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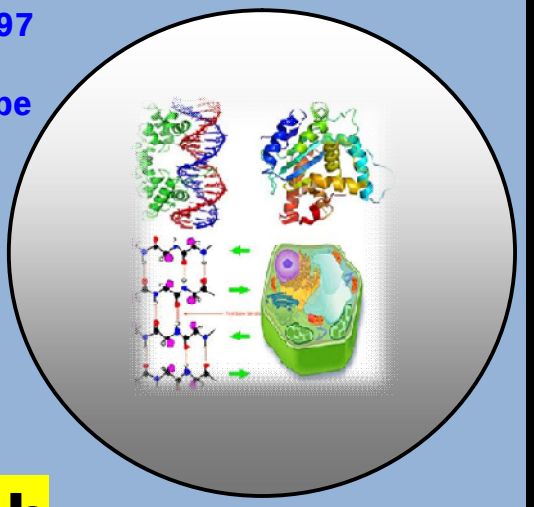

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Effects of Monosodium Glutamate on Some Biochemical and Hematological Parameters in Adult Rabbits and Potential Protective Effect of Soybean Oil

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ABSTRACT

Monosodium glutamate (MSG) is the most commonly used flavoring agent all over the world. In order to enjoy food, taste and flavor are necessary. Several reports of toxicity studies on MSG are contradictory. Therefore, there is growing concern regarding the safety of MSG as food additive. In the present study, the effect of orally administered MSG on some biochemical and hematological parameters were investigated in adult rabbits. Following a 21-day treatment with 3 mg/kg body weight of MSG daily, and 3 mg/kg body weight of MSG + 1ml /kg body weight soybean oil. There were significant changes in RBC, MCV, MCH, WBC counts and platelets. Significant changes were also observed in some biochemical parameters studied, including indices of Triglycerides, albumin, urea, uric acid, creatinine, calcium concentration and alkaline phosphatase, AST and ALT activities. In the present study soybean oil administration concomitantly with MSG reduced MSG- induced lesions in a significant manner.

Key words: Monosodium glutamate, Biochemical and Hematological Parameters, Liver Function, Kidney Function, Soyabean Oil and Rabbits.

INTRODUCTION

Monosodium glutamate (MSG)is the sodium salt of the non-essential amino acid glutamic acid, one of the most abundant amino acids found in nature. MSG contains 78% of glutamic acid, 22% of sodium and water Samuels, (1999).

A tasteless food even when composed of required nutritional elements is usually rejected. Therefore food flavoring is important especially in elderly persons who have irreversible chemosensory deficit. In such individuals, it is necessary to improve appetite to ensure adequate dietary intake. Flavor enhancers are also useful in situations where salt intake needs to be reduced such as in hypertension Chi, and Chen, (1992).Glutamate is also produced in the body and plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state of animal such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato IFIC, (1994).

Monosodium glutamate (MSG) is a compound popularly used to enhance food taste and flavor. It is one of the non-essential amino acids that are synthesized endogenously. It is a component of many proteins and peptides. When bound to proteins, glutamate is tasteless. The sweet umami taste and flavor becomes perceptible only when free glutamate dissociates from proteins during the processes of fermentation, ripening and cooking Yoshida, (1998). Tomatoes, cheese and mushrooms contain large quantity of glutamate and are thus used to enrich the taste and flavor of foods. However, the use of excess amount of glutamate actually makes food more distasteful rather than impart good taste Jinap, and Hajeb (2010) .MSG is produced commercially by fermentation of molasses; it is used in home cooking, restaurants and for industrial food production around the worldFarombi, and Onyema, (2006). Consumption of glutamate has been linked to obesity andmetabolic syndrome independent of physical activity andcalorie intake He, et al.,(2008) . Some researchers have also reportedneurotoxic effects of glutamateCortese, and Phan., (2005).Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animalBiodun and Biodun(1993). MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches. In addition to these MSG symptom complex, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfortGeha, et al,. (2001).When MSG is added to food, itprovides a flavoring function similar to naturallyoccurring free glutamate which differ from the fourclassic tastes of sweet, sour, salt and bitter.MSG has a toxic effect on the testis by causing a significant oligozoospermia and increase abnormal sperm morphology in a dose-dependent fashion in male Wistar rats Onakewhor et al., (1998). It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphologyOforofuo et al., (1997) .The Food and Drug Administration (FDA) of the United States reports that MSG is safe and that it should be maintained on the “Generally Recognized as Safe” (GRAS)-list of foods. MSG is thus reportedly permitted as a safe food additive that needs no specified average, daily intake or an upper limit intake requirement Rogers and Blundell., (1990).Considering the discrepancies in the literature and the growing safety concern for the use of monosodium glutamate, there is need for further studies on this important food additive.

In this study, the effects of monosodium glutamate and the protective effects of soybean oil in some hematological and biochemical parameters in adult domestic rabbits were studied

MATERIAL AND METHODS

This research study was carried out between March to April 2014 in the Biology Laboratory of the faculty of Science, Islamic university- Gaza.

Chemicals

Monosodium glutamate (MSG) was purchased from Sigma chemical company (USA). All other chemicals used were of analytical grade.

Experimental Animals

Healthy rabbits weighing between 1000 and 1200g were purchased from the local markets. They were kept in a well-ventilated and hygienic laboratoryenvironment. Rabbits were kept in plastic cages with wire mesh covers and maintained under the following conditions: temperature (200C– 210C), relative humidity (40% - 60%) and a light /dark cycle of 14 and 10 hours. The rabbits were allowed free access to standard rabbits feed pellets and clean water ad libitum.

Experimental Procedure

Rabbits were randomly divided into 3 groups of 6 rabbits each. Group 1 served as the control group and received normal saline (3ml/kg body weight/ day). Group 2 were treated with 3 mg/kg body weight of MSG daily for 21 days and Group 3 were treated daily with 3 mg/kg body weight of MSG + 1ml /kg body weight soybean oil for 21 days. All drugs were administered orally by gastric tube. On the 21st day (D21) of the dosing period, all the animals were sacrificed by bleeding using dissecting kit and blood samples collected in plastic test tube and allowed to stand for 30 min. to ensure complete clotting. The clotted blood samples were then centrifuged at 3000 rpm for 10 minutes with the clear serum samples was subsequently used for biochemical assays. Whole blood was used for hematological study.

Hematological and Biochemical Studies

Hematological parameters

Determination of hematological parameters was carried out using an 18 automated parameter hematology analyzer. ABX Micros 60 from Horiba ABX. France.

Biochemical Parameters

Serum glucose, triglycerides and total cholesterol were determined using the method described by Trinder,(1969); Fossati and Prencipe (1980) and Allain et al., (1974) respectively. Serum urea measurement was based on the cleavage of urea with urease (Berthelot's reaction) according to Fawcett and Scott(1960), Serum Uric Acid was determined by Uricase /POD method using Bio Systems kit, Spain Fossati, P. and Prencipe, L. (1982). Serum creatinine was measured without protein precipitation according to Bartels et al., (1972). Serum total protein was determined according to biuret reaction ass designed by Armstrong and Carr, (1969). The kits were purchased from Biotech laboratories, UK. Serum albumin was determined using RANDOX reagent kits, following their instruction manual according to the method of Doumas et al., (1971).The concentrations of globulins (g/dL) were equal to Total protein –Albumin. The activities of serum AST and ALT were determined according to the method of Reitman and Frankel, (1957). The measurement of serum ALP activity was based on the method of Bessey et al., (1946). Estimation of serum Na+ and K + was conducted by Flame Photometric method (Tietz, 1990). Estimation of serum Ca++ was done by Gitelman (1967) method.

Data analysis

Data were computer analyzed using SPSS version 11.0 for windows (Statistical Package for the Social Sciences Inc. Chicago, Illinois, USA). Means were compared by independent-samples test, $p<0.05$ was considered as significant. Percentage change was also calculated Tello, and Crewson, (2003).

RESULTS

The present study demonstrated that MSG at the doses administered did not cause any significant change in packed cell volume (PCV), hemoglobin concentration (HB) and MCHC. While, total red blood cell(RBC) and platelets counts were significantly decreased (<0.01) and MCV, MCH and white blood cell (WBC) countswere significantly increased (<0.01) in both treated with MSG alone and treated with MSG+ soybean oil compared to controlas shown in table (1).

Data in table(2) showed that, anon-significant increase in the glucose of the rabbits were observed after administration of MSG alone($p>0.05$) and decreased non-significantly after administration of MSG and Soybeanoil ($p>0.05$).A significant increase in the Triglyceridesof the rabbits was observed after administration of MSG alone and MSG + Soybean Oil (<0.01).Whereas,a non-significant changes inCholesterolconcentrationof the rabbits were observed after administration of MSG alone and MSG + Soybean Oil (>0.05).

The determined mean value of total protein in serum of control rabbits was 8.88 g/dl. A serum sample of treated animals was 8.11 and 7.10 g/dl. The 8.67% and 20.05% reduction in serum total protein of MSG alone and MSG + soybean oil administration to rabbits failed to reach the statistical significance level (>0.05) (Table 3). Albumin concentration decreased significantly (<0.01) after administration of MSG alone and MSG + Soybean Oil compared to control. Globulin concentration showed a significant increase after administration of MSG alone (8.29%), and a non-significant decreaseafter administration of MSG+ soybean oil(-19.60%). Blood serum urea of the rabbits administered with MSG alone and MSG + Soybean Oil showed significant increase(<0.01) as compared with urea mean values of the control. Concentration of creatinine in serum samples of control and treated animals were, respectively, 0.90, 1.10 and 1.0mg/dl. Serum creatinine of the treated rabbits was increasedsignificantlyas compare to control (<0.01). Uric acid concentrations were also increased significantly with percentage change of 97.59 and 71.33% respectively as compared to control.

Table (4) showed that there was no significant variation obtained in theserum sodiumand potassiumtested when compared with control.Whereas, serum calcium showed significant increases in both treated groups as compared with control.

The activity of the serum enzyme alkaline phosphatase increased significantly by MSG administration at concentration of 40.1 U/L and increased non-significantly by MSG +soybean oil administration at concentration of 35.95. U/L. On the other hand,highly significant ($p<0.01$) increase in the serum aspartate aminotransferase(AST) and ALT were observed in the MSG-treated rabbits compared to the control rabbits (Table 5). AST had activity means of 31.90, 40.55 and 38.90 U/L in serum of the control, MSG and MSG + soybean oil treated adult rabbits respectively.

DISCUSSION

The literature is full of contradictory reports on the level of toxicity of MSG in animal and human experiments. Previous studies suggest that MSG increases, decreases orhas no effect on the body weight of rodents. Kondoh and Torii (2008), reported that MSG treated rats had a significantly smaller weight gain, reduced abdominal fat mass, and lower plasma leptin levels, compared to control rats. Likewise there are reports suggesting that MSG could be toxic to erythrocytes and also cause deleterious changes in hematological and biochemical parameters Ashaolu, et al.,(2011) and Meraiyebu, et al., (2012). The present study demonstrated that MSG and MSG + soybean oil at the dose administered did not cause any significant change in packed cell volume (PCV), hemoglobin concentration (HB) and MCHC. These results are in agreement with the work of Maluly et al., (2013). On the other hand, there were a significant increase in white blood cell (WBC) counts, MCV, MCH and a significant decrease in red blood cell (RBC) counts and platelets (PLT) were found in rabbits administered with MSG and that with MSG + soybean oilas shown in table (1)

Table 1 Effects of oral administration of MSG with or without soybean oil on blood picture of rabbits.

	Control		**Treated with MSG**	**Treated with MSG + Soybean Oil**
RBC ($X10^6$ cell/µl)	5.50±0.18	**Mean ± S.E % of change p**	3.4±0.17 -38.18 <0.01	3.10±0.16 -43.64 <0.01
Hb (g/dl)	11.8±0.17	**Mean ± S.E % of change p**	12.12±0.15 2.71 >0.05	11.15±0.14 -2.54 >0.05
PCV (%)	38.0±0.16	**Mean ± S.E % of change p**	35.20±0.18 -7. 37 >0.05	33.51±0.14 -11.82 <0.05
MCV (μm^3)	69.09±0.15	**Mean ± S.E % of change p**	103.53±0.16 49.85 <0.01	108.10±0.13 56.46 <0.01
MCH (Pg)	21.46±0.11	**Mean ± S.E % of change p**	35.65±0.12 66.12 <0.01	37.10±0.19 72.88 <0.01
MCHC (%)	31.05±0.14	**Mean ± S.E % of change p**	34.43±0.15 10.86 <0.05	33.27±0.16 6.67 >0.05
WBC ($X10^3$ cell/µl)	7.50±0.12	**Mean ± S.E % of change p**	13.1±0.11 74.67 <0.01	11.11±0.13 48.13 <0.01
PLT ($X10^3$ /µl)	390.90±30. 13	**Mean ± S.E % of change p**	230.3±25.50 -41.09 <0.01	215.90±29.90 -44.77 <0.01

RBC count suggests that it probably reduces the life span of red blood cells in the blood which might be as a result of direct toxicityAshaolu et al.,(2011). This might also have been mediated through a deleterious effect on the hemopoietic stem cells in the bone marrow. No ameliorating effect was observed, but the increased parameters observed are more indicative of anemia. Increased mean corpuscular volume (MCV) indicates the cells to be macrocytic, while the increased MCH is indicative of macrocytic anemia. These results corroborate findings by Sembulingham, (2005). Increase in MCV is seen in pernicious anemia (normochromic) and megaloblastic anemia (hypochromic) Richards (1993). Under normal circumstances, the acute toxicity of glutamate is very low. No significant change was observed in glucose , cholesterol , total protein and globulin parameters, table (2,3). These results are again in agreement with the work of Maluly et al.,(2013) who reported similar findings. The concentration of triglycerides, was significantly increased ($p < 0.001$), in comparison with controls. These results are in agreement with Savcheniuk et al., (2014). Albumin concentration decreased significantly in the serum of MSG dosed rabbits. The synthetic function of liver was altered by MSG, so albumin level decreased. Moreover, partial recovery occurred in the MSG + soybean oil treated group. Similar results were demonstrated by El-Nahrawy et al., (2012).

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

Table 2. Effects of oral administration of MSGwith or without soybean oil on some chemical constituents of rabbit'sserum.

	Control		**Treated with MSG**	**Treated with MSG + Soybean Oil**
Glucose (mg/dl)	88.0±0.24	**Mean ± S.E** **% of change** **p**	90.90±0.22 3.30 >0.05	80.80±0.23 -8.18 >0.05
Triglycerides (mg/dl)	108.8±0.26	**Mean ± S.E** **% of change** **p**	133.13±0.24 22.36 <0.01	150.15±0.23 38.01 <0.01
Cholesterol (mg/dl)	203.5±0.33	**Mean ± S.E** **% of change** **p**	220.1±0.35 8.16 >0.05	200.3±0.29 -1.57 >0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

In the present study, administration of MSG resulted in impairment of some renal biomarkers reflected by the significant increase in urea and creatinine levels. The significant increase in creatinine content of the serum following the administration of MSG may be attributed to compromise of the renal functional capacity. These results are in agreement with Vinodini et al., (2010); Abass and Abd El-Haleem (2011) and El-Nahrawy., et al (2012) who showed an increase in BUN and creatinine that proved that the damages caused by MSG even compromised the kidney function.

However, no significant variation recorded between the electrolytes of the MSG treated Rabbits; MSG + soybean oil treated and controls, which indicate normal renal capacity to excrete the electrolyte which might possibly reflect the preservation to some extent of the renal integrit .Results of the present study are in accordance with the findings of Ramadan et al., (2010) and El-Nahrawy et al., (2012).

Table 3.Effects of oral administration of MSGwith or without soybean oil on some chemical constituents of rabbit'sserum.

	Control		Treated with MSG	Treated with MSG + Soybean Oil
Total protein (gm/dl)	8.88±0.31	**Mean ± S.E** **% of change** **p**	8.11±0.27 -8.67 >0.05	7.10±0.29 -20.05 <0.01
Albumin (gm/dl)	4.90±0.25	**Mean ± S.E** **% of change** **p**	3.80±0.26 -28.9 <0.01	3.9±0.24 -20.41 <0.01
Globulin (gm/dl)	3.98±0.22	**Mean ± S.E** **% of change** **p**	4.31±0.18 8.29 >0.05	3.20±0.19 -19.60 <0.01
Urea (mg/dl)	32.20±0.51	**Mean ± S.E** **% of change** **p**	48.1±0.33 49.38 <0.01	47.12±0.31 46.34 <0.01
Uric acid (mg/dl)	4.15±0.17	**Mean ± S.E** **% of change** **p**	8.2±0.16 97.59 <0.01	7.11±0.15 71.33 <0.01
Creatinine (mg/dl)	0.90±0.03	**Mean ± S.E** **% of change** **p**	1.10±0.05 22.22 <0.01	1.0±0.04 11.11 <0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

Table 4. Effects of oral administration of MSGwith or without soybean oil on serum electrolytes constituents of rabbits.

	Control		Treated with MSG	Treated with MSG + Soybean Oil
Ca^{2+} (mEq/L)	9.15±0.12	**Mean ± S.E** **% of change p**	11.11±0.11 21.42 <0.01	10.10±0.13 10.38 <0.05
Na^{+} (mEq/L)	140.99±0.15	**Mean ± S.E** **% of change p**	140.12±0.16 -0.62 >0.05	135.5±0.14 -3.89 >0.05
K^{+} (mEq/L)	3.33±0.14	**Mean ± S.E** **% of change p**	3.55±0.17 6.60 >0.05	3.41±0.16 2.40 >0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

The health of the liver can assess by estimating the activities of serum AST, ALT and ALP. The AST & ALT enzymes are a sensitive marker of liver damage Al-Mamary, et al., (2002). Therefore, the increase in the serum AST and ALT activities might perhaps be an indication of liver damage. The present result seemingly agrees with the reports of Farombi and Onyema (2006) and Onyema et al. (2006) demonstrated that the activity of serum AST increased in male rats that were fed MSG probably due to the finding that MSG induced oxidative stress in the liver.

Table 5. Effects of oral administration of MSGwith or without soybean oil on serum enzyme activities of rabbits.

	Control		**Treated with MSG**	**Treated with MSG + Soybean Oil**
Alkaline phosphatase (U/L)	34.0±0.19	**Mean ± S.E** **% of change** **p**	40.1±0.21 17.94 <0.01	35.95±0.22 5.74 >0.05
AST (U/L)	31.90±0.23	**Mean ± S.E** **% of change** **p**	40.55±0.24 27.12 <0.01	38.90±0.26 21.94 <0.01
ALT (U/L)	34.0±0.19	**Mean ± S.E** **% of change** **p**	40.1±0.21 17.94 <0.01	35.95±0.22 5.74 >0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

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