



Effect of some food additives on lipid profile, kidney function and liver function of adult male albino rats

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Abstract

One of the most important problems in the human health nutrition field is the use of food additives. Monosodium glutamate (MSG) as a flavor enhancer and aspartame (ASP) as a non-nutritive sweetener are two of the most widely used additives all over the world. The objective of the present study is to evaluate the effect of daily oral administration of MSG (13 mg/kg b.w.) and ASP (13 mg/kg b.w.) for one month either individually or in combination on different hematological parameters, lipid profile, liver and kidney functions of adult male albino rats. The data revealed that there was a significant reduction in platelets count and WBC count in both of MSG and ASP treated groups. While the combination of the two additives resulted in significant reduction in Hb, PCV, MCV, MCH and WBC values. There is a highly significant increase in HSP-70 levels in all treated groups when compared to the control group. The data revealed also that, both of ASP+MSG treated rats showed highly significant increase in lipid profile parameters (TG, TC, HDL, LDL, VLDL) and liver enzyme activities (ALT, AST, ALP). The levels of serum urea, creatinine and uric acid revealed a significant increase in all treated groups especially the group of (ASP+MSG) treated rats. In conclusion administration of ASP and MSG to adult male albino rats caused disturbances in hematological parameters, lipid profile, liver and kidney functions of adult male albino rats. Getting more attention to the constituents of food products is more recommended to protect people from these additives.

Keywords: Aspartame, Monosodium glutamate, Hematology, HSP70, Liver, kidney.

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1. Introduction

Food additives are chemical compounds, which are added to food to improve color, flavor, taste or sweetened [1]. The most commonly components added to foods include sorbic acid and its salts, benzoic acid, nitrates, monosodium glutamate (MSG) and aspartame (ASP). Monosodium glutamate (MSG) is the sodium salt of glutamic acid. It is used as a flavor enhancer and increases the sapidity of food. It is present in many-packaged food as salad dressing, processed meat and soups [2]. The estimated average daily intake of MSG is ranged from 0.3-1.0 g in industrialized countries, but can be higher occasionally [3]. It has great vital contribution inside the body as a metabolic intermediate and protein constituent [4]. Of which glutamate is the main excitatory neurotransmitter in the central nervous system of mammals [5]. Also, glutamate plays an important role in the integration of sensory perception and motor output and is involved in the coordinating and control of voluntary movement [6]. Although of this, it has been reported that glutamate has a very low acute toxicity under normal circumstances [7]. Monosodium glutamate at lower

doses induces toxicity signs on histological and immunohistochemical of features cerebellar cortex of albino rats [8]. Many studies reported that excessive consumption of MSG could produce many symptoms as weakness, diarrhea, numbness, headaches, vomiting and flushing. Alalwani [9] found that oral doses of MSG (30, 60 g/kg b.wt) for 2 months induces testicular lesions in rats. Also, it has deleterious effects on various organs in experimental animals [10, 11].

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is one of low-calorie artificial sweeteners, which is used in many countries worldwide. It is an odorless, white, crystalline powder and having strong sweet taste [12]. After ingestion and absorption in the intestinal lumen, aspartame is hydrolyzed to phenylalanine (50%) -the precursor for two neurotransmitters of the catecholamine family-; aspartic acid (40%) -an excitatory amino acid-; and methanol (10%) - which is oxidized to cytotoxic formaldehyde and formic acid [13]. Aspartame has a neurological and behavioral disturbance. Also cause some clinical disturbance as

blindness, metabolic acidosis and cause an elevation in methanol levels [14]. Most of its toxicity was related to generation of aspartame metabolite, particularly to methanol metabolites as formaldehyde and format [15]. Investigators found that ASP administration induces disruption of liver function in albino rats associate with elevated levels of ALT, AST and ALP and oxidative stress [15, 16]. In Europe and USA, 50 mg/kg body weight and 40 mg/kg b.wt are acceptable for aspartame daily intake, respectively [16]. Even though, the acceptable daily intake of aspartame or below induce side effects in adults, children and in fetal life exposure [16, 17].

The data available about the effect of ASP and MSG at or below the acceptable daily intake is insufficient. Furthermore, that of their combination is unavailable. Therefore, the present study was carried out to investigate the effect of daily intake of ASP and MSG at doses below acceptable on hematological parameters, lipid profile, liver and kidney function individually or in combination.

2. Materials and Methods

Animals and Experimental Groups:

The present study was carried out on adult male albino rats, (one-month age and weighing 120±10g). They were housed for 10 days in well-ventilated room under controlled laboratory conditions. After acclimatization, rats were randomly divided into 4 experimental groups (5 in each) away from any stressful stimuli and supplied with diet and water *ad libitum*.

Group I (control group): rats in this group received a daily oral dose of 1ml of distilled water for one month.

Group II (ASP treated group): rats in this group received a daily oral dose of ASP 0.13 g dissolved in 1ml of distilled water per kg of body weight for one month.

Group III (MSG treated group): rats in this group received a daily oral dose of MSG 0.13 g dissolved in 1ml of distilled water per kg of body weight for one month.

Group IV (ASP+MSG treated group): rats in this group received a daily oral administration of ASP and MSG (ASP 0.13 g + MSG 0.13 g dissolved in 1ml distilled water per kg of body weight for one month.

Monosodium Glutamate and Aspartame were obtained from EL-Gomhoria Company for pharmaceuticals. Egypt. Oral administrations of rats were carried out by using metal intra-gastric tube. At the end of the experimental period, rats were deprived of food but not water over night. Blood samples were collected on the day of sacrifice from post caval vein using a syringe and collected in dry glass tube. Serum was separated by centrifugation at 3000 rpm for 15 min, then serum was stored at -20 °C until analysis.

Hematological parameters

Hemoglobin Hb and Hematocrit values Hct% were determined by the method described by Drabkin [18]. Red

blood cells (RBC) count, white blood cells (WBC) count, platelets and differential count of leucocytes were determined according to Dacie and Lewis [19]. The calculated blood indices were estimated using the following formulae according to Benjamin [20]:

$$\text{MCV (fl)} = \text{HCT} \times 10 / \text{RBCs count}$$

$$\text{MCH (pg)} = \text{Hb} \times 10 / \text{RBCs count}$$

$$\text{MCHC (\%)} = (\text{Hb}/\text{HCT}) / 100$$

Heat shock protein 70 (hsp-70) was assessed by sandwich ELIIZA using monoclonal antibody to hsp-70 [21].

Biochemical assay

Lipid profile:

Serum triglycerides (TG) high density lipoprotein (HDL) low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were determined spectrophotometrically by using commercial kits from Biodiagnosticco. Egypt according to the method of Satoh [22]. Serum total cholesterol (TC) level was determined spectrophotometrically by using Linear Chemicals S.L kits according to the method of Jama [23].

Concentration of LDL and VLDL were calculated according to Friedewald [24] equation as follows:

$$\text{LDL} = [\text{Total Cholesterol} - (\text{HDL} + \text{triglycerides}/5)].$$

$$\text{VLDL} = \text{triglycerides}/5.$$

Liver function:

Serum aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) activities were determined by using Centronic GmbH kits according to the method of Klin [25]. Serum alkaline phosphatase (ALP) activity was determined by using Stan Bio-Kits according to the method of Tietz [26].

Kidney function:

Serum uric acid level was determined by using Linear Chemicals S.L kits according to the method of Tietz [27]. Serum urea and creatinine levels were determined by using Bio. Med Diagnostics kits according to Young [28].

Statistical analysis

Results obtained from the experiment were expressed as mean ± SE and analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test [29]. Differences were considered significant at $P < 0.05$. This was performed using the Statistical Package for Social Science (SPSS) computer program software; Version 20.00 produced by IBM Software, Inc. Chicago, USA.

3. Results

The data presented in table (1) showed effect of oral doses of ASP, MSG or their combination on hematological parameters of male albino rats for one month. Oral administration of ASP induced non-significant fluctuations in tested hematological parameters, except white blood cells (WBC), which was significantly reduced from those of the control.

Table (1): Haematological parameters of male albino rats orally administered with aspartame (0.13 g/Kg b.w), monosodium glutamate (0.13 g/Kg b.w) and their combination for one month.

Parameters \ Groups	Control	Aspartame (ASP)	Monosodium glutamate (MSG)	ASP + MSG
RBC (10 ⁶ /cmm)	7.62 ± 0.14 ^a	7.74 ± 0.44 ^a	7.56 ± 0.20 ^a	7.08 ± 0.27 ^a
Hb (g/dl)	15.22 ± 0.19 ^a	15.84 ± 0.42 ^a	14.72 ± 0.20 ^a	13.08 ± 0.50 ^b
Hct (vol%)	47.20 ± 0.54 ^a	51.66 ± 2.03 ^a	47.30 ± 1.17 ^a	40.24 ± 1.80 ^b
MCV (fl)	62.02 ± 0.89 ^{ab}	65.52 ± 3.53 ^a	62.56 ± 1.76 ^{ab}	56.96 ± 1.18 ^b
MCH (pg)	20.02 ± 0.39 ^{ab}	20.68 ± 1.08 ^a	19.54 ± 0.54 ^{ab}	18.52 ± 0.23 ^b
MCHC (%)	32.26 ± 0.66 ^a	31.58 ± 0.40 ^a	31.14 ± 0.38 ^a	32.48 ± 0.42 ^a
Platelets (10 ³ /cmm)	637 ± 41.86 ^{ab}	695 ± 41.78 ^b	506 ± 19.41 ^c	1179 ± 82.94 ^a
WBC (10 ³ /cmm)	15.26 ± 0.31 ^a	10.64 ± 1.57 ^b	12.72 ± 0.59 ^{ab}	14.78 ± 0.90 ^a
Bands	2.60 ± 0.60 ^a	3 ± 0.44 ^a	2.60 ± 0.40 ^a	3 ± 0.31 ^a
Segmented	35 ± 11.50 ^a	43.80 ± 12.28 ^a	28 ± 11.13 ^a	51.20 ± 16.43 ^a
Neutrophils	37.60 ± 11.72 ^a	46.80 ± 12.70 ^a	30.60 ± 11.07 ^a	54.20 ± 16.63 ^a
Lymphocytes	53.80 ± 13.89 ^a	46.80 ± 14 ^a	64.60 ± 11.43 ^a	39.60 ± 17.74 ^a
Monocytes	6.20 ± 1.74 ^a	4.40 ± 1.28 ^a	2.80 ± 0.91 ^a	4.60 ± 1.20 ^a
Eosinophil's	2.20 ± 0.58 ^a	1.60 ± 0.24 ^a	1.60 ± 0.24 ^a	1.60 ± 0.24 ^a
Basophils	0.20 ± 0.20 ^a	0.40 ± 0.24 ^a	0.40 ± 0.24 ^a	0.00 ± 0.00 ^a

Number of animals in each group = 5 In the same row:
 Data were presented as mean ± SE Different letters mean significant difference at P < 0.05

Regarding MSG effect, the presented data recorded significant reduction in platelets count compared to those given saline solution or ASP. The other tested hematological parameters were non-significantly changed from the control group.

Rats treated with ASP and MSG mixture exhibited general reduction in most of the tested hematological parameters

compared to control table (1). Only hemoglobin content, Hct value of ASP+MSG treated group showed significant reduction from the control. Compared to the control group the rest of the tested hematological parameters were non-significantly varied. MCV, MCH and WBC values for ASP+MSG treated group showed significant reduction from those given only ASP.

Table (2): Heat shock protein (hsp-70) of male albino rats orally administered with aspartame (0.13 g/Kg b.w) monosodium glutamate (0.13 g/Kg b.w) and their combination for one month.

Parameters \ Groups	Control	Aspartame (ASP)	Monosodium glutamate (MSG)	ASP + MSG
HSP70 (ng/ml)	5.68 ± 0.28 ^c	7.22 ± 0.30 ^b	7.95 ± 0.26 ^b	9.72 ± 0.28 ^a

Number of animals in each group = 5 In the same row:
 Data were presented as mean ± SE Different letters mean significant difference at P < 0.05

Heat shock protein (hsp-70) showed significant elevations in ASP, MSG and ASP+MSG groups over those recorded for control group table (2).

By analysis of varies variance lipid profile parameters (table 3) showed a general increase in ASP, MSG and ASP+MSG groups in relation to those of control, being mostly significantly varied from that of the control group. Serum TG level increased significantly in all treated groups compared to control one. Treatment with ASP induced non-

significant increase in TC and LDL levels, but MSG and ASP+MSG treated groups showed significantly increased values in serum TC and LDL levels. There was no significant difference between all groups in serum HDL level. Serum VLDL level increased significantly and non-significantly in ASP+MSG and ASP treated groups, respectively. On contrary, treatment with MSG caused significant decrease in VLDL level.

Table (3): Serum lipid profile parameters of male albino rats orally administered with aspartame (0.13 g/Kg b.w), monosodium glutamate (0.13 g/Kg b.w) and their combination for one month.

Parameters	Control	ASP	MSG	ASP + MSG
Triglycerides TG (mg/dl)	76.68 ± 2.09 ^b	84.24 ± 2.17 ^a	82.80 ± 1.87 ^a	88.14 ± 1.85 ^a
Total cholesterol TC (mg/dl)	110.80±7.88 ^b	120.52 ± 3.59 ^{ab}	129.06 ± 0.29 ^a	128.88 ± 0.41 ^a
High density lipoprotein HDL (mg/dl)	17.70 ± 0.17 ^a	18.72 ± 0.19 ^a	19.24 ± 0.17 ^a	18.96 ± 0.20 ^a
Low density lipoprotein LDL (mg/dl)	78.42 ± 5.94 ^b	82.80 ± 5.36 ^{ab}	93.24 ± 0.45 ^a	92.21 ± 0.43 ^a
Very low density lipoprotein LDL (mg/dl)	15.33 ± 0.41 ^{ab}	16.85 ± 0.43 ^{ab}	16.36 ± 0.14 ^c	17.71 ± 0.44 ^a
Number of animals in each group = 5		In the same row:		
Data were presented as mean ± SE		Different letters mean significant difference at P < 0.05		

Data in table (4) showed serum liver functions of male albino rats after treatment with ASP, MSG and their combination. In the last group, serum AST activity showed significant increase compared to control group. Rats treated with, ASP, MSG and ASP+MSG showed significantly increased values ALT activity compared to control group. A significant increase in serum AST activity in ASP and ASP

+ MSG treated group as compared to the control group. Meanwhile serum AST activity showed significantly decrease in MSG group. The combination of ASP+MSG caused highly significant increase in ALP activity, while oral administration of ASP and MSG individually caused non-significant decrease in ALP activity compared to the control group.

Table (4): Serum liver functions "Serum alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) of male albino rats orally administered with aspartame (0.13 g/Kg b.w), monosodium glutamate (0.13 g/Kg b.w) and their combination for one month.

parameters	Control	ASP	MSG	ASP + MSG
ALT (U/L)	141.42±2.76 ^b	194.36±14.70 ^a	193.37±17.77 ^a	184.20±5.76 ^a
AST (U/L)	58.92±3.59 ^a	76.82±6.49 ^a	49.76±4.06 ^b	62.42±4.80 ^{ab}
ALP (U/L)	79.86±5.44 ^b	60.47±2.96 ^b	68.84±9.17 ^b	121.11±14.79 ^a
Number of animals in each group = 5		In the same row:		
Data were presented as mean ± SE		Different letters mean significant difference at P < 0.05		

Rats treated with ASP showed significant increase in serum urea and uric acid levels compared to control group. Treatment with MSG and ASP+MSG combination caused

significant increase only in serum creatinine level compared to control group (table 5).

Table (5): Serum kidney functions parameters of male albino rats orally administered with aspartame (0.13 g/Kg b.w), monosodium glutamate (0.13 g/Kg b.w) and their combination for one month.

Groups parameters	Control	ASP	MSG	ASP + MSG
Urea (mg/dl)	16.14 ± 0.47 ^b	17.48 ± 0.78 ^a	16.28 ± 0.36 ^b	17.20 ± 0.73 ^{ab}
Creatinine (mg/dl)	0.31 ± 0.01 ^b	0.31 ± 0.00 ^b	0.38 ± 0.00 ^a	0.37 ± 0.11 ^a
Uric acid (mg/dl)	8.58 ± 0.18 ^b	9.22 ± 0.16 ^a	8.60 ± 0.16 ^b	8.76 ± 0.22 ^{ab}
Number of animals in each group = 5 Data were presented as mean ± SE		In the same row: Different letters mean significant difference at P < 0.05		

4. Discussion

Many of chemical substances are used as food additives. Of which the monosodium glutamate and aspartame are added to many foods as food additives. The present study reported that daily oral administration of MSG and ASP caused significant alterations in some physiological parameters as lipid profile, liver and kidney functions.

The hematological parameters are used to assess the general healthy status as well as toxicity evaluations. This is because they respond to any changes or disturbances of the overall body performance [30].

The present study showed that ASP administration generally induced non-significant fluctuations in all examined hematological parameters, only WBCs showed significantly reduction levels that of the control group. [1] reported that ASP induced non-significant hematological disturbance in male albino rat. So, the results recorded here are in agreement with those recorded by [1], except WBCs for which significantly reduced. This indicate a reduction of defense due to ASP administration. However, [1] recorded non-significant reduction in rat treated with ASP. This is an agreement with those reported earlier for other food additives [30].

The obtained data for hematological parameters of MSG administrated group showed non-significant reduction compared to that of the control. This is agreeing with the previous study of who recorded significant reduction in hematological parameters due to MSG administration [30, 31]. Such differences may be related to differences in MSG route of administration that is oral in the present undertaken and subcutaneously injection for [30]. The reduction in erythroid series (RBCs, Hb, Hct%) with variation in blood indices may be due to increased hemolysis or / inhibited erythropoiesis and release from bone marrow [30, 32]. Our data showed that MSG induced significant reduction in platelets count with the mean value of 506±19.41 compared to 637±41.86 for the control. This is an indication of the reduced clotting ability as harmful effect of MSG. This is in

contradictory with those recorded by Ajibola [33] for MSG. They recorded increase in platelet count, clotting time and bleeding time. The increase in the number of platelet cells in the blood is known as thrombocytosis or thrombocythemia. It was suggested this increase might be due to excessive intake of one of MSG component (sodium and glutamate) that affect blood and body fluid compartments and water balance of the body [33]. They also add that it could be related to the biosynthesis of the thrombopoietin hormone, that stimulates the production of platelets. Similar to the data recorded by Ajibola [33], our study evoked elevated platelet ASP treated group.

The present undertaken data for rat given mixture of ASP+MSG showed more potent action in hematological parameters more than those given single dose of either ASP or MSG. This indicates the add effect of the tested food additives.

The data for hsp-70 obtained in the present study for rats treated with ASP+MSG for the two additives showed that (MSG+ASP) group evoked an additive affection hsp-70.

The obtained data in the present study similar potency for ASP and MSG treated groups. While, for those given mixture of ASP+MSG group it was significantly highly with ASP or MSG. Therefore hsp-70 data evoked the additive effect of ASP and MSG when presented in mixture. These data are in parallel with those reported for the impact of ASP and MSG [34]. The greater increase in hsp70 expression evoke greater susceptibility of immune organs to oxidative stress, this is explained by immuno-regulatory functions of hsp70 [35]. Heat shock proteins 70 (hsp-70), function as molecular chaperones, assisting in the folding and transport of proteins and their assembly into complexes. They protect cells from stress, and provide cytoprotection against a wide number of stressors and stress hormones such as corticosterone [36].

Daily oral administration of MSG and ASP to adult male albino rats for 30 days either individually or in combination

caused elevations in serum levels of TG, TC and LDL. This may be due to that MSG and ASP are able to increase the activity of 3-hydroxy-3-methylglutaryl-Co enzyme A reductase “the rate limiting enzyme in biosynthesis of cholesterol” and also increase lipogenesis and impaired TC and TG metabolism [37, 38]. Also, it may be attributed that these food additives may cause liver oxidative stress, which obtained in the present study. By the recorded elevations in ALT and AST serum levels which evoked liver damage. This is in agreement with data recorded by [1, 10]. The disturbed liver metabolism reported in the present undertaken caused consequently disturbance in total body cholesterol metabolism. These results are in harmony with [37, 38] who found that MSG causes significant elevation in serum TG, TC and LDL-cholesterol levels in rats and non-significant difference in HDL-cholesterol level.

Also, these results agree with those reported for rats received ASP in drinking water [39] and for zebra fish fed on ASP [40]. While these results are not in support with Sani [41] who reported that MSG caused a decrease in levels of serum TC and LDL-C in rats but induced an increase in TG level.

Aspartate and alanine aminotransferase are sensitive marker enzymes for liver damage. Treatment the rats with ASP and MSG or their combination caused an elevation in serum ALT and AST levels. MSG could dissociate easily to release free glutamate, which produces ammonium ions. This ammonium ion overload could damage the liver, therefor releasing transaminases [42, 43]. The elevation in enzyme activities may be attributed oxidative stress, which cause liver damage, which include structural damage and necrosis of hepatocytes. Oxidative stress combined with free radical generation, lipid peroxidation and imbalance between production of reactive oxygen species (ROS) and anti-oxidant defense. These changes caused alternation in membrane integrity, which result in leakage of intracellular enzymes [44].

Toxic effect of glutamate may be due to its ability to raise the level of intra-cellular calcium resulting in activation of some enzymes that responsible for cell death by different mechanisms [45]. The results in the present study are similar to those of [42] whom found that male albino rats treated

with MSG showed increased in levels of AST and ALT enzymes.

Also, the obtained data herein agreed with those reported previously for rats treated with MSG for 10 consecutive days [30] and rats treated with ASP for 15 consecutive days [39, 46] as they found significant increase in serum levels of ALT, AST and ALP. Also, the present data are agreement with [1] found that ALT, AST and ALP activities increased significantly in male albino rats with ASP treatment.

It has recorded that MSG cause alteration in hepatic parenchyma even in low doses [45]. This is supported by our data for alkaline phosphatase, which increased significantly for rats treated with combination of ASP+MSG. This is an explanation of the oxidative stress potency induced by the interactive effect off both MSG and ASP rather than single effect of each one.

Treatment with ASP caused and increased in urea and uric acid levels but induced non-significant change in creatinine level. While treatment with MSG caused increased in creatinine level and non-significant change in urea and uric acid levels. These results indicate impaired kidney function due to single action off the ASP and MSG. The combination between ASP and MSG caused increase in all measured kidney function parameters. This is an indication of the interactive toxicity effect off both ASP and MSG which is confirmed by the obtained data for the liver function.

The elevated levels of urea, creatinine and uric acid are indicator for renal dysfunction. This may be due to the oxidative stress which caused by treatment with ASP and MSG. Renal halt reported earlier by [45] also found that MSG caused pathological changes in renal tissue such as necrosis and degeneration of epithelium lining renal tubules.

These obtained disturbances of kidney function are in a harmony with results of [30] as they found that rats treated with MSG for 10 consecutive days showed an increased in levels of urea, creatinine and uric acid. Also, Abd EL-Reheim [47] found that administration of MSG to rats caused an elevation in kidney functions parameters.

The present results also agreed with those reported recently by EL-Sokkary [48] and Abd EL-Wahab [49] who found that adult male albino rats treated with ASP showed elevation in levels of urea and creatinine.

5. Conclusion:

The present study concluded that administration of ASP and MSG either individually or in combination caused many alternations in lipid profile, kidney functions and liver functions. It also explores the potentiality of the ASP and

MSG mixture to induce interactive toxicity. So, the present study recommends staying away from using MSG and ASP in our foods.

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