

Full Length Research Paper

Effect of monosodium glutamate and aspartame on behavioral and biochemical parameters of male albino mice

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The present study aimed to investigate the individual and combined effect of mono-sodium glutamate (MSG) and aspartame (ASM) on biochemical, blood parameters and neuro-behavioral aspects of mice. The results indicated that exposure induced many changes in fear and anxiety behavior. The non-social and social behavior of the exposed mice was significantly affected, showing an increase in the former and a decrease in the later stages, respectively. The elements of social behavior including attack, numbers of fights and bites, naso-nasal and naso-genital contacts were decreased significantly. The latencies to threat and attack and first bite were increased significantly. Locomotor activity and neuromuscular coordination (grip strength) were decreased in treated animals as compared to the control group. There was a significant decrease in the red blood cell count, packed cell volume, hemoglobin concentration, white blood cell count platelets count and testosterone hormone in the treated males. The activity of acetylcholinesterase enzyme decreased as compared to the control. In conclusion, the current study indicated that exposure to food additives MSA and ASM was dangerous to mice in relation to behavior and biochemical analysis. In addition, these food additives need more scientific researches to investigate their effect on other parameters.

Key words: Mono-sodium glutamate, mono-sodium aspartame, fear and anxiety, locomotory behavior, grip strength, acetylcholinesterase.

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 to cause toxic effects in humans and to

determine if safe conditions of use can be established. 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).

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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 learning difficulty (Abu-Taweel et al., 2014), 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 (Robinson 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, 1980). Acute ingestion of MSG has been associated with adverse symptoms that include general weakness, muscle tightness or tenderness, flushing or sweating, headache, paresthesias, arrhythmias and tachycardia in healthy individuals (Shimada et al., 2015).

Aspartame (ASM) is a dipeptide (L-aspartyl-L-phenylalanine methyl ester) and is used as an artificial sweetener that is now in wide and frequent use. It was discovered by chance over 25 years ago and was first used as a food additive 13 years ago (Lajtha et al., 1994). ASM is used in a variety of 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 (Nagasawa et al., 1974; Abu-Taweel et al., 2014). Aspartame is metabolized in the gastrointestinal tract into 50% of phenylalanine, 40% of aspartic acid and 10% of methanol. The degradation of aspartame may be due to both temperature changes and the time spent before usage. d-Phenylalanine, d-aspartic acid and methanol may be formed on exposure to heat, during shipping, baking, heated foods and beverages. Effect of aspartame consumption on behavior is the most important aspect to be considered because controversial reports do exist for aspartame (Ashok et al., 2014). The present study aimed to investigate the individual and combined effect of monosodium glutamate and mono-sodium aspartame on biochemical and neuro-behavioral aspects in mice.

MATERIALS AND METHODS

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 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.

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 naive 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 with 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. 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.

Behavioral studies

Social behavior in all male animals was measured in the "standard opponent" test and the "tube restraint test".

Anxiety behavior in the elevated plus-maze test

The elevated plus-maze (with 2 opened and 2 enclosed arms) is frequently used as a measure for evaluating the risk assessment and anxiety behavior of an ethologically derived animal model (Wall and Messier, 2001). The plus-maze was elevated to a height of 80 cm above the floor. The mice were individually placed onto the central platform facing one of the open arms and were observed for 5 min while freely exploring the maze. The animal was considered to have entered an arm when all four limbs were inside the arm. Duration of time spent in open and enclosed arms and number of entries in open and enclosed arms were measured during the test period. On completion of the test, the maze was cleaned with a 10% ethanol solution for control.

Standard opponent test

All males from each treatment category and control group were individually housed in new cages for 14 days. After this isolation period, these male animals were subjected to "standard opponent" tests under dim red lighting (ca. 8 lux). The docile and age-matched male "standard opponents" were rendered anosmic by applying 25 µl of 4% zinc sulphate solution to the nasal tract under ether anesthesia for three days prior to encounters. The anosmic 'standard opponent' intruders were introduced in the home cages of 'test animals' and the "standard opponent" test of each 'test animal' was observed visually for 500 s. The opponents were used to assess the selected "elements" of behavior as studied earlier by standard procedures (Brain et al., 1987; Ajarem and Ahmad, 1991; Abu-Taweel and Ajarem, 2008).

Tube restraint test

The males from treated and control group were used for the 'tube

restraint test'. The apparatus was based on the equipment described by standard procedure (Abu-Taweel et al., 2006) and consisted of a cylindrical transparent perspex tube 13 cm in length and with an internal diameter of 3.1 cm. One end of the tube was blocked by a perforated perspex wall through which a 2 cm long metal target was attached to a telegraph key/electronic counter arrangement. This enabled recording the number of bites directed by the restrained mouse towards the target. The test was conducted visually for 500 s under normal laboratory white lighting and temperature.

Motor activity test in automated activity meter

Spontaneous motor activity has been used extensively in rodents to study the pharmacological and toxicological effects of chemicals. Motor activity was measured using automated electronic activity meter (Ugo Basile, Comerio-Varese, Italy) (Kim et al., 2015). The horizontal and vertical motor activities were detected by arrays of infrared beams located above the floor of the testing arena. Each interruption of the beams on the x or y axis generated an electric impulse which was recorded on a digital counter. Each animal was tested separately and the motor activity was recorded for a period of 2 min in the activity meter (Abu-Taweel et al., 2013).

Neuromuscular coordination in grip strength meter

Neuromuscular coordination was measured using automated electronic grip strength meter (U.S. company products Biocompare). Device consists of two parts; first a square base is composed of Perspex material, ending with rectangular column length of 15 cm, on the column cylinder fixed by another column, and out of brass pieces length of about 5 cm (catching the mouse during the test). Test maintenance of mouse was started gently in the tail; it was placed in front of a copper piece. It remained in the front by raising the hind limbs when measuring the power base forelimbs. It was laid at the rear base to measure the tensile strength of the parties Quartet. Grip strength was measured by kg/m after two minutes (Ali et al., 2004).

Blood parameters

After completion of behavioral tests, the blood was collected from the retro-orbital plexus of the animals in heparinized tubes at the end of the experiments. Blood parameters namely, red blood count, packed cell volume, hemoglobin content, total white blood count and blood platelets were measured using the automated parameter hematology analyzer (T 450, USA).

Estimation of testosterone in plasma

The collected blood was centrifuged at 4000 rpm/min for 10 min and plasma was obtained and kept at -30°C until it was used for hormones estimation. Testosterone was estimated using hormone analysis instrument (Hitachi-Eleceys 2010-Roche Diagnostic, USA) by the method of electrochemiluminescence immunoassay.

Biochemical studies in brain tissue

The brain of animals were removed and gently rinsed in physiological saline (0.9% NaCl), and then blotted on Whatman filter paper. Their fresh weights were recorded, and organs were then frozen.

Brain homogenate preparation

A 10% (w/v) homogenate of each frozen brain was prepared in teflon-glass homogenizer at $4 \pm 1^\circ\text{C}$, centrifuged at 1000 xg for 10 min to remove cell debris and the supernatant was used for enzyme assays. The brain homogenate was prepared in an ice-cold phosphate buffer (0.067 M, pH 7.2) solution.

Estimation of AChE

The AChE activity in the homogenised brain tissue was estimated by the method of Ellman et al. (1961), utilizing acetylthiocholine iodide (ATCI) as substrate. The rate of production of thiocholine was determined by the continuous reaction of the thiol with 5,5-dithiobis-2-nitrobenzoate (DTNB) ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Spectrophotometric assay of enzyme activity was performed by adding 0.4 ml of the supernatant to a cuvette containing phosphate buffer (2.6 ml, pH 8) and 0.2 ml of 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (Sigma Chem. Co., St. Louis, MO, USA). After adjusting the absorbance to zero, 0.02 ml of the substrate acetylthiocholine iodide (Sigma Chem. Co., St. Louis, MO, USA) was added and change in absorbance over 5 m was recorded. The specific activity of AChE was expressed as μ moles of acetylthiocholine iodide hydrolyzed/min/g of wet tissue.

Statistical analysis

The data of standard opponent and tube restraint tests were compared within the experimental groups by the analysis of variance (ANOVA) and were subsequently analyzed using Mann-Whitney U tests. Data of fear and anxiety in Plus-Maze, locomotory behavior, neuromuscular activities in grip strength meter, blood parameters and biochemical analysis were compared within the experimental groups by the analysis of variance (ANOVA) using minitab computer programme and were subsequently analyzed by Student's t-test (Yamane, 1973).

RESULTS

Anxiety behavior in the elevated plus-maze test

Exposure to MSG and ASM both (individually and in combination) caused many disorders in fear and anxiety behavior. The treated animals spent more time in the closed arm ($P < 0.001$) while the time spent in opened arm was shorter ($P < 0.001$) in the control animals (Figure 1). Figure 2 shows that the number of entries of open arm decreased ($P < 0.001$) while the number of entries of closed arm increased ($P < 0.001$) in the treated animals as compared to the controls.

Social behavior (standard opponent and tube restraint tests)

The results indicate that exposure to MSG and ASM led to changes in social behavior of mice. The data on standard opponent and tube restraint tests (Tables 1A, B and 2) showed that MSG and ASM increased significantly nonsocial investigation, number of wall rears and rears,

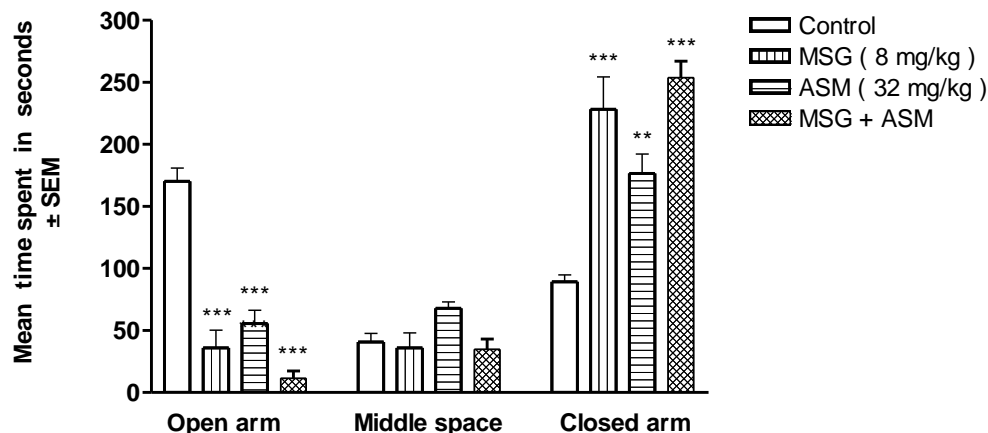


Figure 1. Effects of MSG and ASM on time spent in closed and open arms. ** and *** statistically significant at $p < 0.01$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

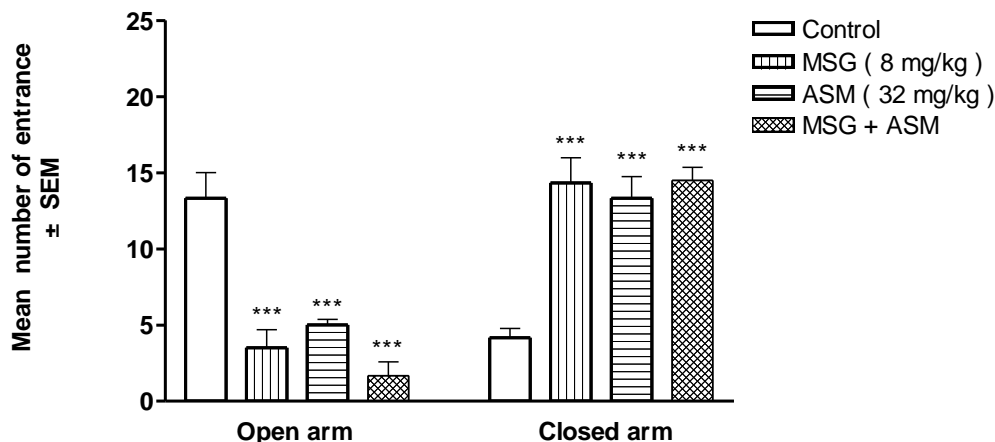


Figure 2. Effects of MSG and ASM on entries number of closed and open. *** statistically significant at $p < 0.001$, as compared to the control group by ANOVA and student's t-test.

latency to threat ($p < 0.05$, $p < 0.001$), attack and latency to first bite ($p < 0.05$, $p < 0.01$, $p < 0.001$), while it decreased significantly social investigation, time of defense, attack and displacement ($p < 0.05$, $p < 0.01$, $p < 0.001$), threat ($p < 0.05$), number of fights, naso-nasal and naso-genital contacts, and number of bites ($p < 0.001$). Furthermore, the data in these tables show that exposure in combination has more effect than individual toxicity of MSG and ASM.

Motor activity test in automated activity meter

Figure 3 shows the decrease in motor activity induced by exposure (individually and in combination) to MSG and ASM. Horizontal and vertical activities were reduced significantly ($p < 0.001$ and $p < 0.05$, $p < 0.001$ and $p < 0.001$,

respectively) as compared to the control group.

Neuromuscular coordination in grip strength meter

Exposure to MSG and ASM (individually and in combination) impact the neuromuscular coordination in mice. MSG and ASM reduced significantly neuromuscular coordination in treated animals ($p < 0.05$, $p < 0.01$ and $p < 0.001$), respectively as compared to their control (Figure 4).

Blood parameters

MSG and ASM exposure individually or in combination led to significant depletion in some of the observed blood parameters like the red blood cell count, packed cell

Table 1A. Effect of monosodium glutamate (MSG) and aspartame (ASM) on social behavior of male mice.

| Group | Median number (with ranges) of seconds allocated to behaviours like | | | | | |
|----------------|---|--------------------------|----------------------|-----------------------|------------------------|---------------------|
| | Nonsocial investigation | Social investigation | Defense | Threat | Attack | Displacement |
| Co | 150.00(94.30-170.60) | 224.00(214.10-241.30) | 18.00(15.20-20.70) | 20.00(13.30-30.00) | 64.00(50.40-100.20) | 20.00(4.90-56.60) |
| MSG (8 mg/kg) | 349.00*** (323.80-350.30) | 50.00*** (4.50-53.30) | 4.00*** (3.20-18.80) | 50.00** (37.40-70.80) | 30.00** (23.90-55.00) | 24.00 (12.00-46.60) |
| ASM (32 mg/kg) | 290.00*** (250.90-335.10) | 100.00*** (88.20-147.20) | 2.00*** (0.70-8.80) | 42.00 (19.00-56.80) | 30.00** (0.00-58.90) | 14.00* (9.50-46.80) |
| MSG + ASM | 362.00*** (320.00-418.00) | 41.00*** (28.100-100.60) | 2.00*** (0.00-7.40) | 14.00* (10.00-42.20) | 16.00*** (0.00-100.10) | 9.00** (2.10-25.30) |

*, ** and *** significantly different ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) from the control groups by ANOVA and Mann-Whitney U test.

Table 1B. Effect of monosodium glutamate (MSG) and aspartame (ASM) on social behavior of male mice.

| Group | Median number (with ranges) of acts and postures | | | | | | |
|----------------|--|-----------------------|--------------------|-------------------------------|---------------------------------|-----------------------|------------------|
| | Latency to threat (s) | Latency to Attack (s) | Number of fights | Number of Naso-Nasal contacts | Number of Naso-Genital contacts | Wall rears | Rears |
| Co | 15.00(5.00-30.00) | 62.00(20.00-70.00) | 20.00(15.00-21.00) | 27.00(23.00-29.00) | 18.00(14.00-22.00) | 13.00(10.00-16.00) | 9.00(8.00-11.00) |
| MSG (8 mg/kg) | 50.00(15.00-80.00) | 70.00(50.00-100.00) | 11.00(4.00-20.00) | 15.00(0.00-18.00) | 8.00*** (3.00-12.00) | 10.00(8.00-19.00) | 7.00(5.00-8.00) |
| ASM (32 mg/kg) | 120.00* (90.00-200.00) | 200.00(120.00-220.00) | 27.00(0.00-43.00) | 43.00** (42.00-81.00) | 7.00*** (6.00-9.00) | 30.00*** (2.00-52.00) | 7.00(4.00-20.00) |
| MSG + ASM | 350.00*** (40.00-70.00) | 212.00(0.00-365.00) | 5.00(0.00-55.00) | 25.00(15.00-42.00) | 3.00*** (1.00-4.00) | 10.00(9.00-20.00) | 6.00(5.00-10.00) |

*, ** and *** significantly different ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) from the control groups by ANOVA and Mann-Whitney U test.

volume, hemoglobin content, white blood cell count and platelets count in the males (Figure 5A, B, C, D and E).

Testosterone

Testosterone in male (Figure 6) mice depleted significantly ($p < 0.01$, $p < 0.001$) due to MSG and ASM exposure, respectively. This depletion increased significantly in the combination ($p < 0.001$) than individually as compared to the control.

Acetylcholinesterase enzyme

Figure 7 shows that the activity of acetyl-

cholinesterase (AChE) enzyme decreased significantly ($p < 0.001$) in MSG and ASM animals as compared to the control.

DISCUSSION

Food additives are substances that are part of a food product when added (intentionally or unintentionally) during the processing or production of that food. They include using salt to preserve meats, adding herbs or spices to foods, or pickling foods in vinegar solutions. However, concerns about food additives most often relate to artificial ingredients added to them (Rangan and Barceloux, 2009). The safety of the two food additives, L-glutamic acid or mono-sodium

glutamate (MSG) and Aspartame, 1-methyl N-L-alpha-aspartyl-phenylalanine (ASM) were examined widely and fears of toxic effects were expressed (Rothman and Olney, 1995).

Monosodium-glutamates or MSG carries the number 621 in food additives. MSG occurs naturally in many plants such as tomatoes, spinach and grapes. This form of MSG is bound to proteins and is in low concentration. MSG is a flavor-enhancing food additive used in Asian cooking and also commonly found in fast foods as well as commercially packaged food products such as chips, crackers, soups and soup mixes, lunch meats, salad dressings and many others (Yu et al., 1997). Some people find that consuming MSG, especially in large quantities, can trigger various side effects and symptoms,

Table 2. Effect of monosodium glutamate (MSG) and aspartame (ASM) on social behavior of male mice.

| Treatment group | Measures (median values with ranges) | |
|-----------------|--------------------------------------|-------------------------|
| | Latency to first bite (s) | Number of bites |
| Control | 15.0(10.0 - 40.0) | 70.0(45.0 - 96.0) |
| MSG 8 mg/kg | 85.00 ** (0.00 - 150.00) | 14.00 ** (0.00 - 54.00) |
| ASM 32 m/kg | 132.0 *** (0.0 - 180.0) | 13.0 *** (0.0 - 54.0) |
| MSG + ASM | 35.00* (0.00 - 150.00) | 5.00 *** (0.00 - 15.00) |

*, ** and *** significantly different at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, from the control group by ANOVA and Mann-Whitney U test.

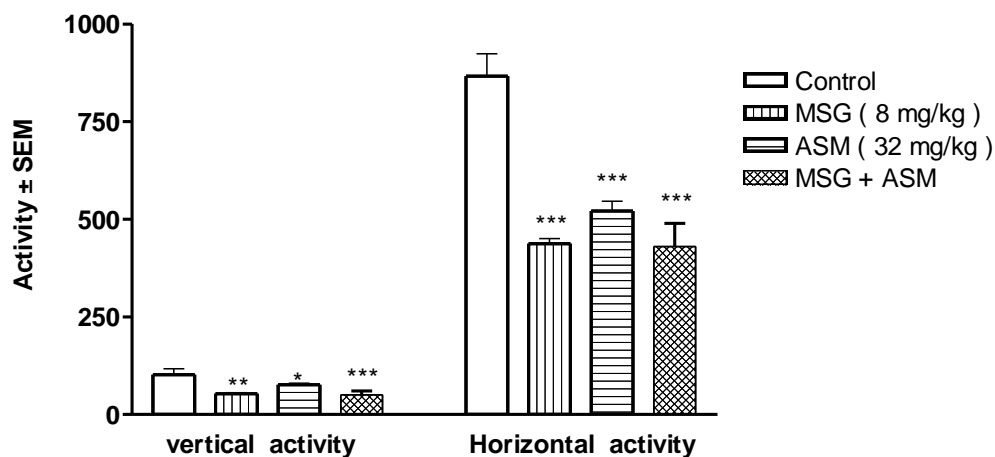


Figure 3. Effects of MSG and ASM on motor activity of mice. *, ** and *** statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

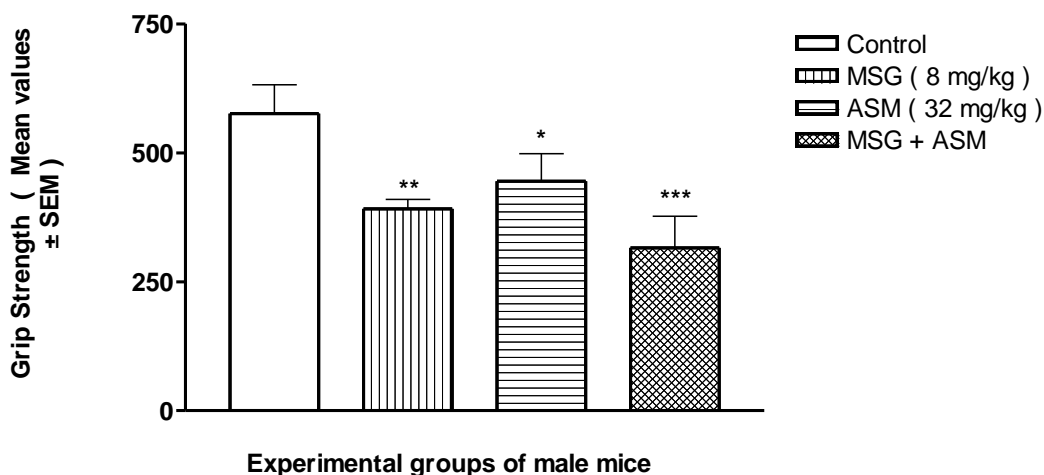


Figure 4. Effects of MSG and ASM on neuromuscular coordination of mice in grip strength meter. *, ** and *** statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

including headaches, nausea, dizziness, rapid or irregular heartbeat, flushing or excessive sweating, skin rash, numbness, intense thirst, lethargy or sleepiness, ringing

ears and tingling in the mouth (MSG Standard, 2007).

The number of aspartame in food additives is 951 and it took 20 years of debate before it was approved by the US

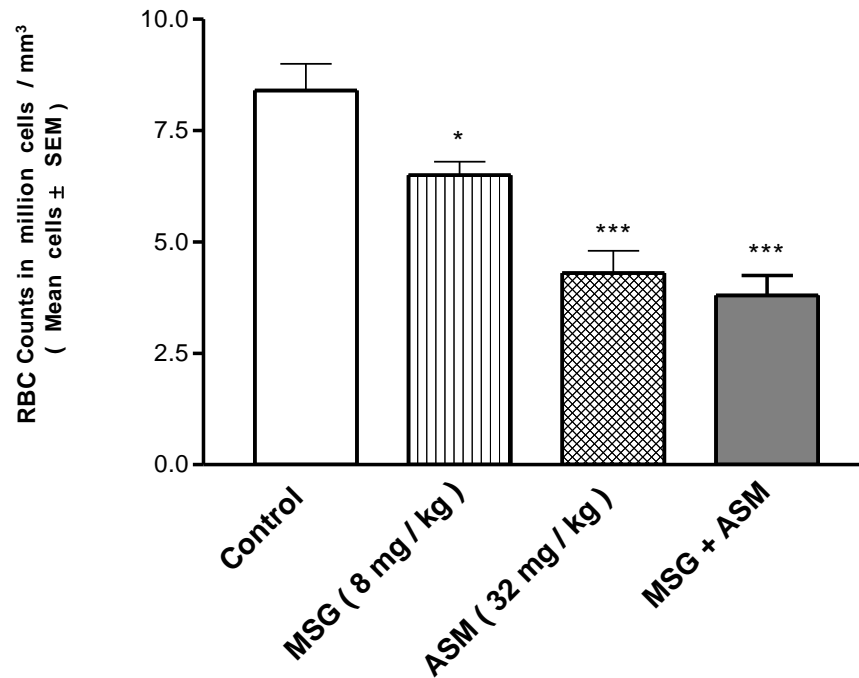


Figure 5A. Effects of MSG and ASM on RBC counts. * and *** statistically significant at $p < 0.05$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

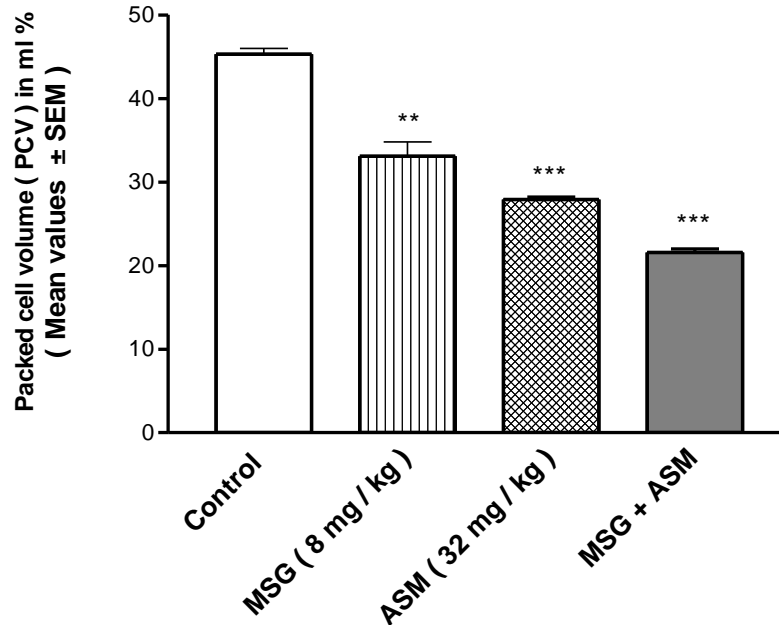


Figure 5B. Effects of MSG and ASM on packed cell volume. ** and *** statistically significant at $p < 0.01$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

Food and Drug Administration (FDA). Aspartame is made up of methanol (10%), phenylalanine (50%) and aspartic acid (40%). ASM is a widely used artificial sweetener in

soft drinks and low calorie food. There have been reports of adverse neurological effects such as headache, insomnia and seizures after ingestion of aspartame

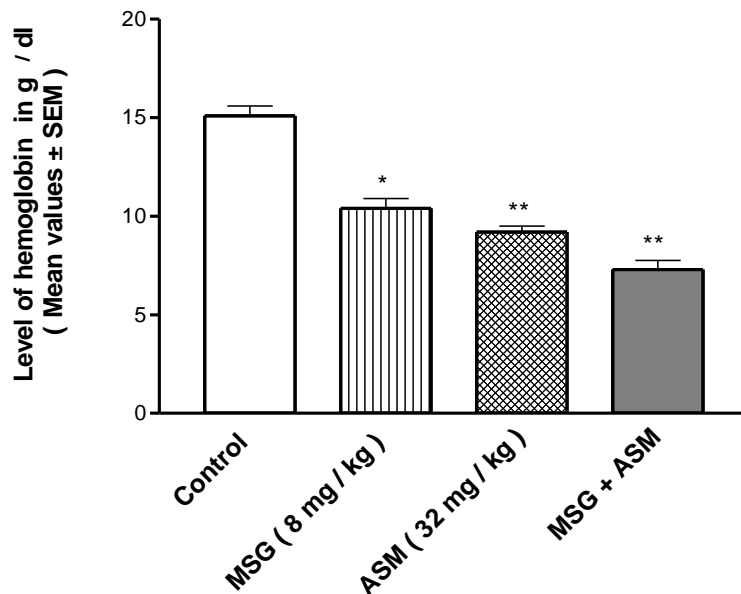


Figure 5C. Effects of MSG and ASM on hemoglobin concentration. * and ** statistically significant at $p < 0.05$ and $p < 0.01$, respectively, as compared to the control group by ANOVA and student's t-test.

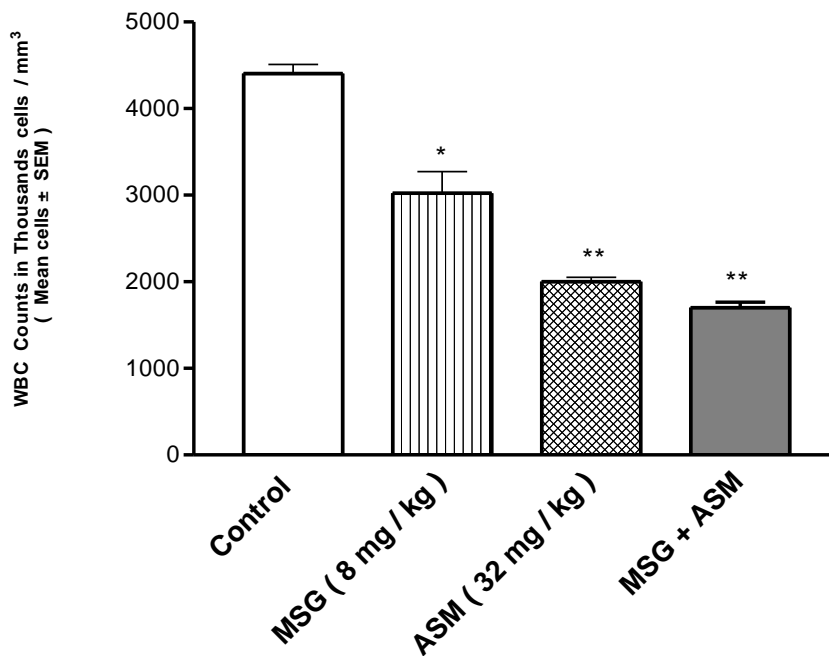


Figure 5D. Effects of MSG and ASM on white blood cell count. * and ** statistically significant at $p < 0.05$ and $p < 0.01$ respectively, as compared to the control group by ANOVA and student's t-test.

(Bergstrom et al., 2007). The present study and previous studies suggested that exposure to monosodium glutamate (MSG) or aspartame (ASM) is very dangers.

The term anxiety covers four aspects of experiences an individual may have: mental apprehension, physical tension, physical symptoms and dissociative anxiety

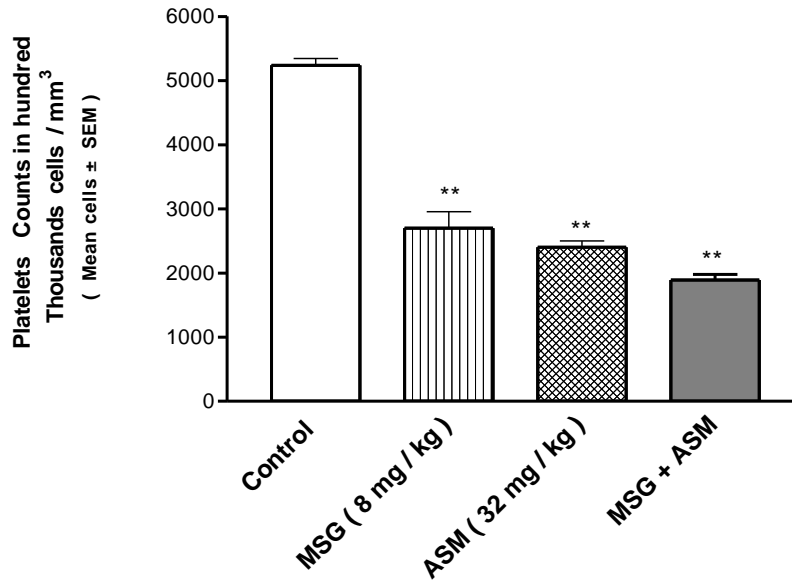


Figure 5E. Effects of MSG and ASM on platelets count. ** statistically significant at $p < 0.01$ as compared to the control group by ANOVA and student's t-test.

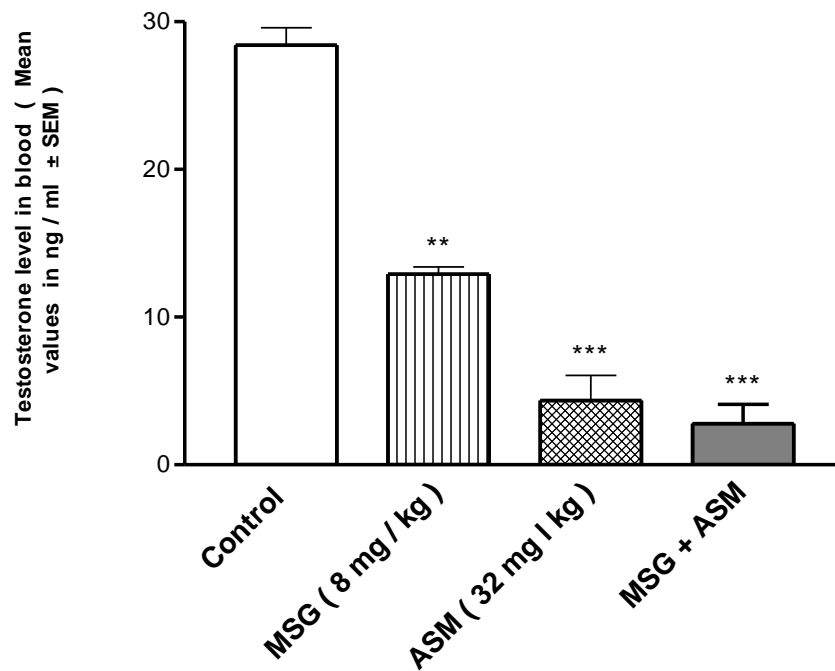


Figure 6. Effects of MSG and ASM on testosterone level in plasma. ** and *** statistically significant at $p < 0.01$ and $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

(Pizzi and Barnhart, 1975). The single exposure to food additives (MSG, ASM) or synergistic led to many disturbances in the behavior of fear and anxiety. The residence time in the closed arm and the number of times

it enter closed arm increased, while the residence time in the open arm and the number of times it entered the open arm decreased as compared to the control group. The present study results agreed with that of Caputo

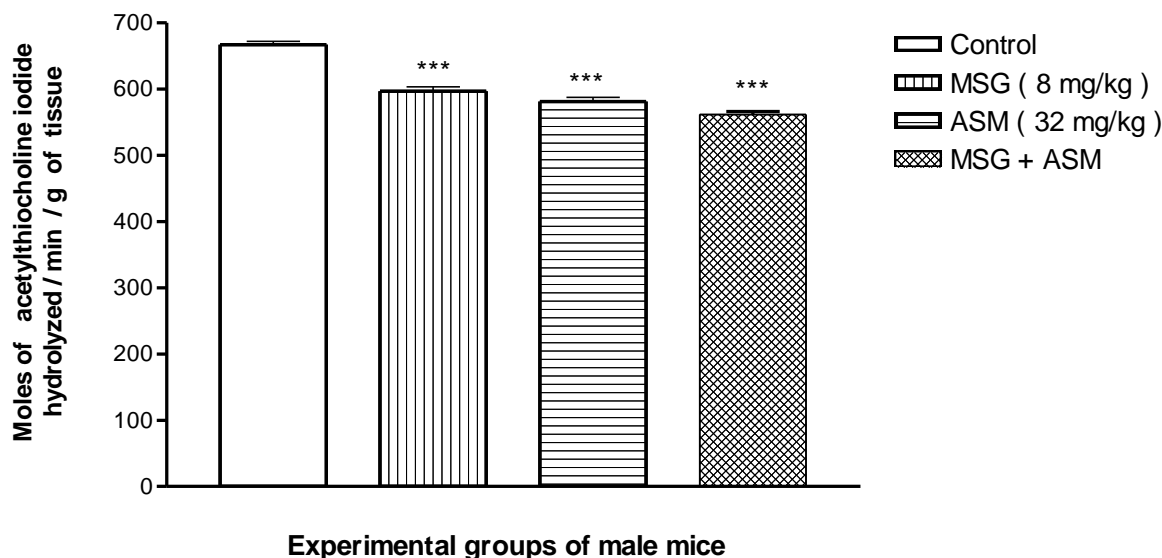


Figure 7. Effects of MSG and ASM on Acetylcholinesterase activity. *** statistically significant at $p < 0.001$ respectively, as compared to the control group by ANOVA and student's t-test.

et al. (1996).

Rosen and Schulkin (1998) justified the changes in the behavior of concern to the imbalance in the hippocampus and amygdala regions which play an important role in the onset of symptoms as upset of their electroencephalography (EEG) and increases in their blood flow when diagnosing the disease in those infected. Studies carried out by researchers proved that aluminum affects the hippocampus area (Beas-Zaratea et al., 2002), and discovered that the area of mice brain die due to cellular apoptosis and necrosis when exposed to these food additives commonly available in the market (Gonzalez-Burgos et al., 2001). The changes in the anxiety behavior may be caused by alternations of essential elements for body growth like iron and zinc that result from exposure to MSG and ASM (Collison et al., 2010).

Similar mechanisms of these food additives and lead influence adrenocorticotrophic hormone (ACTH). Lead increases this hormone that is produced by the pituitary gland and activates the adrenal cortex to increase the secretion of corticosterone hormone. These hormones increase pressure and anxiety (Nieto-Fernandez et al., 2006). This is done through overlapping element in the axis that reaches the hypothalamus with pituitary and adrenal glands called hypothalamic-pituitary-adrenal gland axis (Virgolini et al., 2006). Lack of weight of adrenal gland might lead to the secretion of corticosterone hormone. Some scientific reports indicated that exposure to food additives (MSG, ASM) reduced relative weight of adrenal, pituitary and thyroid glands (Zelena et al., 1998).

Some scientists showed the existence of a potential link between anxiety disorders and imbalance in the dynamic equilibrium of several neurotransmitters of the

brain, such as serotonin, dopamine, noradrenaline and amino acids (Tsunoda, 1998). Cecci et al. (2002) pinpointed the overlapping imbalance in neurotransmitters like mental disorders, cognitive and neurological disorderly attention, anxiety and aggression. Some research works show anxiety disorders change serotonin, which is linked to changes in mood and anxiety (Shinagawa, 1994). Studies have shown those changes in serotonin in cortex, hippocampus, striatum, hypothalamus, the olfactory lobe, cerebellum and brain stem in rats exposed to MSG and ASM (Kristova et al., 1998).

It could be that alternations in some brain peptides cause increased anxiety in mice treated with food additives in the current study, such as peptides of bombesin (BB). Merali et al. (1999) found that bombesin (BB), responsible for loss of appetite and increased sense of false satiety (Sidhu and Nehru, 2003), also encourages anxiety and stress by inducing secretion of the ACTH hormone. This hormone activates adrenal cortex hormone, which helps the emergence of fear, anxiety, loss of appetite and lack of physical activity (Koob, 1999). Among other neuropeptides causing anxiety peptides factor prompts adrenal cortex Corticotropin-releasing factor (CRF), which is composed of forty-one amino acids that spark at the initial excitation of HPA to counter pressures. It also represents the link between the nervous system of self-autonomic nervous system and some components of behavior to control and avoid damage. It also participates in the feedback process for hypothalamus response to external pressures (Spiga et al., 2006).

Social behavior reflects group of animals' relationship and the extent of their ability to interact and exchange

things within the group. Our results indicated that the single exposure to food additives (MSG, ASM) or synergistic interaction led to many disturbances in social behavior. The non-social and social behavior of the exposed mice was significantly affected; there was an increase in the former and a decrease in the later, respectively. The elements of social behavior including attack, number of fight, naso-nasal and naso-genital contacts were decreased significantly. The latencies to threat and attack were also increased significantly. Overall, the results indicate that social behavior is significantly decreased due to exposure to MSG and ASM. Conversely, anti-social behavior and its elements like fears, in treated animals were increased significantly. Our results agreed with studies of Ramanathan et al. (2007).

Changes in social behavior caused by MSG and ASM in the nervous system affect the olfactory nerve which controls the sense of smell in mice (Park et al., 2000). It has been shown also that exposure to food additives leads to the appearance of many behavioral changes, like aggressiveness and changes in activity as a result of damage caused by MSG and ASM in the hippocampus.

Food additives work to reduce androgens which indirectly affect the axis connecting the pituitary gland. This has negative effects on social behavior and the relative weight of members of the sex-producing hormone (Sun et al., 1991). Deficiencies of the testosterone hormone might cause social behavioral changes because of its importance in the regulation of aggression in mammals (Terry et al., 1981). Studies have indicated that exposure to MSG and ASM negatively affects the concentration of neurotransmitters. This suggests it causes changes in social behavior, increases isolation and lack of social movements in the current study. Current study indicated that treatment with food additives led to decreased locomotion activity and neuromuscular coordination (Grip strength) in treated animals compared to the control group.

Food additives affect the composite structure and function of neurons in the hands and feet, hurt the process of molecular adhesion between nerve cells, disrupt the process of cellular communication between neurons, astrocytes. These happen through the influence of the cellular communication channels with gaps or by changes in the function or installation of structural proteins (Sakr, 2004), resulting in imbalance in the transfer of nerve impulses, or reduction in the speed of nerve impulses and conduction velocity (NCV). This is because myelin membrane is removed (Demyelination) which covers axes neurons. The composite materials that lead to the breakdown of nerve cells in the nervous system reduce or delay access commands to the muscles, resulting in a slow response.

Aspartic acid, another ASP hydrolysis product, is a dicarboxylic amino acid that may exert toxic effects when administered at very high doses; although species

susceptibility varies considerably (Simintzi et al., 2007). The observed lowered enzyme activities may be due to an increase in reactive oxygen species and intracellular Ca^{2+} concentrations by the metabolite, as reported previously (Sureda et al., 1996). Free radical attacks on unsaturated bonds of membrane fatty acids result in an autocatalytic process called membrane lipid peroxidation, which can impair the function of membrane AChE. MeOH is totally absorbed and may interact with lipids of the mice forebrain cell membrane and/or protein parts of mice forebrain AChE, resulting in a reduction of its activity (Oyama et al., 2002).

Overall, the present study concluded that MSA and ASM were dangerous to behavioral and biochemical analysis in mice. In addition, these food additives need more scientific researches to investigate their harmful effects on other parameters.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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