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Antagonistic Effects of Chinese Salt and Folic Acid on Developing Swiss Albino Mice

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ABSTRACT

One of the most often utilized taste enhancer in commercial meals is monosodium glutamate (MSG) commonly called Chinese salt. MSG utilization has been increasing over time and linked with toxicity in liver and other organs. **Objective:** To determine the teratogenic and toxic effects of Chinese salt and folic acid on developing mice. **Methods:** In this study, 20 female pregnant albino mice were divided into four groups, each consisted 5 mice. The control group was supplied with water. To check the teratogenicity and toxicity of Chinese salt and folic acid the treated groups such as Group-I, Group-II and Group-III were supplied with Chinese salt and/or folic acid of concentration 7.50 ug/g of their body weight. The dose was administered orally on daily basis during 6th to 12th day of gestation. This was accomplished through an 18 days trial. On the eighteenth day of gestation, the pregnant mice underwent dissection and the fetuses were retrieved. Fetuses were taken from all groups for histopathological findings and morphometry. **Results:** Vast range of morphological, morphometric and histological abnormalities in mice were observed in pregnant mice and fetuses. **Conclusions:** The findings of this study clearly revealed that Chinese salt and folic acid overdose are potentially toxic to liver and stomach.

INTRODUCTION

Monosodium glutamate (MSG) also known as Chinese salt in everyday usage and commonly used as flavor enhancers are natural component of foods that are rich in proteins such as meat, cheese and vegetables, which is also used worldwide for enhancing food palatability. L-Glutamic acid is an amino acid, which is a component of MSG. [1, 2]. The typical daily consumption of MSG in developing nations is believed to be between 0.3 and 1.0 g, but this might vary depending on the food items a person prefers and how much they eat [3]. It is an essential component in the body's protein constituents and metabolic intermediates [4]. High glutamate activation causes neurological problems in fetuses and long-term depression in rats [5]. MSG excitotoxins cause affects that result in overstimulation of nerve cell till damage point and cause death of these neurons [6]. Brain damage, epilepsy, oligozoospermia, degeneration of retina, development of hepatic inflammation are all neurotoxic effects caused by MSG [7, 8]. Monosodium glutamate enhances the production of free radicals, proteases, phospholipases and transcriptional endonucleases resulting in genotoxicity and apoptosis in mice and rat [9, 10]. In case of neonatal mice and rats, treated with MSG it causes damage to the arcuate hypothalamic nucleus, which effect neuro-endocrine function and induces intolerance to glucose, causes obesity, resistance to insulin, accumulation of fat, dyslipidemia, diminished responsiveness of vascular systems and reduced growth hormone secretion leading to

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stunted growth and obesity [11, 12]. In an interesting research, cross fostering reduced many of these side effects in rats whose mothers where fed on MSG, which caused them to become obese [13]. According to [14] folic acid controls cancer progression in new developing tumors as well as in old tumors. Deficiency of folic acid resulted in intestinal tumors in mice due to altered expression of G2-M phase regulators [15]. Deficiency of folic acid causes DNA repair instability, irregular gene expression and proliferation of cell and also supports tumor development [16]. In women, folic acid protected against breast cancer with a daily intake of>456ug/d as compare to 160ug/d. Hypomethylation of the genomic DNA is caused by folic acid deficiency [17, 18]. Neural tube defects are caused due to deficiency of folic acid [19]. 10% of the US population faces folate deficiency, which causes chromosomal damage and breaking of DNA strands leading to multiple birth defects in their children. Relationship between folic acid and methylation is so strong that it has a basic role in fetus development, and in synthesis of phospholipids, myelin sheath, basic proteins and neurotransmitters establishing a proper mechanism for embryogenesis [20]. In a study, it was found that folic acid supplementation during pregnancy in substantial concentrations, resulted in a 90% decrease in NTD (neural tube defects) and other congenital abnormalities [21]. During pregnancy folate deficiency causes low birth weight of infants, retardation of fetus growth, blood homocysteine level increase, placental abruption and pre-eclampsia. Folate is necessary for male fertility, contributing to spermatogenesis [22]. Pregnant women who take food rich in folic acid tend to have reduced serious birth defects but folic acid intake in more than normal potency may cause serious complications in fetuses [23].

The current investigation attempts to investigate the antagonistic effects of folic acid and Chinese salt on Swiss albino mice which may be inducing hepato and gastric toxicity.

METHODS

All animal trial investigations have been carried out using international and regional protocols. These were carried out under the Wet op de dierproeven (article 9) of Dutch law on animal testing. The NIH document "Guide for the care and Use of Experimental Animals" was used for animal testing and rearing [24]. Housing: A group of 10 albino mice (10 females and 2 males) were obtained from Veterinary Research Institute, Lahore. These animals were kept in a controlled condition in an animal room at 25±1°C temperature featuring steel racks and cages with a 12-hourly light/dark cycle and a relative humidity of between forty and fifty percent. Two females were kept with one male in five different cages. Each cage had wood shavings as bedding material, which was replaced daily. During this research, Folic acid and Chinese salt (Monosodium Glutamate) were tested for their toxic effects and teratogenicity. Different dose groups were managed as follows and elaborated in figure 1.

C (Control): Having 0.1 ml Nestle's water

G (Experimental Groups); G-I: 0.1 ml Nestle's water mixed with, Folic acid 7.50µg/g body weight (B.W)

G-II: 0.1 ml Nestle's water with Chinese salt 7.50µg/g B.W

G-III: 0.1 ml Nestle's water with Folic acid 3.75µg/g B.W + Chinese salt 3.75µg/g B.W

The pregnant mice were weighed on the eighteenth day of pregnancy and given ether for anesthesia. Following a cesarean section, the uterus's two horns had been taken from the body and placed under weight. After the fetuses were counted and removed from the uterus, they were fixed for 48 hours in Bouin's fixative. Fetuses were stored in 70% alcohol after 48 hours. The tissues underwent a series of procedures including a 0.9 percent saline solution wash, 10% formalin solution fixation, graded ethanol dehydration for clarifying, xylene treatment, and paraffin wax embedding. Sections of the liver and kidney with a thickness of four micrometres were created using a microtome, and they were then stained with eosin and haematoxylin in accordance with the recommended methodology [25]. Following full drying, the produced slides were examined under a microscope at 10X and 40X for further histological investigation. Microphotography was then carried out. Both Control and treated fetuses after dosage administration were observed for morphological and anatomical studies. Morphological and morphometric studies involved wet weight, crown rump (CR), length of each fetus as well as circumference of head and eye, which were calculated using the computer-based program "Ellipse Circumference Calculator" which was utilized by downloading from the CSG network website [26]. The entire set of data underwent mathematical computations and was examined using the computer application SPSS. The One-Way ANOVA Duncan test was employed to analyze the data.

RESULTS

The morphometric analysis of the fetuses yielded interesting results. The number of fetuses recovered after dosage administration is depicted here. In control group, the average body weight of fetuses was 1470 ± 35.32mg, eye circumference was 6.01 ± 1.09 mm, the average forelimb size was 6.24 ± 0.48 mm respectively. While in G-I group treated with the 7.50 µg/g B.W folic acid, the average body weight was 963.54 ± 50.01mg and head circumference was23.37 ± 1.23 mm, average eye circumference was 4.27 ±

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0.31 mm, average forelimb and hind limb size was 5.45 ± 51 mm and 5.99 ± 0.30 mm. In G-II, Chinese salt and folic acid, the average body weight was 777.74 ± 75.56 mg, head circumference was 14.87 ± 1.07 mm, average eye circumference was 3.81 ± 0.38 mm, average forelimb and hind limb size was 98 ± 0.31 mm and 4.17 ± 0.40 mm, which were significantly reduced in comparison to the control group. In G-III, (Chinese salt) the average body weight was 480.42 ± 98 mg, head circumference was 13.00 ± 1.03 mm, average eye circumference was 2.84 ± 0.45 mm, average forelimb and hind limb size was 3.08 ± 0.41 mm and 3.96 ± 0.34 mm, which were significantly reduced as compared to control group (Table 1).

Table 1: Developmental abnormalities in fetuses of 18th-day-old mice that had been obtained from pregnant mice by the administration oral doses of folic acid and Chinese salt between days 6th and 12th of gestation

Dose GroupsΔ	Number of Implantations (N)	Body Weight (mg ± S.E.)	Head Circumference (mm^2 ± S.E.)	Eye Circumference (mm^2 ± S.E.)	Forelimb Size (mm ± S.E.)	Hindlimb Size (mm ± S.E.)
Control	42	1470 ± 35.33***	27.62 ± 1.09	6.01 ± 0.81	6.24 ± 0.48	7.59 ± 0.32
Folic acid	40	963.54 ± 50.01***	*23.37 ± 1.23	*4.27 ± 0.31	5.45 ± 0.51***	**5.99 ± 0.30
Chinese salt and folic acid	36	777.74 ± 75.56***	***14.87± 1.07	*3.81 ± 0.38	3.98 ± 0.31***	***4.17 ± 0.41
Chinese salt	30	480.42 ± 98.00	***13.00 ± 1.03	***2.84 ± 0.45	3.08 ± 0.41	***3.96 ± 0.34

*The asterisks indicating a significant difference from the controls; ***= p<0.001, **= p <0.01 and *= p <0.05*

4.3 Histological studies

Histological examination of cranio-visceral fetal organs including spinal cord, heart, lungs and liver was carried out. This study was done in order to understand Chinese salt and folic acid related histopathological changes. Transverse section through cranial region revealed no major derangements upon histological examination of fetuses in control group (Figure 1A). Group-I treated with folic acid revealed improper formation of spinal cord, pharynx and tongue (Figure 1B). Group-II treated with Chinese salt and folic acid showed damaged spinal cord, pharynx and tongue (Figure 1C). While Group-III treated with Chinese salt showed poorly formed spinal cord as well as no pharynx was observed (Figure 1D).

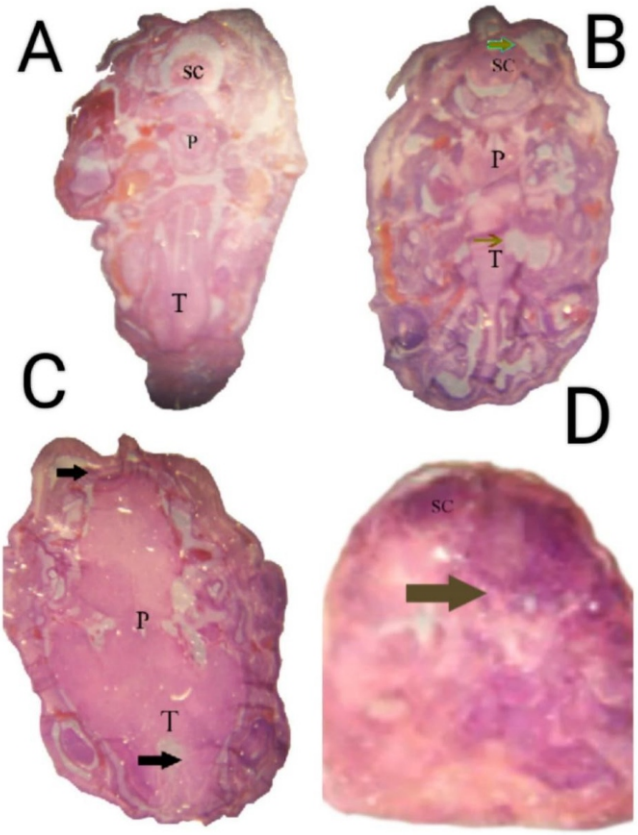

Figure 1: Transverse section through cranial region of (A): Fetus from control group (B): Fetus from treated group with folic acid; (C): Fetus from treated group with Chinese salt and folic acid; (D): Fetus from treated group: spinal cord (SC), pharynx (P), tongue (T)

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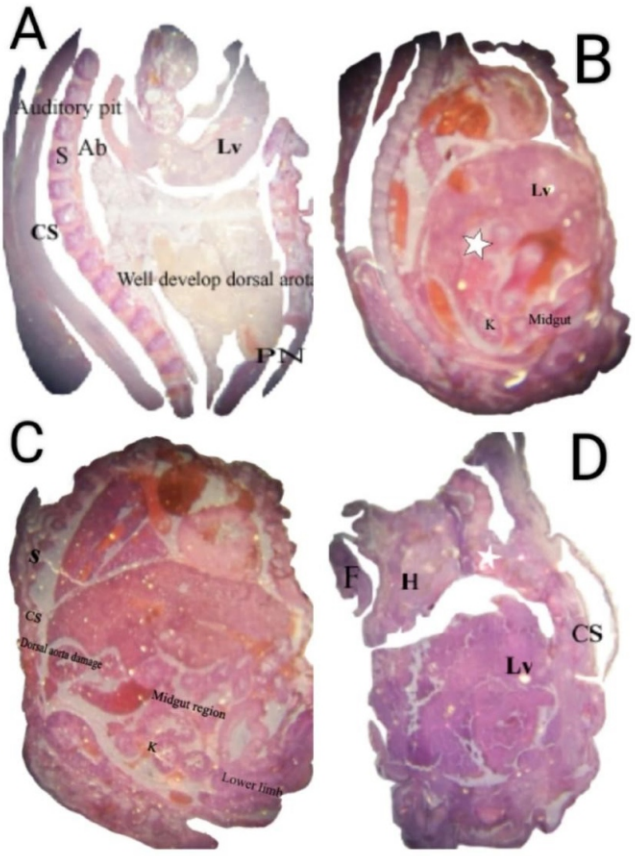

Figure 2: Longitudinal sections of trunk region (A): Fetus from control group, (B): Fetus from Group-I treated with folic acid (C): Fetus from group treated with Chinese salt and folic acid cervical spine (CS), (D): heart (H), liver (LV) and cervical spine (CS):and tongue (T), somite (S). Histopathological sections of fetus having Longitudinal sections of trunk region in Control Group showed well developed aortic bulb, liver and cervical spines and neuropore (Figure 2A). In Group-I (Figure 2B) treated with folic acid showed abnormal liver, kidney and mid gut (white star showing necrosis in lower region of trunk). In Group-II treated with Chinese salt and folic acid showed formation of formed abnormal somite of cervical spine, damaged dorsal aorta and necrosis apparent in lower limb region (Figure 2C). In Group-III treated with Chinese salt showed abnormal formation of heart liver and cervical spine (white star) showing necrosis in medullary regions of kidneys (Figure 2D).

DISCUSSION

The purpose of this study was to investigate the opposing effects of folic acid and Chinese salt on the growth of mice. Oral dosages were administered to pregnant mice on days 6–12 of gestation every day for a period of 18 days. Fetuses were recovered, fixed and analysed on morphological, morphometric and histological bases. The results obtained in this study agree with available data, and show a decrease in the body weights of new born mice affected by MSG. In the referenced study, they also performed the same experiment but did not test the antagonistic effect of folic acid [27]. In a similar experiment by It was seen that the offspring born from MSG treated female mice were quite weak, often did not pull through the pregnancy or had lower body weights as compared to the control groups [28]. Interestingly the body weights of the mothers had gained significantly after prolonged intake of MSG indicating an increasing effect of MSG on normal body weight. The proposed explanation is that the MSG adversely effects the hunger controlling parts of the brain and may lead the rats towards obesity. Our studies also show different morphological abnormalities in fetuses such as Morphometric studies of fetuses like Control Group fetuses remained healthy while Treated Groups such as Group-I, showed over use of folic acid revealing distorted axis, mild reduction in head circumference, eye circumference, forelimb, hindlimb size and in Group-II both Chinese salt and folic acid were used revealing folic acid compensate the teratogenic effects of Chinese salt indicating that Chinese salt has teratogenic nature but folic acid overcomes effects of Chinese salt if used in correct proportion based on requirement. While in Group III fetuses exposed with Chinese salt revealed drastic reduction in head circumference, eye circumference, limbs size as well as cardiac and neural tube defects.

CONCLUSIONS

On the basis of this study, it can be concluded that Chinese salt and folic acid do indeed show teratogenic effects in developing mice fetuses. They caused a vast range of morphological, morphometric and histological abnormalities in mice. This study will provide awareness about the toxic effects of Chinese salt and folic acid particularly from the stand point of teratogenic and embryotoxic effects that it has on developing mice.

Authors Contribution

Conceptualization: AA
Methodology: AA, SK, AUMF
Formal analysis: AA, SK
Writing-review and editing: AUMF, HF, FA, RB, ML

The author has read and agreed to the published version of the manuscript.

Conflicts of Interest

The author declares no conflict of interest.

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REFERENCES

[1] Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC *et al*. Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo-controlled study. The Journal of Nutrition. 2000 Apr; 130(4): 1058S-62S. doi: 10.1093/jn/130.4.1058S.

[2] Zai H, Kusano M, Hosaka H, Shimoyama Y, Nagoshi A, Maeda M *et al*. Monosodium L-glutamate added to a high-energy, high-protein liquid diet promotes gastric emptying. The American Journal of Clinical Nutrition. 2009 Jan; 89(1): 431-5. doi: 10.3945/ajcn.2008.26180.

[3] Goldsmith PC. Neuroglial responses to elevated glutamate in the medial basal hypothalamus of the infant mouse. The Journal of Nutrition. 2000 Apr; 130(4): 1032S-8S. doi: 10.1093/jn/130.4.1032S.

[4] Hashem HE, El-Din Safwat MD, Algaidi S. The effect of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immunohistochemical study). Journal of Molecular Histology. 2012 Apr; 43: 179-86. doi: 10.1007/s10735-011-9380-0.

[5] Vinodini NA, Nayanatara AK, Gowda KM, Ahamed B, Ramaswamy C, Bhat RM. Effect of monosodium glutamate-induced oxidative damage on rat testis. Journal of Chinese Clinical Medicine. 2008 Jan; 3(7): 370-3.

[6] Nakanishi Y, Tsuneyama K, Fujimoto M, Salunga TL, Nomoto K, An JL *et al*. Monosodium glutamate (MSG): a villain and promoter of liver inflammation and dysplasia. Journal of autoimmunity. 2008 Feb; 30(1-2): 42-50. doi: 10.1016/j.jaut.2007.11.016.

[7] Eweka AO and Om'Iniabohs FA. Histological studies of the effects of monosodium glutamate on the small intestine of adult Wistar rat. Electronic Journal of Biomedicine. 2007; 2: 14-8.

[8] Babai N, Atlasz T, Tamás A, REGL? DI DO, Toth G *et al*. Search for the optimal monosodium glutamate treatment schedule to study the neuroprotective effects of PACAP in the retina. Annals of the New York Academy of Sciences. 2006 Jul; 1070(1): 149-55. doi: 10.1196/annals.1317.003.

[9] Stafstrom CE. Dietary approaches to epilepsy treatment: old and new options on the menu. Epilepsy Currents. 2004 Nov; 4(6): 215-22. doi: 10.1111/j.1535-7597.2004.46001.x.

[10] Farombi EO and Onyema OO. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Human & experimental toxicology. 2006 May; 25(5): 251-9. doi: 10.1191/0960327106ht621oa.

[11] Collison KS, Makhoul NJ, Zaidi MZ, Al-Rabiah R, Inglis A, Andres BL *et al*. Interactive effects of neonatal exposure to monosodium glutamate and aspartame on glucose homeostasis. Nutrition & metabolism. 2012 Dec; 9: 1-3. doi: 10.1186/1743-7075-9-58.

[12] Dief AE, Kamha ES, Baraka AM, Elshorbagy AK. Monosodium glutamate neurotoxicity increases beta amyloid in the rat hippocampus: a potential role for cyclic AMP protein kinase. Neurotoxicology. 2014 May; 42: 76-82. doi: 10.1016/j.neuro.2014.04.003.

[13] Miranda RA, da Silva Franco CC, de Oliveira JC, Barella LF, Tófolo LP, Ribeiro TA *et al*. Cross-fostering reduces obesity induced by early exposure to monosodium glutamate in male rats. Endocrine. 2017 Jan; 55: 101-12. doi: 10.1007/s12020-016-0965-y.

[14] Winkels RM, Brouwer IA, Siebelink E, Katan MB, Verhoef P. Bioavailability of food folates is 80% of that of folic acid. The American Journal of Clinical Nutrition. 2007 Feb; 85(2): 465-73. doi: 10.1093/ajcn/85.2.465.

[15] Knock E, Deng L, Wu Q, Leclerc D, Wang XL, Rozen R. Low dietary folate initiates intestinal tumors in mice, with altered expression of G2-M checkpoint regulators polo-like kinase 1 and cell division cycle 25c. Cancer Research. 2006 Nov; 66(21): 10349-56. doi: 10.1158/0008-5472.CAN-06-2477.

[16] Wang Q, Zhao J, Chang H, Liu X, Zhu R. Homocysteine and Folic Acid: Risk Factors for Alzheimer's Disease—An Updated Meta-Analysis. Frontiers in Aging Neuroscience. 2021 May; 13: 665114. doi: 10.3389/fnagi.2021.665114.

[17] Fogde DL, Xavier CP, Balnytė K, Holland LK, Stahl-Meyer K, Dinant C *d*. Ursolic acid impairs cellular lipid homeostasis and lysosomal membrane integrity in breast carcinoma cells. Cells. 2022 Dec; 11(24): 4079. doi: 10.3390/cells11244079.

[18] Mathers JC. Reversal of DNA hypomethylation by folic acid supplements: possible role in colorectal cancer prevention. Gut. 2005 May; 54(5): 579-81. doi: 10.1136/gut.2004.059881.

[19] Bohnsack BL and Hirschi KK. Nutrient regulation of cell cycle progression. Annual Review of Nutrition. 2004 Jul; 24: 433-53. doi: 10.1146/annurev.nutr.23.011702.073203.

[20] Shookhoff JM and Gallicano GI. A new perspective on neural tube defects: folic acid and microRNA misexpression. Genesis. 2010 May; 48(5): 282-94. doi: 10.1002/dvg.20623.

[21] Czeizel AE and Vargha P. Periconceptional folic acid/multivitamin supplementation and twin pregnancy. American Journal of Obstetrics and Gynecology. 2004 Sep; 191(3): 790-4. doi: 10.1016/j.

DOI: https://doi.org/10.54393/mjz.v4i02.76

ajog.2004.02.018.
[22] Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Human Reproduction Update. 2007 Mar; 13(2): 163-74. doi: 10.1093/humupd/dml054.
[23] Wilton DC and Foureur MJ. A survey of folic acid use in primigravid women. Women and Birth. 2010 Jun; 23(2): 67-73. doi: 10.1016/j.wombi.2009.09.001.
[24] Institute of Laboratory Animal Resources (US). Committee on Care, Use of Laboratory Animals. Guide for the care and use of laboratory animals. US Department of Health and Human Services, Public Health Service, National Institutes of Health; 1986.
[25] Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Elsevier health sciences; 2018.
[26] CSGNetwork. Ellipse Circumference Calculator. [Last Cited: 8th Jan 2024]. Available at: http://www.csgnetwork.com/circumellipse.html.
[27] Shosha HM, Ebaid HM, Toraih EA, Abdelrazek HM, Elrayess RA. Effect of monosodium glutamate on fetal development and progesterone level in pregnant Wistar Albino rats. Environmental Science and Pollution Research. 2023 Apr; 30(17): 49779-97. doi: 10.1007/s11356-023-25661-x.
[28] George KR, Shibija NG, Malini NA. Monosodium glutamate (MSG) induced developmental dysfunction in female albino rats (Rattus norvegicus). The Bioscan. 2013; 8: 73-6.