Formaldehyde as a trigger for protein aggregation and potential target for mitigation of age-related, progressive cognitive impairment. Current topics in medicinal chemistry, 16(5), 472-484.

Sun-Edelstein C, Mauskop A. (2009). Foods and supplements in the management of migraine headaches. Clin. J. Pain 25 (5), 446–452

Taylor, S.L. (1993): Possible adverse reactions to hydrolyzed vegetable protein. Paper submitted to the Federation of American Societies for Experimental Biology review panel.

Tesoriere, L.; Attanzio, A.; Allegra, M.; Gentile, C.; Livrea, M.A. (2014) Indicaxanthin inhibits NADPH oxidase (NOX)-1 activation and NF-κB-dependent release of inflammatory mediators and prevents the increase of epithelial permeability in IL-1β-exposed Caco-2 cells. Br. J. Nutr., 111, 415–423.

Tordoff, M G, & Alleva A M,(1990) Oral stimulation with aspartame increases hunger. Physiology & behavior, 47(3), 555-559.

Trocho C, Pardo R, Rafecas I, Virgili J, Remesar X, Fernandez-Lopez JA, Alemany M (1998). Formaldehyde derived from dietary aspartame binds to tissue components in vivo. Life Sci; 63:337–349.

Waer H F, Edress E (2006). The Effect of Monosodium Glutamate (MSG) On Rat Liver And The Ameliorating Effect Of "Guanidino Ethane Sulfonic acid (GES)" (Histological, Histochemical and Electron Microscopy Studies). Egyp. J Hosp. Med., 24:524 – 538.

DeWit M, Nel P, Osthoff G, Labuschagne MT (2010) The effect of variety and location on cactus

pear (Opuntia ficus-indica) fruit quality. Plant Foods Hum Nutr 65:136–145**.**

Wolfram R, Budinsky A, Efthimiou Y, Stomatopoulos J, Og-uogho A, Sinzinger H (2003). Daily prickly pear consumption improves platelet function. Prostaglandins Leukot Essent Fatty Acids 69:61–66.

Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, Qian G, Zheng JC.(2013) IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. J Neurochem. 125(6):897-908.

Zai H, Kusano M, Hosaka H, Shimoyama Y, Nagoshi Mori KO (2009) Monosodium L-glutamate added to a high-energy, high-protein liquid diet promotes gastric emptying. Am J Clin Nutr 89:431–435.

Zhang Y, Babcock S A, Hu N, Mari J R, Wang H, Ren J, (2012) Mitochondrial dehydedehydrogenase (ALDH2) protects against streptozotocin-induced diabetic cardiomyopathy: role of GSK3 beta and mitochondrial function, BMC Med. 1040.