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Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice

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ABSTRACT

The present study was designed to investigate the in vivo effects of monosodium glutamate (MSG) and aspartame (ASM) individually and in combination on the cognitive behavior and biochemical parameters like neurotransmitters and oxidative stress indices in the brain tissue of mice. Forty male Swiss albino mice were randomly divided into four groups of ten each and were exposed to MSG and ASM through drinking water for one month. Group I was the control and was given normal tap water. Groups II and III received MSG (8 mg/kg) and ASM (32 mg/kg) respectively dissolved in tap water. Group IV received MSG and ASM together in the same doses. After the exposure period, the animals were subjected to cognitive behavioral tests in a shuttle box and a water maze. Thereafter, the animals were sacrificed and the neurotransmitters and oxidative stress indices were estimated in their forebrain tissue. Both MSG and ASM individually as well as in combination had significant disruptive effects on the cognitive responses, memory retention and learning capabilities of the mice in the order (MSG + ASM) > ASM > MSG. Furthermore, while MSG and ASM individually were unable to alter the brain neurotransmitters and the oxidative stress indices, their combination dose (MSG + ASM) decreased significantly the levels of neurotransmitters (dopamine and serotonin) and it also caused oxidative stress by increasing the lipid peroxides measured in the form of thiobarbituric acid-reactive substances (TBARS) and decreasing the level of total glutathione (GSH). Further studies are required to evaluate the synergistic effects of MSG and ASM on the neurotransmitters and oxidative stress indices and their involvement in cognitive dysfunctions.

1. Introduction

Food additives that are intended for human use are generally approved after testing for their toxicity through animal toxicity tests (Kokoski et al., 1990). The overall goal of such tests is twofold: to assess the additive's potential for causing toxic effects in humans and to determine if safe conditions of use can be established (Kokoski et al., 1990). However, evaluation for the safe consumption of such food additives is usually based on their toxicity data obtained from animal studies since human data are scantily available (Lin et al., 1992).

Monosodium glutamate (MSG) is one of the most popular flavoring agents of modern time 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 many effects like learning difficulty (Olvera-Cortes et al., 2005), obesity (Nagasawa et al., 1974), and gonadal dysfunction (Pizzi et al., 1978). Brain damage induced by the neurotoxicity of MSG has also been established in experimental chicken (Robinzon et al., 1974). MSG injected i.p. at 2 and 4 mg neonatally in mice produced lesions in the arcuate nucleus region of the brain affecting the regulation of water drinking (Morley and Flood, 1989). Some of the neurotransmitters like norepinephrine, serotonin, dopamine and their metabolites in the hypothalamus region were found to be depleted in MSG treated rats (Nakagawa et al., 2000). MSG administration (4 mg/g) has also been associated with oxidative stress in the hepatic tissue of young rats (Diniz et al., 2004). Elevation of serum alanine aminotransferase (ALAT) and aspartic aminotransferase (ASAT) with degenerative changes in hepatocytes after a single high dose intraperitoneal injection of MSG was noted in rats (Ortiz et al., 2006).Hepatocellular damage due to long term exposure to MSG (2 mg/g body weight) was also reported in albino mice after neo-natal exposure (Bhattacharya et al., 2011). On the contrary, some researchers reported that MSG taken with food showed no adverse effect (Stegink et al., 1985).

Aspartame (ASM) is a dipeptide (L-aspartyl-L-phenylalanine methyl ester) and is used as an artificial sweetener. ASM is used in a variety of

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food products; however, ASM-related neurological disturbances such as dizziness, headaches, gastrointestinal symptoms, mood alterations, allergic type reactions and alterations in menstrual patterns have also been reported (Coulombe and Sharma ,1986). Studies in mice have reported that alterations in brain neurotransmitters are responsible for the behavioral effects associated with ASM consumption at varying doses of 13, 130 or 650 mg/kg (Coulombe and Sharma, 1986). ASM consumption has also been reported to affect motor behavior in rats (Dourish et al., 1983). Furthermore, consumption of ASM by rats during pregnancy and lactation also affects their offspring's morphological and reflex development (Brunner et al., 1979). Oral intake of ASM in mice has been reported to be the cause of neuronal necrosis in several regions of the brain including the hypothalamus (Reynolds et al., 1976; Olney et al., 1980). In another study, Holder and Yirmiya (1989) reported that ASM had adverse effects in rats when injected intraperitoneally and not when administered orally. Possible epileptogenic or neurotoxic effects of ASM (34 mg/kg) have also been reviewed in experimental models (Stegink, 1987; Janssen and van der Heijdan, 1988).

As we can see from the above literature survey, plenty of studies on MSG and ASM individual exposures have been reported in experimental animals at neonatal stages and have looked for various deleterious effects at the adolescent and/or adult stages. However, the combined effects of MSG and ASM have not been studied in experimental models as widely as their individual exposures; and the combined effects of MSG and ASM still remain unclear. Olney and Ho (1970) and Olney et al. (1980) reported in neonatal mice that MSG and ASM in combination doses of 34 mg/kg each produced neuronal necrosis in brain tissue. Very recently, Collison et al. (2012) reported that MSG and ASM (120 and 50 mg/kg body weight/day respectively) administered in neonatal mice impaired their glucose and insulin homeostasis. On the contrary, little hazard has been reported from injection of combined doses of MSG and ASM in rodents and primates (Reynolds et al., 1976). Studies related to exposures to food additives in combined doses at adulthood stages are wanting. Furthermore, studies on the effects of MSG and ASM exposures (singly or in combination) on behavior and neurotransmitters and oxidative stress in brain tissue are also much needed in order to understand their biochemical correlation with the memory retention system.

Thus, it was hypothesized that consumption of MSG and ASM in combination could be comparatively more deleterious than exposure to them individually. Although no effort has been made to compare the doses of MSG and ASM used herein with doses that a human would be exposed to, the present study used doses that fall within the range of the doses used for a previous study conducted in adult humans (Stegink et al., 1982). Furthermore, the present study was hypothetically designed to investigate the in vivo toxic effects of MSG and ASM individually and in combination on cognitive behavior and to find out their correlation with biochemical parameters like some neurotransmitters and some oxidative stress indices in forebrain tissue regions that are reportedly responsible for cognitive activities.

2. Materials and methods

2.1. Experimental animals

Forty male Swiss–Webster strain mice (8–10 weeks old, bred and reared under controlled conditions) were housed in opaque plastic cages measuring 30 × 12 × 11 cm (5 animals per cage) under hygienic conditions in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. All animals were maintained under reversed lighting conditions with white lights on from 22.00 to 10.00 h local time. The ambient temperature was regulated between 20 and 22 °C. Food (Pilsbury's Diet) and water were available ad libitum, unless otherwise indicated. All procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals, and all protocols were approved by the local Ethics and Care of Experimental Animals Committee.

2.2. MSG and ASM administration

All animals were randomly divided into four different groups with ten animals each. Group I consisted of untreated mice and served as naïve controls since they were given only plain tap water. Group II was treated with monosodium glutamate (MSG) at a dose of 8 mg/kg body weight/day, dissolved in drinking water. Group III was treated with aspartame (ASM) at a dose of 32 mg/kg body weight/day, dissolved in drinking water. Group IV was treated with MSG and ASM together in the same doses as in groups II and III dissolved together in drinking water. The doses were selected on the basis of our pilot studies and from available literature. All exposures were through oral administration in their drinking water that formed the only source of drinking fluid for a period of one month. Our pilot studies have shown that a normal adult mouse on average consumes 30 ml of water per day. Thus, all doses of MSG and ASM were prepared in such a manner that the required doses of MSG and ASM (individually and in combination) were consumed by the animals per day through their daily consumption of water. MSG and ASM of analytical grade, from Sigma Chemical Company, USA, were used in this study. After the exposure period of one month, the animals were subjected to cognitive behavioral tests in a shuttle box and a water maze. Subsequently, the animals were sacrificed and the neurotransmitters and oxidative stress parameters were estimated in their forebrain tissue.

2.3. Body weight observation

Throughout the exposure period, all animals were subjected to body weight observations and their body weight was recorded on day 1, day 6, day 18, day 24 and day 30 of the treatment period.

2.4. Cognitive behavioral studies

The learning capabilities of all animals were measured in the same order in the shuttle-box followed by the water maze test.

2.4.1. Shuttle-box test (active avoidance responses)

The active avoidance responses were measured in the animals using a shuttle-box (Ugo Basile, Comerio-Varese, Italy). The rectangular shaped shuttle-box was divided into two chambers of equal size by a stainless steel partition with a gate providing access to the adjacent chambers. Before starting the trial sessions, each animal was allowed to adapt and acquaint itself with the shuttle-box for 2 min without any stimulus. A light bulb (21 W) for 6 s duration and a buzzer (670 Hz and 70 dB) were switched on consecutively and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s. The US was an electric scrambler shock (1 mA for 4 s) applied to the metallic grid floor that was hinged in the middle with a fulcrum (8 cm height) located in the middle half of the floor below the metallic gate. Because of the fulcrum the entire metallic grid floor worked like a see-saw. The floor was lowered on the side where the animal entered through the gate. Thus, the floor was a two way procedure and the shock (US) was delivered on either side of the metallic grid floor after the light and sound stimuli (CS). If the animal avoided the US by running into the other compartment within 5 s after the onset of the CS, the microprocessor recorder unit of the shuttle-box recorded an avoidance response and this was considered as a conditioned avoidance response to avoid the electric shock. Each animal was given 50 trials with a fixed intertrial interval of 15 s. During the 50 trial sessions of the individual animals, the total number of avoidance was measured. The total time taken until the animal entered the other chamber to avoid the shock treatment (latency of avoidance response or escape latency in seconds) was also measured for each animal. The recorder unit

of the automated shuttle-box continuously recorded these parameters during the whole experimental period (50 trials) for each animal.

2.4.2. Morris water maze test

The animals were subjected to cognitive behavioral studies over a period of 6 days. Animals were allowed to acclimatize to the testing room for 2 h before testing. All tests were performed between 10:00 and 15:00 h. The Morris water maze test has been extensively used to assess cognitive functions in animal models (Faverjon et al., 2002; Rutten et al., 2002). Thus, the mice in the present study were tested for visual–spatial memory performance using a water maze (Morris, 1984). The water maze consisted of a galvanized white circular water tank (90 cm diameter, 45 cm height) filled with clear tap water (26 ± 1 °C) to a depth of 30 cm. A 10 cm diameter, and 29 cm high stainless steel, white, escape platform was placed 1 cm below the water level and the water was made opaque by the addition of 1 l of milk, which prevented visualization of the platform. Four points on the rim of the tank were designated as north (N), south (S), east (E) and west (W), thus dividing the pool into four quadrants (NW, NE, SE and SW).

On the first day, each mouse was allowed to swim freely in the pool for 60 s without the platform present in the pool. This free swim enabled the mouse to become habituated to the training environment. On days 2–5, mice were trained for 24 trials (six trials a day, with an intertrial interval of 30 s) to locate and escape onto the submerged platform. At the start of each trial, the mouse was held facing the perimeter of the water tank from different directions and dropped into the pool to ensure immersion. The latency from immersion into the pool to escape onto the hidden platform (maximum trial duration of 120 s) was recorded. On mounting the platform, each mouse was given a 30 s intertrial interval for rest and for learning and memorizing the spatial cues to reach the platform for escape. The testing procedure used during the four days of locating the hidden platform provides a measure of hippocampus-dependent spatial reference memory (Spiers et al., 2001).

On day 6, the mouse was subjected to a 120 s probe trial in which the platform was removed from the pool. The time spent in each quadrant (within a 120 s probe test) was recorded. In such probe trials of the water maze test, normal animals typically spend more time in the quadrant where the platform had been primarily located (days 2–5) than in other quadrants. Such probe trial is a measure of the strength of spatial learning or memory recall, the closest parallel to episodic memory in humans (Jeltsch et al., 2001).

2.5. Biochemical studies

Immediately after completing the behavioral tests, the animals were sacrificed by decapitation and the brains were dissected on ice. The complete forebrain was isolated (including the cerebral areas with the hippocampus and striatum) and after recording their wet weight, was frozen in liquid nitrogen and stored at −70 °C for determination of monoamines, lipid peroxides (TBARS) and glutathione content.

The forebrain weight and the relative forebrain weight with respect to the body weight after 30 days of experimental exposure to the food additives were also taken into consideration to record any deleterious effect on the forebrain weight.

2.5.1. Determination of monoamines

The monoamines were estimated using the modified method of Patrick et al. (1991). A 10% homogenate of forebrain tissue was prepared by homogenizing the tissues for 10 s in 0.1 M $HClO_4$ containing 0.05% EDTA, centrifuged at 17,000 rpm at 4 °C for 5 min. The supernatants were filtered using 0.45 μm pore filters and analyzed by high performance liquid chromatography (HPLC). The mobile phase consisted of 32 mM citric acid monohydrate, 12.5 mM disodium hydrogen orthophosphate, 7% methanol, 1 mM octane sulfonic acid and 0.05 mM EDTA. The mobile phase was filtered through a 0.22 μm filter and degassed under vacuum before use. μBondpak C18 column was used at a flow rate of 1.2 ml/min and the injection volume of the sample was 20 μl. The levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and serotonin or 5-hydroxytryptamine (5-HT) were calculated using a calibration curve and results were expressed as ng/mg tissue weight.

2.5.2. Determination of lipid peroxides

Lipid peroxides (LP) in forebrain tissue were determined spectrophotometrically as thiobarbituric acid-reactive substances (TBARS) according to the method of Ohkawa et al. (1979). The tissue samples were homogenized in 1.15% cold KCl with an Ultraturax homogenizer. After centrifugation at 3000 ×*g* for 5 min, an aliquot of supernatant was mixed with 2 ml of reaction mixture (containing 15% trichloroacetic acid and 0.375% thiobarbituric acid solution in 0.25 N HCl) and heated for 5 min in a boiling water bath. The tubes were cooled at room temperature and centrifuged at 1000 ×*g* for 10 min. The absorbance of supernatant was read at 535 nm against a blank that contained all reagents except the homogenate. Tissue lipid peroxide levels were quantified using an extinction coefficient of $1.56 \times 10^5\ m^{-1}\ cm^{-1}$ and expressed as nanomoles of TBARS formed per g tissue weight.

2.5.3. Determination of glutathione

Total glutathione (GSH) level in forebrain tissue was measured enzymatically by a slightly modified method of Mangino et al. (1991). Briefly, about 50 mg of brain tissues was homogenized with 1 ml 0.1 M perchloric acid plus 0.005% EDTA. The homogenates were centrifuged at 4000 rpm for 10 min and the supernatants were used for GSH assay. The reaction mixture consisted of the following three freshly prepared solutions: solution I, 0.3 mM NADPH; solution II, 6 mM 5, 5′-dithiobis(2-nitrobenzoic acid) and solution III, 50 U/ml glutathione (all chemicals are from Sigma). All three solutions were prepared with a stock buffer consisting of 125 mM NaH_2PO_4 and 6.3 mM EDTA at pH 7.5. At the time of the glutathione assay, 800 μl of solution I, 100 μl of solution II, and 10 μl of solution III were mixed in a quartz cuvette and placed in a dual beam UV–Vis spectrophotometer (Shimadzu UV160) at 30 °C. The enzymatic reaction was started by the addition of 100 μl of the supernatant and the absorbance was monitored for 3 min at 412 nm. The slope of the change in absorbance was used for quantitative estimation of total GSH by comparing the slope of the samples with a standard curve prepared with pure glutathione (Sigma).

2.6. Statistical analysis

The data were analyzed for variance (Bartlett's test for equal variance) and normality (Gaussian-shaped distribution) using the Kolmogorov–Smirnov goodness-of-fit test. As the data passed the normality test ($p > 0.10$), the elements found significantly different from the control groups were compared (within the experimental groups) with respect to the factors of individual and combined doses of MSG and ASM with the ANOVA with post-hoc testing using Tukey–Kramer Multiple Comparisons Test or Student–Newman–Keuls Multiple Comparisons Tests. All results were expressed as means ± SEM and significance was defined as $p < 0.05$ for all tests.

3. Results

3.1. Body weight and brain weight

Animals exposed to the food additives ASM and MSG, individually or in combination for one month caused significant depletion in their body weight gain compared to their respective control groups (Fig. 1). Both food additives individually caused significant ($p < 0.05$) depletion in body weight gain compared with the experimental groups and with their respective control. Depletion in body weight gain was evident on days 18, 24 and 30 of exposures (Fig. 1). However, the doses of MSG and ASM in the combination treatment had the most significant

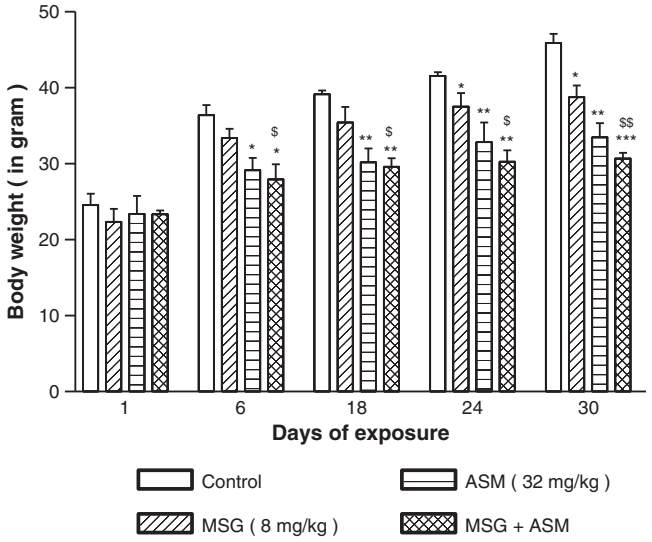

Fig. 1. Effect of the food additives MSG and ASM individually and in combination, on the body weight of the male mice recorded on days 1, 6, 18, 24 and 30 after exposure. *, **, and *** represent statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) compared to the control group and $, $$ and $$$ represent statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) compared with the treated experimental groups by ANOVA followed by Tukey's HSD post-hoc test and Student's t-test. Although differences within the groups of MSG and ASM were not significantly observed, the combined doses of MSG and ASM were highly significant throughout. ASM is aspartame and MSG is monosodium glutamate.

($p < 0.001$) effect throughout compared to their individual exposures and within the experimental groups (Fig. 1).

Observations on the wet weight of the forebrain tissue at the end of the total exposure period of a month showed that MSG had an insignificant effect, and the effect of ASM was slightly significant ($p < 0.05$), whereas the combined dose of MSG and ASM had a significant ($p < 0.01$) depleting effect compared to the control group. Furthermore, the combined dose had a significant ($p < 0.05$) effect on the forebrain weight as also observed by Tukey's test (Fig. 2A). However, reduction in forebrain weight remained insignificantly affected when their relative weight was assessed (Fig. 2B).

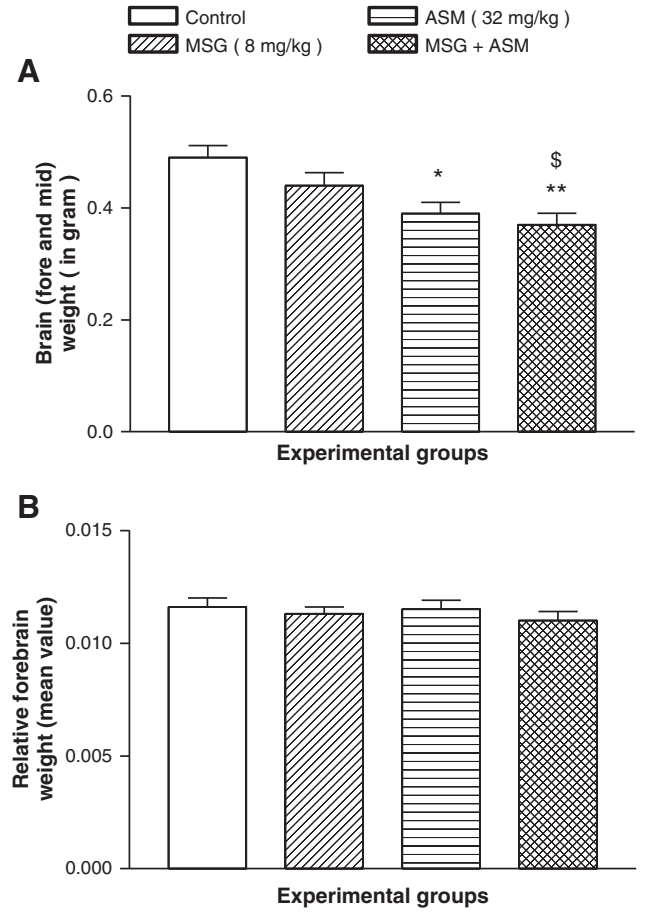

Fig. 2. Effect of the food additives MSG and ASM individually and in combination, on the actual wet weight of the forebrain (A) and on relative weight of the forebrain with respect to the body weight at 30 days after exposure (B) of the male mice. Statistical significance and abbreviations are the same as in Fig. 1.

3.2. Cognitive and learning ability

3.2.1. Active avoidance test in shuttle-box

In the shuttle-box active avoidance test, the total time taken by the food additive exposed animals during the trial period to enter the other chamber to avoid the shock treatment (latency of avoidance or escape latency response in seconds) was significantly ($p < 0.001$) greater in the animals exposed to the combined dose of MSG and ASM than in the animals exposed individually to MSG ($p < 0.05$) and ASM ($p < 0.01$) (Fig. 3A). Furthermore, for the number of avoidance during the reinforced trial period, the animals exposed to the combined dose of MSG and ASM were more significantly ($p < 0.001$) affected compared to the animals exposed to ASM and MSG individually which showed a lesser significant effect (Fig. 3B). Overall, the animals exposed to the food additives were poor learners and took significantly more time in avoiding the shock treatment; however, exposure to both additives in combination had a greater effect on the animals than exposure to the food additives individually (Fig. 3A and B).

3.2.2. Water maze test

Animals treated with ASM and MSG (individually and in combination) exhibited longer escape latencies to reach the platform compared to the control group ($p < 0.001$; Fig. 4A) in the water maze test. The latencies were longer since the treated animals exhibited slower swimming activities and frequently swam around the wall of the swimming tank rather than attempting to find the escape platform in the target quadrant. It was observed that all animals in the control group displayed gradual improvement in performance over the 4 days of testing (training) period whereas the treated groups did not exhibit any improvement and remained confused on all 4 days of training sessions. Animals exposed to the combined dose of MSG and ASM showed a highly significant ($p < 0.001$) cognitive imbalance throughout the 4 days (Fig. 4A).

The probe trial studies showed that food additive treated animals spent the least time significantly ($p < 0.001$) in the target (platform) quadrant relative to the other three quadrants. If the total time spent by the treated animals in the other three quadrants is combined, it is observed that all treated animals spent comparatively more time in the quadrants other than the target quadrant (Fig. 4B).

3.3. Biochemical studies

3.3.1. Levels of monoamines in forebrain tissue

There was alteration of 5-HT (Fig. 5A), DA (Fig. 5B) and DOPAC (Fig. 5C) in the forebrain of mice treated with the food additives in a combination form only. Exposure to ASM and MSG individually had no significant effect on the levels of these neurotransmitters; on the other hand, ASM and MSG in combination caused a significant ($p < 0.05$) depletion in the levels of all the monoamines (Fig. 5A–C) compared to the controls.

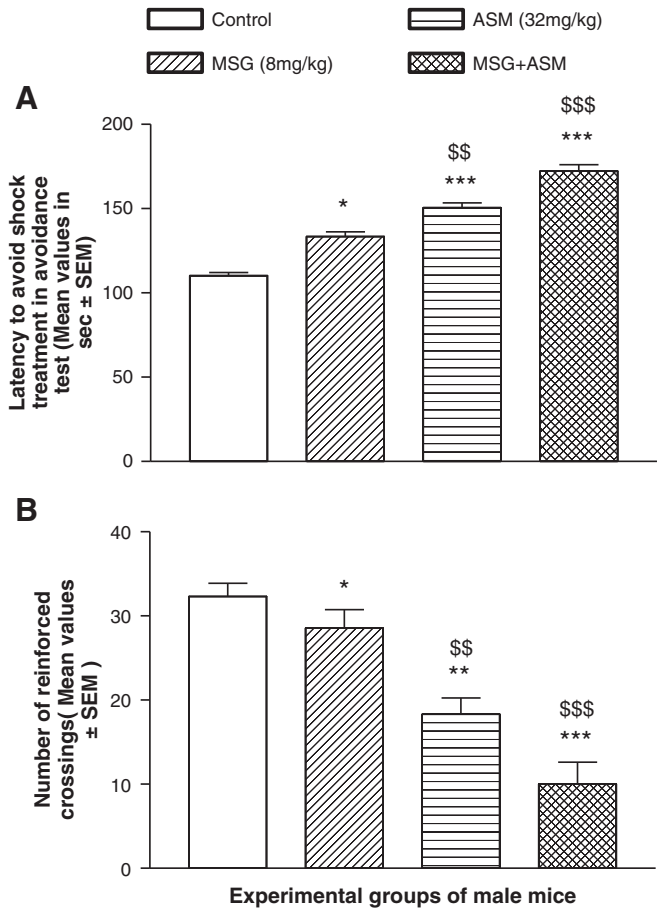

Fig. 3. Effect of the food additives MSG and ASM individually and in combination on the cognitive (learning) performance of the mice in the shuttle-box test showing the total time taken by the animals (total latency) in avoiding the shock treatment (A) and the number of reinforced crossing of the chambers by the animals (B) for avoiding the shock treatment during light and sound stimuli. Statistical significance and abbreviations are the same as in Fig. 1.

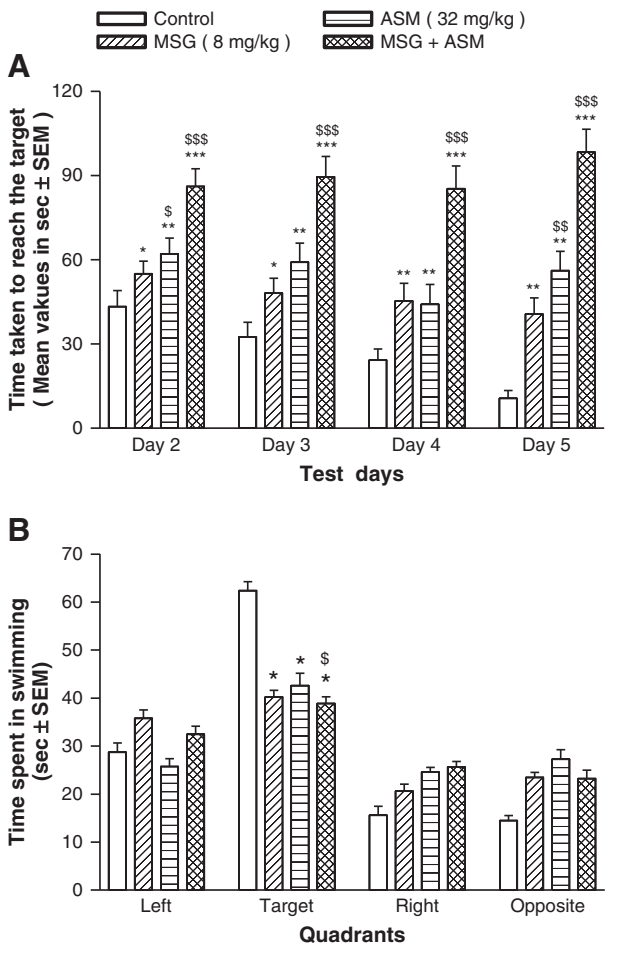

Fig. 4. Performance in the water maze of animals that were exposed to MSG and ASM individually as well as in combination. (A) Mean latency to reach the hidden platform (y-axis) on each testing day (x-axis) showing that animals exposed to food additives were slower in finding the platform (cognitive effect) than the controls on all four testing days. (B) The probe test shows that the treated animals spent lesser time in the target quadrant (showing decreased memory retention) than the control group. However, animals in quadrants other than the target quadrant spent their time in a confused manner showing no significant difference within the experimental groups.Statistical significance and abbreviations are the same as in Fig. 1.

3.3.2. Lipid peroxidation (TBARS) levels in the forebrain tissue

The lipid peroxidation (TBARS) level in the forebrain tissues was insignificantly increased in the ASM and MSG treated groups, whereas the MSG and ASM in a combination dose caused a comparatively significant increase ($p < 0.01$) compared to the control group (Fig. 6A).

3.3.3. Glutathione (GSH) levels in the forebrain tissue

On the other hand, depletion in the GSH level was observed to be significant ($p < 0.01$) in the forebrain tissue of the group exposed to ASM and MSG in combination only (Fig. 6B), whereas exposure to ASM and MSG individually had no significant effect on the level of GSH compared to the control group (Fig. 6B).

4. Discussion

Our results show that ASM and MSG when administered in a combination dose (ASM + MSG) affect behavioral (cognitive) parameters as well as biochemical parameters significantly. However, when ASM and MSG are administered individually, they only affect behavioral parameters significantly, but are unable to produce any significant effect on biochemical parameters. These food additives were toxic and were found to influence the body weight and various cognitive behavioral activities of the animals as well as the neurotransmitter levels and oxidative stress levels in their brain tissues. The forebrain wet weight apparently seemed to be affected by ASM and the combination of MSG and ASM (Fig. 2A), but taking into account the relative forebrain weight, it remained unaffected in all groups (Fig. 2B). Thus, it is most likely that due to the well known phenomenon of brain-sparing, the forebrain weight may have actually remained unaffected. But due to a significant effect on the forebrain wet weight (Fig. 2A) it will be presumed that the food additives may also affect the brain weight. Overall, it was observed that MSG and ASM were comparatively more toxic when administered in a combination dose (ASM + MSG together).

Animals treated with MSG and ASM in the present study had a reduced body weight compared to the control group and our results are in agreement with other reported studies (Lamperti and Blaha, 1978; Pizzi et al., 1978; Fisher et al., 1991; Zelena et al., 1998; Mistlberger and Antle, 1999; Park et al., 2000; Hlinak et al., 2005; Collison et al., 2010). However, some studies are in disagreement with these reported findings (Yu et al., 1997; Wong et al., 1997). The differences might be due to the variations in dose regimen and perhaps to different periods or duration of exposure and the type or strain of the animals used in these studies. The reduction in forebrain weight as observed in the present study is supported by another study where consumption of ASM by rats had demonstrated reduced body and brain weights (Brunner et al., 1979). Oral intake of ASM in mice results in

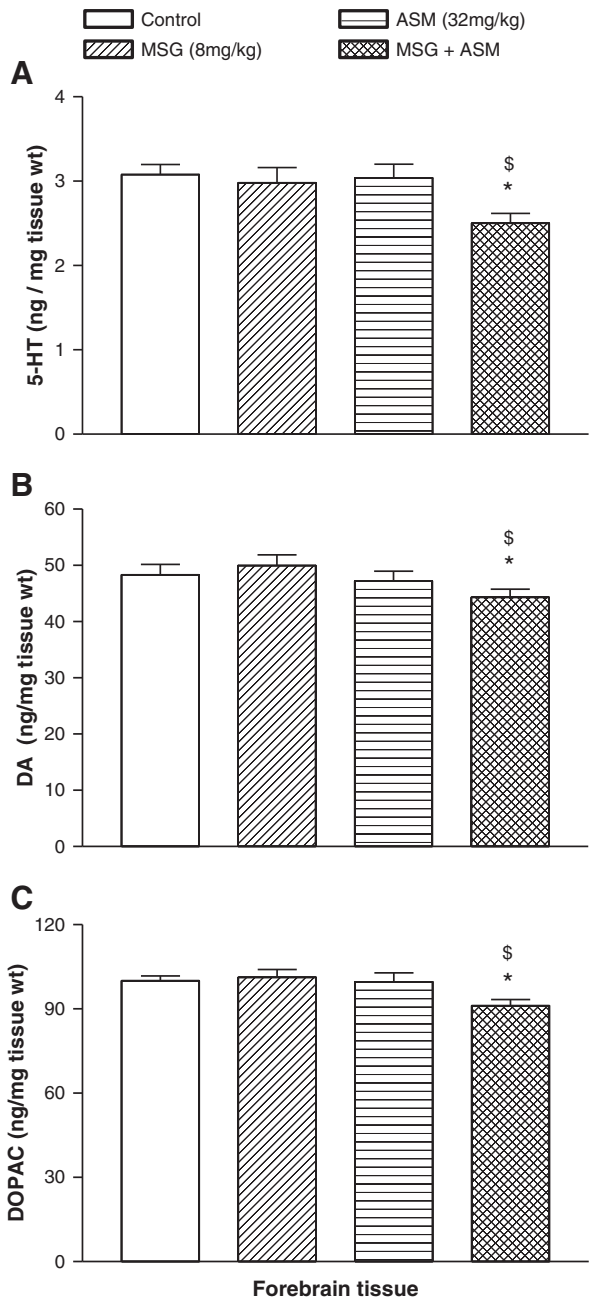

Fig. 5. Effect of the food additives MSG and ASM individually and in combination, on the levels of the neurotransmitters like (A) serotonin (5-HT), (B) dopamine (DA) and (C) the by-product of DA, dihydroxyphenylacetic acid (DOPAC), in the forebrain of the male mice. Only the animals exposed to the combined dose of MSG and ASM were significantly affected. Statistical significance and abbreviations are the same as in Fig. 1.

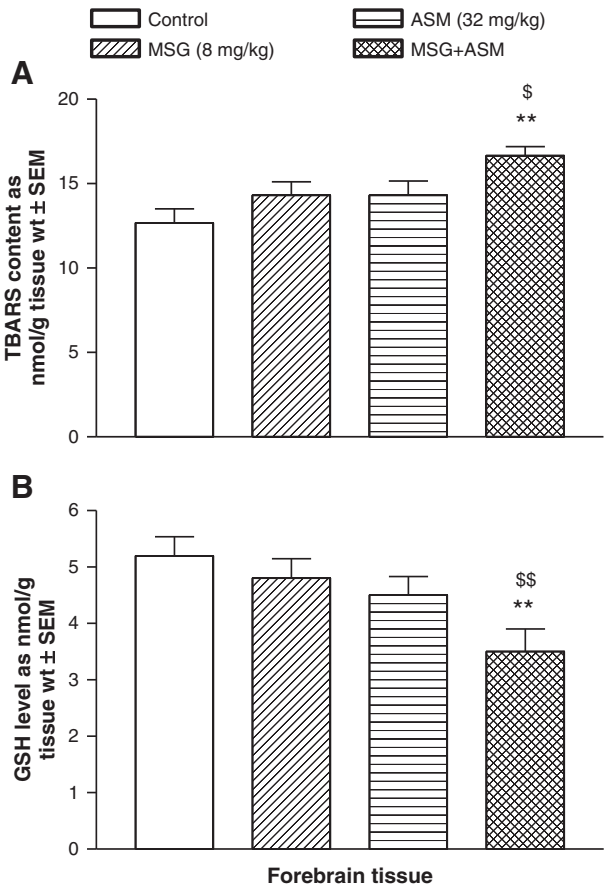

Fig. 6. Effect of the food additives MSG and ASM individually and in combination, on the oxidative stress depicted by increased level of TBARS (A) and decreased level of GSH (B) in the forebrain of the mice. In both indices, treatment with the combination dose of MSG and ASM had a more significant effect compared with the other treatments. Statistical significance and abbreviations are the same as in Fig. 1.

neuronal necrosis of several brain regions including the hypothalamus (Reynolds et al., 1976; Olney et al., 1980). This might be one of the possible factors in the present study behind the reduction in wet weight of the forebrain. However, the phenomenon of brain-sparing cannot be ruled out.

Mice exposed to food additives (MSG, ASM) in the present study showed deprivations in their learning and memory retention capabilities as observed from the active avoidance responses and from water maze tests. The present study is also supported by earlier findings (Fisher et al., 1991; Dubovicky et al., 1997; Wong et al., 1997; Engelmann et al., 1998; Ali et al., 2000; Sanabria et al., 2002; Olvera-Cortes et al., 2005). It is stated that the hippocampus in the brain plays a major role in controlling memory and regulating learning functions (Abu-Taweel et al., 2013). The forebrain region that includes the hippocampus area also plays a role in regulating mental processes that include advanced mental capacities such as mental learning, thinking and information storage and expressed mental or cognitive capacities (Jeltsch et al., 2001; Spiers et al., 2001). Thus exposure to MSG and ASM individually as well as in combination could be toxic by causing cognitive dysfunctions.

Significant alterations in the monoamines and oxidative stress were induced only when the two food additives MSG and ASM were administered in a combination dose where monoamines like DA, DOPAC and 5-HT and oxidative stress parameters like GSH were decreased, while TBARS were increased. Such neurotransmitters and oxidative stress indices have also been reported to be affected by MSG and ASM exposures in earlier studies (Dawson, 1983; Johnston et al., 1984; Dawson et al., 1989; Shinagawa, 1994; Bamforth et al., 1993) and these studies support the present findings keeping in mind the fact that the synergistic effects of MSG and ASM when administered in combination may be due to the interaction and cumulative effects of their individually caused effects that statistically remained insignificant in the present study. MSG and ASM alone showed no significant changes in biochemical parameters, most likely due to variations in the time period and age of exposures and the doses used herein. According to the literature, some of the effects of MSG may differ from the effects of ASM. For instance on the one hand Coulombe and Sharma (1986) reported alterations in neurotransmitter concentrations including DA, DOPAC and 5-HT in various brain regions of mice including the cerebellum, midbrain and other regions, which may be responsible for the reported clinical and behavioral effects associated with ASM ingestion. On the other

hand, treatment of mice with MSG produced lesions in the form of cell loss in the arcuate nucleus of their brain (Lorden and Caudale, 1986; Morley and Flood, 1989). Thus, overall, it is evident from the present study that MSG and ASM are significantly toxic when used in combination compared to their individual exposures, possibly due to their interactive and cumulative effects which, however, need further studies to confirm this possibility.

DA is one of the most prevalent catecholamine neurotransmitters in the brain, especially in parts responsible for movement, motivation and learning, such as the corpus striatum (Marinho and Manso, 1994). On the other hand, other neurotransmitters like 5-HT and the by-product of DA in the hippocampus and striatum areas in the forebrain region are reportedly involved in cognitive activities (Freitas et al., 2003, 2004; Tariq et al., 2008). Oxidative stress is a condition characterized by elevated levels of intracellular reactive oxygen species (ROS). Either ROS are free radicals or they break down to form free radicals (Xu et al., 2005). Thus, the oxidative stress along with disruptions in the neurotransmitters due to MSG and ASM exposures in a combination dose might be one of the possible reasons for the dysfunctions produced in the cognitive retention capacity of the treated animals in the present study.

It is concluded from the present study that although the effects of MSG and ASM found herein are in accordance with earlier findings, the present study differs from earlier reports from the results found for the combination dose point of view of MSG and ASM. The use of ASM and MSG in a combined dose is more significantly and synergistically effective than their individual use, and may add toxicity when taken jointly. However, the present study lays emphasis for further studies in micro-dissected and specific brain regions including more biochemical as well as histopathological parameters to establish the involvement of synergistic effects of MSG and ASM exposures in cognitive dysfunctions.

Conflict of interest statement

The authors have no conflicts of interest of any kind.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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