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The Effect of Monosodium Glutamate (MSG) on Brain Tissue, Oxidation State, True Cholinesterase and Possible Protection against Health Hazards Using Natural Spices

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ABSTRACT

Monosodium Glutamate (MSG) is the most widely used flavor enhancer that exerts serious health hazards to consumers particularly children and elder. Perhaps, brain is the most affected organ. This study deals with the effect of MSG consumption on oxidation state, cholinesterase concentration and brain histopathology. The experiment was done on rats given a diet containing 70 g MSG/ kg diet. The protective action of spices such as cinnamon, ginger, sumac, rosemary and thyme either individual or in combination was investigated. Results revealed an increase in plasma malonedialdehyde due to MSG ingestion which was corrected by addition of spices. The activities of RBCs superoxide dismutase, glutathione peroxidase and plasma catalase were decreased due to MSG ingestion. Addition of spices rendered enzymes activities near normal. The increased brain and plasma true cholinesterase concentration can be symptoms of Alzheimer's disease. Addition of spices reduced brain and plasma cholinesterase concentrations. Brain of rats given MSG showed focal gliosis, cellular oedema, focal hemorrhage and neuronal necrosis which became minor when adding spices.

In conclusion, long term consumption of MSG exerts serious health hazards on oxidation state, antioxidant enzymes and the neurotransmitter cholinesterase which affects brain tissue structure. These symptoms are prevented to a great extent when spices are included with MSG.

Keywords: MSG, Brain, Oxidation state, Cholinesterase, Histopathology

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INTRODUCTION

Monosodium Glutamate (MSG) is now used widely in most countries of the world as a food additive to enhance flavor of the food products in addition to its use by house wives during preparation of meals. Numerous health hazards were reported to prolonged consumption of MSG that even reached to the finding that MSG exerts genotoxic effects on lymphocytes in humans [1]. However, it remains that the most serious hazardous action of MSG is the neurotoxic effect. This is suggested to be related to over activation of an excitatory amino acid receptor that cause an increase in intracellular calcium which in turn promote a series of enzymatic reaction which lead to cell death [2]. Moreover, it has been shown that MSG consumption causes pathological changes in brain structure associated with neuronal injury and pronounced oxidation stress [3].

Cholinesterases are distinguished into 2 types, one that splits acetylcholine into acetic acid and choline known as true or acetylcholinesterase, the latter called pseudo or butyrylcholinesterase hydrolyzes choline esters and produces choline and carboxylic acid. It is known that the brain of vertebrates contain true cholinesterase only. However, when enough amount of brain tissue is used for estimation, a noticeable activity for pseudo cholinesterase may be detected. Among the factors contributing to Alzheimer's disease are the occurrence of amyloid-β-peptide [4] hyperphsphorelated tau protein [5] and increased acetylcholinesterase and

butyrylcholinesterase. The increased activities of these two enzymes have been implicated in the occurrence of Alzheimer's disease [6]. It has been shown that a disturbance of cholinergic synaptic transmission plays a major role in development of Alzheimer's disease. The relation between the activities of cholinesterases and Alzheimer's disease was studied before in several models, however, no single article reported the effect of MSG on the activities of cholinesterases and in turn the possibility of occurrence of Alzheimer's disease. In a study by [7], it was found that plasma cholinesterase activity was not changed in Alzheimer's patients and was significantly decreased in Parkinson patients. Then, it can be stated that two main phenomena, that will be highlighted in this study, can affect brain behavior namely oxidation stress and change of the cholinesterase activity.

Spices are known to contain a number of bioactive compounds that possess antioxidant characters. Wilson [8] studied more than 30 plants commonly used as spices and reported that most of them have antioxidant properties due to the presence of bioactive compounds such as polyphenols. Spices such as ginger [9], rosemary [10], thyme [11], were reported to alleviate conditions such as memory impairment, oxidation stress and inflammation.

Therefore, the aim of the present study was to investigate the extent of damage and metabolic disturbance that occurs in the brain tissue and the body due to MSG consumption. The effect on oxidation state of the brain and blood, together with the effect on the concentration of plasma and brain true cholinesterases were investigated. The possible protective action of certain spices such as cinnamon, ginger, sumac, rosemary and thyme either individual or in combination, with certain proportions, against the health hazards were investigated.

MATERIALS AND METHODS

MATERIALS

Monosodium glutamate used in the present study was purchased from LOBA Chemie Ltd., Mumbai, India. Spices; cinnamon, ginger, sumac, rosemary, oregano, garlic, cardamom, thyme, nutmeg and coriander were purchased from Production and Marketing Unit of Medicinal Plants Research Department, National Research Centre (NRC), Egypt. Most of the constituents of the standard diet were purchased from the local market. Casein was obtained from Scerma Co., France. Chemicals used for the preparation of vitamin and salt mixtures were obtained from LOBA Chemie Ltd., Mumbai, India. Chemicals which were used for determination of lipid peroxide product in blood samples (malondialdhyde); Tricholoroacetic Acid (TCA) and Thiobarbituric Acid (TBA) were obtained from BDH (England) and Merck (Germany) Companies, respectively. Kits used for determination of blood hemoglobin, plasma catalase, RBCs and brain Superoxide Dismutase (SOD) and RBCs and brain glutathione peroxidase (GPx), were obtained from Biodiagnostic Co., Egypt. Kit used for estimation of true cholinesterase either in plasma or in brain tissue by ELISA technique was purchased from Glory Co., USA.

This study was carried out on Sprague Dawly male Albino rats obtained from the Central Animal House of the National Research Centre. Animal experiment was conducted according to the guidelines of Animal Care and Ethics Committee of the National Research Centre (NRC), Egypt. The study protocol was approved by the Scientific Committee at NRC.

METHODS

Biochemical parameters either in blood or in brain tissue homogenate were determined as follows: Blood hemoglobin as described by Betke and Savelsberg [12]. The antioxidant enzymes namely RBCs SOD, plasma catalase and RBCs GPx either in blood or in brain tissue homogenate according to the methods of Nishikimi et al.; Aebi and Paglia; Paglia and Valentine [13-15], respectively. Plasma Malondialdehyde (MDA) was measured as lipid peroxide product by the Thiobarbituric Acid Assay (TBA) according to the method of Drapper and Hadley [16]. True cholinesterase concentration was determined in plasma or brain tissue homogenate according to Den Blaauwen et al. [17].

EXPERIMENTAL DESIGN

A mixture of spices with certain proportions was formulated as descried in a previous publication [18]. The effect of MSG on the antioxidant state and on true cholinesterase either that of the plasma or the brain tissue was assessed. The protective role of the used spices either individual or in combination was biologically evaluated on animals as follows:

Fifty four Sprague Dawley male albino rats with body weight ranging from 90-110 g were used. They were kept individually in stainless steel cages in a temperature controlled room at 25ºC with food and water allowed *ad libitum*. All rats were left for accommodation for a period of one week before the beginning of the actual experiment. A standard control diet was formulated according to Reeves et al. [19], and introduced to rats. Rats were divided into 9 groups each comprising 6 litters and each was treated as follows:

Group 1: Fed on standard diet and considered as control negative group.

Group 2: Fed on standard diet+70 g MSG /kg diet (control positive).

Group 3: Fed on standard diet+MSG+spices mixture (11.2 g/kg diet).

Group 4: Fed on standard diet+MSG+spices mixture (22.4 g/kg diet).

Group 5: Fed on standard diet+MSG+cinnamon (11.2 g/kg diet).

Group 6: Fed on standard diet+ MSG+ginger (11.2 g/kg diet).

Group 7: Fed on standard diet+MSG+sumac (11.2 g/kg diet).

Group 8: Fed on standard diet+MSG+rosemary (11.2 g/kg diet).

Group 9: Fed on standard diet+MSG+thyme (11.2 g/kg diet).

The experiment lasted for 8 weeks, during which the body weight and the daily food consumption were estimated and recorded. At the end of the experimental period, rats were fasted overnight and in the morning, they were subjected to blood withdrawal by open heart puncture under slight ether anesthesia. Blood samples were collected on heparin. One portion of the heparinized blood samples was centrifuged at 3500 rpm for 15 min to separate plasma which was stored in the deep freeze at -70ºC till analysis of the studied parameters. The other portion of the heparinized blood samples was also centrifuged at 3500 rpm for 15 min to separate RBCs which were subjected to washing three times by saline solution. The washed RBCs were immersed in cold redistilled water for the lyses of RBCs, and then kept at -70ºC till analysis of the antioxidant enzymes.

Brain was separated from each rat and washed with saline, plotted between 2 sheets of filter paper and then weighed. A portion of each of the separated brain was embedded into 10% formalin solution for further histopathological examination. The other portion was further subdivided into two portions one for the estimation of brain antioxidant enzymes and the other for the estimation of brain true cholinesterase. Both of them were kept at -70ºC till analysis.

Brain tissue was homogenized and the homogenate was used for the determination of antioxidant state as described by Farbiszewski et al. [20]. For the estimation of cholinesterase concentration in brain; the brain tissue samples were homogenized according to Ciro et al. [21]. Samples were thawed on ice and sonicated at medium power in 50 mM phosphate buffer containing 10 mM EDTA and 0.5% Tween or Triton-X100 to yield 5-10 mg of tissue per ml of the sonicate. Then, samples were centrifuged at 10000 rpm for 30 min; the clear supernatant was then aspirated and used for the determination of the true cholinesterase activity within 24 hours.

Histopathological analysis

Brain specimens were histopathologically examined after being cleared in xylol, embedded in paraffin, sectioned at 4-6 micron thickness and stained with Heamatoxylin and Eosin according to Carleton [22]. Finally, they were examined under microscope.

Statistical analysis

Results were analyzed statistically according to Williams [23] and Bailey [24] using the computerized program SPSS version “20”. The one way ANOVA test was done followed by Duncan test. Data were represented as mean ± SE. Significance was considered at a level of 0.05.

RESULTS

Oxidation state and antioxidant enzymes

The state of oxidation and antioxidant enzymes in plasma of rats of different groups was estimated and results obtained are tabulated in Table 1. As shown in the table, the plasma Malonedialdehyde (MDA) was significantly increased due to addition of monosodium glutamate to the diet. The control value for MDA was 1.31 ± 0.036 nmol/ml and increased to 2.13 ± 0.129 nmol/ml after addition of monosodium glutamate. By addition of spices to the diet of different groups, the value of MDA more or less returned back to near the normal value of the control negative group. The most notable effect was that of ginger given with monosodium glutamate in group 6. It was noted that the activities of RBCs Superoxide Dismutase (SOD), RBCs Glutathione Peroxidase (GPx) and Plasma

Table 1: Concentration of blood hemoglobin, plasma malonedialdehyde (MDA) and activities of plasma catalase, RBCs superoxide dismutase (SOD) and RBCs glutathione peroxidase (GPx) in control rats and those fed on diets containing MSG alone or in combination with spices

Groups	Hemoglobin (g/dl)	MDA (nmol/ml)	Catalase (U/L)	SOD (U/g Hb)	GPx (mU/ml)
Group 1	13.27 ± 0.^{24}ac	1.31 ± 0.04^{a}	731.51 ± 26^{a}	506.73 ± 12^{a}	515.11 ± 15^{a}
Group 2	11.30 ± 0.^{70}b	2.13 ± 0.13^{c}	583.73 ± 16^{b}	346.40 ± 8^{b}	368.11 ± 8^{b}
Group 3	11.97 ± 0.^{19}ab	1.68 ± 0.12^{b}	470.23 ± 22^{c}	373.93 ± 14^{bc}	494.98 ± 16^{a}
Group 4	14.08 ± 0.^{4}8c	1.40 ± 0.12^{ab}	617.48 ± 12^{bd}	348.45 ± 8^{b}	505.80 ± 17^{a}
Group 5	12.77 ± 0.^{62}ac	1.43 ± 0.09^{ab}	661.90 ± 17^{d}	359.55 ± 16^{bc}	472.65 ± 15^{a}
Group 6	13.98 ± 0.^{4}2c	1.42 ± 0.19^{ab}	583.73 ± 18^{b}	332.52 ± 4^{b}	479.84 ± 13^{a}
Group 7	13.57 ± 0.3^{1}c	1.57 ± 0.10^{ab}	840.24 ± 6^{e}	364.52 ± 12^{bc}	505.77 ± 17^{a}
Group 8	13.19 ± 0.^{63}ac	1.47 ± 0.06^{ab}	375.59 ± 8^{f}	362.78 ± 14^{bc}	492.82 ± 16^{a}
Group 9	14.03 ± 0.^{1}8c	1.43 ± 0 .03^{ab}	775.12 ± 21^{a}	401.09 ± 30^{c}	510.11 ± 16^{a}

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level. *Values that share the same letter at the same column are not significant. *Values that share different letters at the same column are significant. *Group1: Control (-ve); Group 2: Control+MSG (control +ve); Group 3: Mix of low spices dose+MSG; Group 4: Mix of high spices dose+MSG; Group 5: MSG+Cinnamon; Group 6: MSG+Ginger; Group 7: MSG+Sumac; Group 8: MSG+Rosemerry; Group 9: MSG+Thyme

Catalase (CA) were decreased due to addition of monosodium glutamate with diet. The values reported were 506.73 ± 12 U/ml, 731.51 ± 26 U/L and 515.11 ± 15 mU/ml for each of (SOD), (CA) and (GPx), respectively in case of normal rats. The corresponding values for rats given monosodium glutamate alone were 346.40 ± 8 U/ml, 583.73 ± 16 U/L and 368.11 ± 8 mU/ml, respectively. Addition of thyme, mixture with low dose, cinnamon, sumac and, rosemary caused gradual increase in the activities of superoxide dismutase relative to the value obtained for rats given monosodium glutamate alone. In case of catalase the marked effect of spices was reported for rats in group 7 given sumac followed by rats in group 9 given thyme followed by rats of group 5 given cinnamon. Addition of thyme to monosodium glutamate in case of rats of group 9 caused the activity of glutathione peroxidase to return to more or less the normal value.

Activities of brain superoxide dismutase and glutathione peroxidase

The activities of superoxide dismutase and glutathione peroxidase were estimated and the obtained results are shown in Table 2. As shown in the table the activities of both these enzymes in brain were significantly decreased in rats given MSG with the diet. The values reported were 716 ± 31 mU/g tissues for brain glutathione peroxidase and 482 ± 40 U/g tissue for superoxide dismutase. The corresponding values for controls were 1131 ± 36 mU/g tissues and 717 ± 26 U/g tissue, respectively. Adding spices with monosodium glutamate caused increased activities of these enzymes and the most pronounced effect was for group 7 given sumac in case of glutathione peroxidase and for group 9 given thyme in case of superoxide dismutase.

Table 2: Activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) in brain of control rats and those fed on diets containing MSG alone or in combination with spices

Groups	Brain GPx (mU/g tissue)	Brain SOD (U/g tissue)
Group 1	1131 ± 36^{a}	717 ± 26^{a}
Group 2	716 ± 31^{b}	482 ± 40^{bc}
Group 3	919 ± 9^{d}	561 ± 31^{bc}
Group 4	1037 ± 33^{ac}	561 ± 47^{bc}
Group 5	1037 ± 33^{ac}	566 ± 57^{bc}
Group 6	1011 ± 35^{c}	534 ± 28^{bc}
Group 7	1100 ± 7^{ac}	534 ± 19^{bc}
Group 8	1037 ± 33^{ac}	517 ± 20^{bc}
Group 9	1089 ± 40^{ac}	646 ± 72^{ac}

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level. *Values that share the same letter at the same column are not significant. *Values that share different letters at the same column are significant. *Group1: Control (-ve); Group 2: Control+MSG (control +ve); Group 3: Mix of low spices dose+MSG; Group 4: Mix of high spices dose+MSG; Group 5: MSG+Cinnamon; Group 6: MSG+Ginger; Group 7: MSG+Sumac; Group 8: MSG+Rosemerry; Group 9: MSG+Thyme

Plasma and brain true cholinesterase (ACHE)

The concentrations of true cholinesterase in plasma and in brain tissue homogenate were determined and the data obtained is shown in Table 3. As shown in the table a significant increase of plasma true cholinesterase was reported due to the addition of monosodium glutamate with the diet, the value obtained of plasma true cholinesterase amounted to 103.83 ± 13 pg/ml relative to a value of 87.67 ± 5 pg/ml for controls. Addition of any of the used spices caused a reduction in plasma cholinesterase concentration relative to rats in group 2 given monosodium glutamate alone except for rats in group 3 given the mixture with low spices dose and monosodium glutamate where the value obtained was the highest among all groups (133.83 ±11 pg/ml). The highest drop of plasma cholinesterase occurred in rats of group 9 given thyme and monosodium glutamate (46.17 ± 3 pg/ml).

Table 3: Concentration of plasma true cholinesterase and brain true cholinesterase of control rats and those fed on diets containing MSG alone or in combination with spices

Groups	Plasma True Cholinesterase pg/ml	Brain True Cholinesterase pg/g tissue
Group 1	87.67 ± 5^{ab}	568 ± 123^{a}
Group 2	103.83 ± 13^{b}	676 ± 30^{ab}
Group 3	133.83 ± 11^{c}	573 ± 65^{a}
Group 4	81.17 ± 5^{a}	470 ± 98^{a}
Group 5	75.17 ± 5^{a}	560 ± 107^{a}
Group 6	67.67 ± 2^{ad}	570 ± 38^{a}
Group 7	66.17 ± 9^{ad}	513 ± 39^{a}
Group 8	67.67 ± 8^{d}	673 ± 55^{b}
Group 9	46.17 ± 3^{d}	823 ± 61^{b}

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level. *Values that share the same letter at the same column are not significant. *Values that share different letters at the same column are significant. *Group1: Control (-ve); Group 2: Control+MSG (control +ve); Group 3: Mix of low spices dose+MSG; Group 4: Mix of high spices dose +MSG; Group 5: MSG +Cinnamon; Group 6: MSG+ Ginger; Group 7: MSG+Sumac; Group 8: MSG+Rosemerry; Group 9: MSG+Thyme

The concentration of brain cholinesterase was significantly increased in rats given monosodium glutamate alone with the diet. The value obtained for true cholinesterase in the group given monosodium glutamate alone was 676 ± 30 Pg/g tissues relative to a normal value of 568 ± 123 Pg/g tissue. Adding any of the spices to monosodium glutamate in the diet caused a reduction of the true cholinesterase concentration in most of the groups except group 8 that was given rosemary and monosodium glutamate and group 9 that was given thyme with monosodium glutamate. It's worth mentioning that the concentration of true cholinesterase of rats in group 9 was the highest among all values of other groups.

Histopathological changes of brain

Histopathological examination of brain was done (Figure 1a-1q). As shown in a and b; no histopathological changes were reported in normal rats. Brain from group 2 given MSG showed focal gliosis, cellular oedema, focal hemorrhage and necrosis of neurons c and d. Group 3 given the mixture with low spices dose and MSG showed necrosis of neurons and neuronal oedema (f and g). As shown in i; no histopathological changes were reported in group 4 given the mixture with high spices dose and MSG. Brain of rats in group 5 given cinnamon and MSG showed focal gliosis, necrosis of neurons and neuronophagia j and k. As shown in l, brain of rats in group 6 given ginger and MSG showed no histopathological changes. As shown in m and n there was necrosis of neurons in group 7 given sumac and MSG Brain of rats in group 8 given rosemary and MSG showed necrosis of neurons and focal gliosis (o and p). As shown in q brain of rats in group 9 given thyme and MSG showed no histopathological changes.

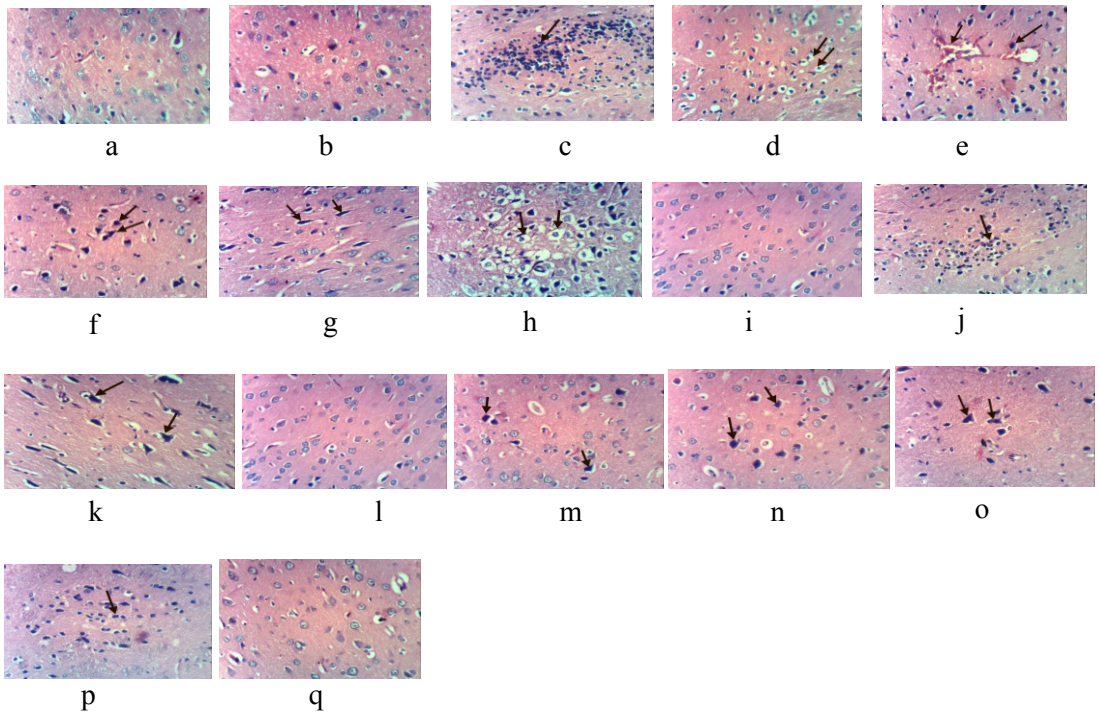

Figure 1: Light Figuremicrographs of brains from rats of different groups (a-q). Brain from control (-ve) group showing the normal histopatholological brain structure (a and b). Brain of rat from group 2 (control+MSG or control +ve) showing focal gliosis, cellular oedema, focal hemorrhage and necrosis of neurons (c-e). Brain of rat from group 3 (mix of low spices dose+MSG) showing necrosis of neurons and neuronal oedema (g-h). Brain of rat from group 4 (mix of high spices dose+MSG) showing no histopathological changes (i). Brain of rat from group 5 (MSG+cinnamon) showing focal gliosis, necrosis of neurons and neuronophagia (j,k). Brain of rat from group 6 (MSG+ginger) showing no histopathological changes (l). Brain of rat from group 7 (MSG+sumac) showing necrosis of neurons (Figures m,n). Brain of rat from group 8 (MSG+rosemary) showing necrosis of neurons and focal gliosis (o,p). Brain of rat from group 9 (MSG+thyme) showing no histopathological changes (q) (H&E X400).

DISCUSSION

Most studies that could be traced in the literature showed that consumption of MSG particularly in case of children and elder induce a state of oxidative stress which is believed to be behind most of the symptoms and health disorders that occur due to excessive use of this compound [25-28].

In the present study the concentration of plasma MDA was significantly increased indicating excessive generation of free radicals all over the body including the brain. A condition which agree with findings by the previous authors. It has been reported by Gebicki [29] that secondary radicals are generated from primary radicals and the latter attack biomolecules particularly proteins. Proteins, being the constituents of all tissues and enzymes profound this effect and result in all health disorders seen in MSG consumers. Natural antioxidants are urgently needed under such condition to eradicate these free radicals and in turn protect from health hazards of MSG. Spices such as cinnamon, ginger, sumac, rosemary or thyme are the most suitable compounds to be added to MSG. It adds more favorable and preferable flavor to the MSG. Also, what is important is that it contains natural compounds with antioxidant characters such as polyphenols which can help to ameliorate health hazards of MSG [30]. A mixture of some common spices which

are mostly used by Egyptians was formulated with certain proportions by our team and registered as a patent (No. 1537/2016). The effect of this mixture and some other individual spices on metabolic parameters of rats given MSG with the diet was investigated. Blood sugar, lipid profile and liver and kidney functions was assessed and the results were published before [18].

In the present study it was found that the activities of SOD and GPx in plasma and brain tissue were decreased due to consumption of MSG. Similar changes in the activities of the antioxidant enzymes associating ingestion of MSG was reported previously in the heart tissue of rats by Singh and Ahluwalia [31]. This is an indication that the toxic effect of MSG extends to all tissues in the body. Free radicals formed in the brain tissue due to ingestion of MSG are known to cause deterioration of most proteins including enzymes [29]. Not only free radicals formed in sito, but also perhaps those due to diffusion from circulation resulting from a defect in blood brain barriers. These may diffuse to the brain and participate in the deleterious action on the brain enzymes.

Although glutamic acid is responsible for triggering neuronal migration and differentiation, synaps remodeling and long-term potentiating, yet excessive concentration of this compound may cause neuronal death [32]. True cholinesterase concentration in brain tissue of rats given MSG was significantly increased. The true cholinesterase concentration in plasma was also significantly increased. It is known that true cholinesterase in plasma is minor; however, it is considered representative to the state of cholinesterase in erythrocytes. In a study made by Phelix and Hartle [33], they found that subcutaneous administration of MSG to rats caused morphological signs of degeneration in the brain including, cytoplasmic vacuolization, chromatin clumping and dendritic hypertrophy. Perhaps this is the reason behind the increased concentration of the true cholinesterase in the brain of rats given MSG. The increased true cholinesterase concentration is also noticed in plasma of rats fed on MSG. Ureña-Guerrero et al. [34], found that MSG neonatal treatment in both cerebral regions caused a gamma aminobuteric acid cells loss. The increased concentration of true cholinesterase in presence of tissue degeneration evidenced by pathological examination seems a little bit surprising. However, it can be a compensating mechanism from the non-affected cells in the brain to substitute the degenerated ones or that the effect of MSG on this neurotransmitter is minimal or nonspecific. According to Cho et al. [35], the increased activities of true cholinesterase are associated with the occurrence of Alzheimer's disease. This means that symptoms of Alzheimer's disease occur when cholinergic synaptic transmission is disturbed. We do not have clear evidence to the occurrence of Alzheimer's disease in animals. Perhaps this is the first time a study points to the possible occurrence of Alzheimer's disease due to ingestion of MSG. However, it deserves further investigations to prove whether or not MSG consumption leads to occurrence of this disease.

Addition of spices to the diet of MSG treated rats caused correction of the activity of brain GPx particularly in case of rats given sumac followed by those given thyme. The activity of brain SOD was also increased towards normal value. Such effect was not so pronounced in plasma as it is in the brain tissue. This indicates that these compounds can protect brain cells from damage caused by ingestion of MSG. This effect is attributable to the antioxidant character of the spices which help to scavenge free radicals thus protect the tissues from damage. The changes that occurred in the activities of the antioxidant enzymes in the brain tissue due to ingestion of MSG, namely the decreased activities of the enzymes were corrected to various extents when different spices were added with MSG. In case of GPx, the most pronounced effect was in case of group 7 given sumac with the MSG. This was followed by group 9, 4, 5 and 8 given thyme, the mixture with high spices dose, cinnamon or rosemary, respectively. In case of SOD, the effect of thyme was the most pronounced followed by cinnamon, then the mixture with low spices dose and the mixture with high spices dose. This shows that the addition of these spices to MSG could prevent the deteriorating action of the free radicals generated due to ingestion of MSG. Also, shows that this protection is not equal to the different spices and also to the different enzymes. This means specificity according to the type of free radical generated and the ability of the bioactive compound in the spices to bind or react with this free radical and stop its deteriorating action. In a previous study made by Shimada et al. [36], it was reported that the neurotoxic effect of MSG is prevented by inclusion of cinnamon in the diet. Gomar et al. [9] showed that when ginger extract was added with the diet of rats treated with morphine, the memory impairment caused by morphine was attenuated. Also, Liu et al. [10] found that addition of rosemary to meat products improved quality with respect to oxidation and acceptability. Thyme was reported to possess an antioxidant characters as evidenced by estimation of the antioxidant power of the extract [11]. In plasma, although the protective effect of spices on activities of antioxidant enzymes of rats given MSG was not pronounced yet it was clear in case of GPX antioxidant enzymes.

It is thus clear that all ingredients used for protecting against hazards of MSG were reported to possess antioxidant characters or can participate in alleviation of symptoms due to ingestion of MSG particularly these affecting the brain. Perhaps this is the first time such herbs are used with MSG to eradicate or prevent MSG hazards.

The histopathological examination of the brain of rats given MSG with diet showed marked changes in the tissue. Severe damage occurred in the brain cell of rats fed on diet containing MSG. This is represented in focal gliosis, cellular edema, focal hemorrhage and necrosis. Such changes also go in parallel with biochemical changes represented in the activities of the brain enzymes namely GPx, SOD and true cholinesterase. The activities of these enzymes were disturbed due to ingestion of MSG. The reason for this change is the degenerative action of MSG on brain cells as shown from pathological examination. As mentioned before in case of the antioxidant enzymes, an improvement of the enzymatic pattern was reported when any of the spices or the mixture of them were added with MSG. However, still this improvement was not complete. Some pathological lesions still persist but to less extent except in group 4 that was given the mixture with high spices dose, group 6 that was given ginger and group 9 that was given thyme, where no histopathological changes in brain tissue were observed. In case of cholinesterase, it can be noticed that the complete protective action of spices occurred in all groups that were given any of the spices or the mixture with MSG except group 8 that was given rosemary and group 9 that was given thyme. This is different from the case of antioxidant enzymes where the best observable effect was reported in rats of group 9 that were given thyme with MSG. This shows that the protective action of spices against the health hazards of MSG ingestion is not similar for all spices and also not similar with regard to the site of action. However, it can be stated that most of the used spices do protect against degenerative action of MSG to a good extent which necessitates adding this spices to MSG products and advice home ladies and cookers to use them for preparation of meals containing MSG.

In brief, it can be stated that ingestion of MSG causes significant changes in the brain tissue and metabolism. This is represented in a change in the oxidation state, antioxidant enzymes and cholinesterase concentration. The histopathological changes coordinated with the biochemical findings and seems to be responsible for the health hazards due to consumption of MSG. Addition of spices with the diet such as cinnamon, ginger, sumac, rosemary, thyme or a mixture of the spices succeeded to correct the metabolic and histopathological state of the brain and in turn protect to a large extent from these health hazards.

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