

Flaxseed Oil Attenuates Monosodium Glutamate-Induced Brain Injury *via* Improvement of Fatty Acids Contents

Eman R. Youness^{1*}, Jihan S. Hussein¹, Amr M M Ibrahim¹ and Fatma E. Agha²

¹Medical Biochemistry Department, Medical Research Division,
National Research Centre, Giza, Egypt.

²Department of Forensic Medicine and Clinical Toxicology,
Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

<http://dx.doi.org/10.13005/bpj/1671>

(Received: 12 February 2019; accepted: 03 May 2019)

Monosodium glutamate (MSG) is immensely globally used as a food aroma and additive, several studies indicated its toxicity in different body organs. Here, we aimed to evaluate brain dysfunctions in experimental animal that administered MSG and appreciate the beneficial role of flaxseed oil in attenuating this effect. In this study, forty male Wistar albino rats were divided into four groups; control, flaxseed oil, MSG and treated groups. Kidney and liver functions were estimated, malondialdehyde (MDA) and paraoxonase (PON1) were measured by colorimetric methods. Blood fatty acids and neurotransmitters parameters were estimated by HPLC. Our results revealed that MSG administration significantly increased oxidative stress and omega-6 fatty acids and decreased brain neurotransmitters as well as omega-3 fatty acids (ω -3 FA). Whereas treatment with flaxseed oil significantly attenuated all these disadvantages. The results of this study indicated that MSG was responsible for brain dysfunction that appeared in disturbances of neurotransmitters levels. In addition, the administration of omega-3 fatty acids in treated group effectively attenuated this dysfunctions through replacing omega-6 fatty acids in the neurocells by omega-3 fatty acids that represent in our study by flaxseed oil.

Keywords: monosodium glutamate, fatty acids, neurotransmitters, HPLC, brain dysfunction.

Glutamate is the chief excitatory neurotransmitter in the mammalian central nervous system (CNS)^{1, 2}. Among plenteous formulae of glutamate, monosodium glutamate (MSG) is immensely used globally as food additive and flavor³.

It was reported that exposure to MSG (4 mg/g body weight) in rodents has many side effects as obesity⁴, learning difficulty⁵, and brain damage⁶. Several studies indicated the reduction of neurotransmitters like norepinephrine, dopamine,

serotonin, and their metabolites after administration of MSG⁷, which is correlated with oxidative stress in the hepatic tissue of experimental rats⁸ in addition to the elevation of serum liver enzymes like alanine aminotransferase (ALT) and aspartic aminotransferase (AST)⁹. Contrarily, others indicated that MSG taken in food displayed no adverse effects¹⁰.

Antioxidant units that derived from food have a robust possibility for long-range used as chemo-preventive factors in several diseases

encompass oxidative stress¹¹. It was reported that, dietary omega-3 fatty acids affect arachidonic acid (AA) metabolism efficiently, thus they dislodge AA from cell membranes and compete with the enzymes catalyzing the biosynthesis of prostaglandins, leukotrienes and thromboxanes. So, supplementation of omega-3 fatty acids (such as flaxseed oil) minimized the potential for cells as eosinophils, monocytes, and neutrophils to synthesize these powerful inflammatory mediators (arachidonic acid-derived mediators). Also, these ω -3 FA diminished the production of the prothrombotic agent thromboxane A₂ by platelets¹².

Previous study indicated that flaxseed oil, the rich source of ω -3 FA comprised a free radical scavenging property; this property exerts a salutary outcome against pathological alterations resulting from the presence of O₂⁻ and OH⁻. Increasing the superoxide dismutase (SOD) activity accelerated dismutation of O₂⁻ to hydrogen peroxide that is removed by catalase. This action might encompass contrivances related to scavenging activity of using treatment¹³.

From this point of view, we hypothetically designed this study to investigate the toxic effects of MSG and to reveal their correlations with biochemical parameters including neurotransmitters and oxidative stress indices that are responsible for cognitive activities and to find out the ability of flaxseed oil supplementation to attenuate all disadvantages that were induced.

MATERIALS AND METHODS

Chemicals

- α -linolenic acid (ALA), AA, linoleic acid (LA), oleic acid (OA), docosahexaenoic acid (DHA) standards (HPLC grade) and neurotransmitters including dopamine (DO), norepinephrine (NOR) and serotonin (5HT) standards were purchased from Sigma Chemicals Co. (Munih, Germany).
- MSG (99% pure) was purchased from the local market, Cairo, Egypt.
- Flaxseed oil was purchased from National Research Centre (NRC), Giza, Egypt.
- All other using chemicals such as acetonitrile, ethanol, and methanol were HPLC grade.

Experimental animals

Forty male Wistar albino rats weighting 180 ± 10 g were obtained from the animal house of NRC, Giza, Egypt. Animals were housed in stainless steel cages at the temperature range of 22±2 °C, under light dark cycle (12/12 hours), and allowed to acclimatize for a period of one week before the experiment; the guidelines of the ethical care and treatment of the animals were followed the regulations of the ethical committee of NRC and the guidelines of National Institutes of Health for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Experimental Design

Animals were divided into four groups, each group contained ten rats, as follow: Group 1: Healthy rats served as control group and received a vehicle (distilled water), group 2: Healthy rats received flaxseed oil by oral gavage in a dose of 1.2 ml/kg body weight, once daily for 6 weeks¹⁴, group3: Healthy rats received MSG in a dose of 0.6 mg/kg. body weight, dissolved in distilled water by oral gavage once daily for 6 weeks¹⁵, group 4: Healthy rats received MSG in a dose of 0.6 mg/kg. body weight, dissolved in distilled water by oral gavage once daily along with flaxseed oil by oral gavage in a dose of 1.2 ml/kg body weight, once daily for 6 weeks.

At the end of the experiment, rats were sacrificed after being anaesthetized with diethyl ether by inhalation; blood samples were collected from the optical vein in a clean dry test tube and let for clotting for 10 min. in 37° C to separate sera.

Biochemical estimations

Serum liver enzymes alanine amino-transferase (ALT) and aspartate amino-transferase (AST) were measured according to the method of Reitman and Frankel¹⁶; blood urea and serum creatinine were estimated according to Fawcett & Scott¹⁷ and Houot¹⁸ respectively.

Lipid peroxidation

The content of lipid peroxidation products in the brain homogenates was assayed by measuring the level of malondialdehyde using the method of Esterbauer and Cheese man¹⁹ where the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having a peak absorbance at 532 nm (UV-VI8 Recording Spectrophotometer, Shimadzu Corporation, Australia).

Paraoxonase 1 activity

The arylesterase activity of paraoxonase (PON1) was measured spectrophotometrically using phenylacetate as a substrate. In this assay, arylesterase/paraoxonase catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The

rate of this formation is measured by monitoring the increase in absorbance at wave length 270 nm at 25 °C. The working reagent consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM CaCl₂ and 4 mM phenyl acetate, as a substrate. Samples diluted 1:3 in buffer were added and the change

Table 1. Liver and kidney functions in different studied groups:

Groups	Control	Flaxseed oil	MSG	Treated
ALT(U/L)	16± 1.2	17 ± 1.5 ^b	24 ± 1.5 ^a	20± 1.4 ^{ab}
AST(U/L)	69± 1.9	72± 1.77 ^b	117± 1.83 ^a	97± 2.01 ^{ab}
Urea(mg/dl)	15± 0.9	17± 0.86 ^b	30± 1.01 ^a	22± 1.2 ^{ab}
Creatinine(mg/ dl)	0.34± 0.2	0.46± 0.11 ^b	0.7± 0.23 ^a	0.6± 0.12 ^a

Significant p value < 0.05

a: significant difference compared to control group

b: significant difference compared to MSG group

Table 2. Oxidative stress parameters in different studied groups

Groups	Control	Flaxseed oil	MSG	Treated
MDA (nmol/ml)	16 ± 0.18	17± 0.20 ^b	44± 0.11 ^a	26± 0.17 ^{ab}
Paraoxonase (IU/ml)	69± 0.20	72± 0.32 ^b	47± 0.31 ^a	69± 0.25 ^{ab}

Significant p value < 0.05

a: significant difference compared to control group

b: significant difference compared to MSG group

Table 3. Blood neurotransmitter in different studied groups

Groups	Control	Flaxseed oil	MSG	Treated
Norepinephrine(pmol/mL)	5.88 ± 1.1	5.91 ± 1.2 ^b	3.71 ± 0.53 ^a	5.22 ± 0.90 ^b
Dopamine(pmol/mL)	2.43 ± 0.71	2.41 ± 0.65	1.54 ± 0.53	2.11 ± 0.41
Serotonin(pmol/mL)	37.9 ± 0.81	40.2 ± 1.1 ^{ab}	20.5 ± 0.76 ^a	31.8± 0.84 ^{ab}

Significant p value < 0.05

a: significant difference compared to control group

b: significant difference compared to MSG group

Table 4. Blood fatty acids in different studied groups

Groups	Control	Flaxseed oil	MSG	Treated
(DHA;22:6x3) µg/ml	0.70± 0.12	0.76± 0.23	0.47± 0.2 ^a	0.49± 0.11 ^a
(ALA; 18:3x3) µg/ml	2.74± 1.9	4.4± 1.77 ^{ab}	1.17± 1.52 ^a	2.6 ± 1.95 ^b
(AA; 20:4x6) µg/ml	8.9 ± 0.58	7.6 ± 0.86 ^b	19.74± 2.31 ^a	14.4± 1.1 ^{ab}
(LA; 18:2x6) µg/ml	11.43 ± 0.9	10.98± 1.1 ^b	15.76± 1.2 ^a	12.61± 0.77 ^b
(OA;18:1 X 9) µg/ml	46.8± 0.23	45.0± 0.36 ^b	69.8± 0.91 ^a	56.8± 0.84 ^{ab}

Significant p value < 0.05

a: significant difference compared to control group

b: significant difference compared to MSG group

in absorbance was recorded following a 20-s lag time. Absorbance at 270 nm was taken every 15 s for 120 s using UV spectrophotometer²⁰.

Determination of neurotransmitters in blood by HPLC

Determination of blood norepinephrine, dopamine and serotonin was carried out using high performance liquid chromatography (HPLC) system Agilent technologies 1100 series, with a quaternary pump (G131A model) according to the method described previously^{21,22}. Briefly, serum sample (500 μ l) was homogenized in phosphate buffer (pH 7.4), centrifuged at 4000 rpm using cooling centrifuge (Laborzentrifugen, 2K15, Sigma, Germany) for 15 minutes at 4°C and the supernatant was removed and injected onto HPLC; separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm x 5 μ m). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1.5 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 μ l. The concentrations of both catecholamines and serotonin were determined by external standard method using peak areas. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations of each standard. The concentrations in samples were obtained from the standard curve.

Blood fatty acids estimation by HPLC

Fatty acids estimation was carried out according to the method described previously¹². Briefly, the sample (400 μ l serum) was homogenized in 2 % acetic acid: ethyl ether mixture (2:1 volume ratio) respectively; the solution was then centrifuged at 500 xg, the organic phase was evaporated to dryness. The extract was dissolved in 400 μ l acetonitrile.

HPLC condition

HPLC column C 18 (260 X 4.6, particle size 5 μ m), mobile phase was acetonitrile / water mixture (70/30) v/v by isocratic elution with flow rate 1 ml / min and 200 nm wave length. Serial dilutions of standards were injected onto HPLC and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from

the standard curve using Agilent Chem Station software for LC&LC/MC system (Agilent Technologies [2001-2010]).

Statistical analysis

Results were expressed as mean \pm standard error. Data were analyzed by independent sample t test (SPSS) version 15 followed by (LSD) test to compare significance between groups. Difference was considered significant when P value <0.05.

RESULTS

In this study MSG increased liver and kidney functions but this elevation is within limit except for AST which is significantly increased in MSG group compared to control group; whereas, treatment with flaxseed oil attenuates this elevation (table 1).

This study revealed that administration of MSG reduced the activity of paraoxonase (PON1), also, increased MDA (table 2).

In addition, neurotransmitters levels in this study are significantly decreased in rats administered MSG compared to control group, whereas:

Flaxseed oil supplementation significantly decreased AA and LA in treated group compared to MSG group but increased ALA significantly compared to MSG group (table 3).

Consequently, Flaxseed oil in the current study significantly increased neurotransmitters levels in the treated group compared to MSG group (table 4).

DISCUSSION

MSG is a food additive that frequently used in fast food. Consumption of fast food has been proposed as an essential factor behind many diseases including metabolic syndrome and brain dysfunction²³.

MSG induced oxidative stress (as was observed in this study) suggesting that MSG induces liberation of free radicals through a central mechanism which depends on the blood brain barrier (BBB). Oxidative stress is characterized by elevation of reactive oxygen species (ROS) levels²⁴.

PON1 is an important antioxidant enzyme that considered one of the detoxification enzymes. Thus, it is the most powerful antioxidant in the cell.

This may indicate the onset of oxidative stress that possibly develops with successive administrations of MSG. In addition, MDA levels were rated as a marker of oxidative damage. Interestingly, MSG caused oxidative stress in the brain of MSG administered rats which could be indicated by the interruption of neurotransmitters levels in this study (table 3).

The reduction of neurotransmitters levels after administration of MSG in this study is in agreement with the study of Abu-Taweel *et al.*²⁵, who indicated that MSG is from toxic food additives that stimulate the body weight and various cognitive behavioral activities of the animals. Also, it affects the neurotransmitter levels and oxidative stress levels in brain tissues.

Dopamine (DA) is one of the richest catecholamines in the brain, particularly in the parts that responsible for movement, stimulus and learning, as in the striatum²⁶. Contrarily, other neurotransmitters such as serotonin are involved in cognitive activities²⁷. Thus, disturbances in the levels of neurotransmitters and the oxidative stress because of administration of MSG might be one of the possible reasons for the brain dysfunctions in MSG treated animals in the present study.

In contrast, the current data appeared that, the treatment with the plant source of omega-3 fatty acids, flaxseed oil, is effectively corrected all biochemical changes to become more or less near the control group.

Administration of flaxseed oil is significantly increased plasma omega-3 fatty acids (ALA) and significantly decreased omega-6 (AA, LA) in treated group compared to MSG group (table 4) ; whereas the mean value of DHA (omega-3) was insignificantly decreased in treated group compared to MSG group indicating the disability of the body to convert ALA to DHA which is a state of several diseases as mentioned before by Hussein (2013) who indicated that hypertensive individuals, some diabetics and patients of several diseases are restricted in their ability to create DHA and eicosapentaenoic acid (EPA) from ALA¹⁴.

Membrane that is composed of saturated fatty acids has different structures and has less fluidity than that includes unsaturated fatty acids. The type of dietary fatty acids has an important role in affecting the neuronal membrane fluidity index. Thus, omega-3 fatty acids have the ability

to decrease the cholesterol level in the neuronal membrane, which would decrease cell membrane fluidity and increase the cell's vulnerability to be injured²⁸.

Brain functions may be affected by fatty acids through several steps including modifications of membrane activity, membrane fluidity, membrane bound enzymes, the number and affinity of receptors, function of ion channels, activity of neurotransmitters & the production and signal transduction which control the neurotransmitters activity and neuronal growth factors¹⁴.

CONCLUSION

From the current data we can conclude that, the food additive MSG is responsible for toxic effect as well as brain dysfunction that appeared in disturbances of neurotransmitters levels. In addition, the administration of omega-3 fatty acids in treated group effectively attenuate these dysfunctions through replacing omega-6 fatty acids in the neuronal cells by omega-3 fatty acids that represent in our study by flaxseed oil.

ACKNOWLEDGEMENTS

Authors are grateful to the National Research Centre, Giza, Egypt for unlimited help and support to carry out this work.

REFERENCES

1. Zhou Y, Danbolt, N.C. Glutamate as a neurotransmitter in the healthy brain. *J. Neural Transm.*, **121**, 799–817 (2014).
2. Lau A., Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.-Eur. J. Physiol.* **460**, 525–542 (2010).
3. Loliger J. Function and importance of glutamate for savory foods. *J. Nutr.* **130**, 915S–920S (2000).
4. Nagasawa H, Yanai R, Kikuyama S. Irreversible inhibition of pituitary prolactin and growth hormone secretion and of mammary gland development in mice by monosodium glutamate administered neonatally. *Acta Endocrinol.* **75**, 249–59 (1974).
5. Olvera-Cortes E, Lopez-Vazquez MA, Beas-Zarate C, Gonzalez-Burgos I. Neonatal exposure to monosodium glutamate disrupts place learning ability in adult rats. *Pharmacol Biochem Behav.*

- 82**, 247–51 (2005).
6. Robinzon B, Snapir N, Perek M. 1974. Age dependent sensitivity to monosodium glutamate inducing brain damage in the chicken. *Poult Sci* **54**, 1539–42.
 7. Nakagawa T, Ukai K, Ohyama T, Gomita Y, Okamura H. Effects of chronic administration of sibutramine on body weight, food intake and motor activity in neonatally monosodium glutamate-treated obese female rats: relationship of antiobesity effect with monoamines. *Exp Anim.* **49**: 239–49 (2000).
 8. Diniz YS, Fernandes AAH, Campos KE, Mani F, Ribas BO, Novelli ELB. Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. *Food Chem Toxicol.* **42**: 313–9 (2004).
 9. Ortiz GG, Bitzer-Quintero OK, Zarate CB. Monosodium glutamate induced damage in liver and kidney; a morphological and biochemical approach. *Biomed Pharmacother.* **50**: 86–91 (2006).
 10. Stegink LD, Filer Jr LJ, Baker GL. Plasma glutamate concentrations in adult subjects ingesting monosodium L-glutamate in consomme. *Am. J Clin Nutr.* **42**: 220–5 (1985).
 11. El-Khayat Z, Rasheed W, Ramzy T, Hussein J, Agaiby M, Morsy S, Morsy F and Shaffie N. Protective effect of garlic oil against liver injury in experimental animals, *Journal of Medicinal Plants Research.* **4**(22): 2359-2369 (2010).
 12. Hussein J S, El-Khayat Z, Morsy S, Oraby Fatma, Snger G. The effect of fish oil on oxidant/ antioxidant status in diabetic rats through the reduction of arachidonic acid in the cell membrane. *Int J Pharm Pharm Sci,* **6** (2): 196-199 (2014).
 13. Hussein J, Abo Elmatty D, Medhat D, Mesbah N, Farrag AR and Fahmy H. Flaxseed oil attenuates experimental liver hepatitis. *Der Pharmacia Lettre,* **8**(8):142-150 (2016).
 14. Hussein J, El-Khayat Z, Taha M., Morsy S., Drees E. and Khateeb S. Dietary omega-3 fatty acids prevent erythrocyte membrane atpase reduction in streptozotocin induced diabetic rats. *Int J Pharm PharmSci,* **5**(4): 211-216 (2013).
 15. Tawfik M.S., Al-Badr N. Adverse effects of monosodium glutamate on liver and kidney functions in adult rats and potential protective effect of vitamins C and E. *Food and Nutrition Sciences.* **3**: 651-659 (2012).
 16. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**(1): 56-63 (1957).
 17. Fawcett JK and Scott J E. Determination of urea. *Journal of Clinical Pathology.* **13**: 156 (1960).
 18. Houot O. Kinetic Determination of Creatinine. In: Interpretation of Clinical Laboratory Tests, Henny, J., G. Siest, F. Schiele and D.S. Young (Eds.). Biomedical Publications, California, USA., 220-234 (1985).
 19. Esterbauer H, Cheeseman K H.. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4- hydroxynonenal. *Methods in Enzymology.* **186**: 407– 421 (1990).
 20. Hussein J, El-Bana M, Refaat E, El-Naggar M E. Synthesis of carvacrol-based nanoemulsion for treating neurodegenerative disorders in experimental diabetes. *Journal of Functional Foods.* **37**: 441–448 (2017).
 21. Abdel-Salam OM.E, Salem N A, El-Shamarka M E S, Ahmed N A, Hussein J S, El-Khyat ZA.. cannabis-induced impairment of learning and memory: effect of different nootropic drugs. *EXCLI Journal.* **12**: 193-214 (2013).
 22. Badawy E A, Rasheed W I, Elias T R, Hussein J, Agaiby M H, Youness E R, El Nemr M. and El-Shamy K A. Exploration of the relationship between adipocytokines, antioxidant vitamins and obese diabetic patients. *Journal of Chemical and Pharmaceutical Research.* **7**(12): 406-411 (2015).
 23. Isganaitis E., & Lustig, R. H.. Fast food, central nervous system insulin resistance, and obesity. *Arterioscler Thromb Vasc Biol.* **25**(12): 2451-2462 (2005).
 24. Xu LC, Sun H, Wang SY, Song L, Chang HC, Wang XR. The roles of metallothionein on cadmium-induced testes damages in Sprague–Dawley rats. *Environ Toxicol Pharmacol.* **20**: 83–7(2005).
 25. Abu-Taweel GM., Zyadah M. A., Jamaan S. A, Ahmad M. Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. *Neurotoxicology and Teratology.* **42**: 60–67b (2014).
 26. Marinho CR, Manso CF. Effect of aluminum on the non-enzymatic oxidation of dopamine. *Acta Med Port.* **7**: 611–5 (1994).
 27. Tariq M, Ahmad M, Al Moutaery K, Al Deeb S . Pentoxifylline ameliorates lithium-pilocarpine induced status epilepticus in young rats. *Epilepsy Behav.* **12**: 354–65 (2008).
 28. Yehuda S, Rabinovitz S, Carasso R L, Mostofsky D I. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. *Neurobiology of Aging.* **23**: 843–853 (2002).