Toxicity of Monosodium Glutamate on Liver and Body Weight with the Protective Effect of Tannic Acid in Adult Male Rats

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KEYWORDS
Monosodium glutamate;
Liver toxicity,
Tannic acid,
Experimental.

ABSTRACT

One of the food additives that are used extensively in the food industry is monosodium glutamate which has a flavor enhancer that is considered a double-edged sword on human health. Tannic acid (TA) is a natural antioxidant that works against many toxic substances. The current study aimed to evaluate the toxicity of MSG on rat liver with a possible protective role of TA. Forty adult male albino rats were used. They were divided into 4 equal groups, 10 rats each. Group, I was the control group, Group II received TA (100 mg/kg) by oral gavage, Group III received MSG (2 g/kg) by oral gavage and Group IV received (TA and MSG) by oral gavage for 4weeks. At the end of the study, biochemical analysis was done and revealed that Group III showed a significant increase in the body weight and significant elevation in the serum levels of ALT and AST enzymes compared to group I with $p > 0.001$. While, there was a non-significant difference in relative liver weight with $p > 0.05$. Histopathological examination revealed that group III showed a severe complete loss in hepatic architecture such as congestion and dilatation in the blood vessels with different cellular changes such as necrosis and apoptosis as compared to group I. All these deleterious effects of MSG were greatly ameliorated by TA administration in group IV as compared to group III.

Introduction

Monosodium glutamate is a flavor-enhancing agent which is one of the most important factors that attract people towards the food and it is used in the food industry with a characteristic umami taste. It is a non-essential amino- acids found abundant in nature (Zanfirescu et al., 2019).

The safety and toxicity of MSG had become controversial in the last few years because MSG has some harmful effect on the human and animal tissues through induction of oxidative stress in different body organs (Husarova and Ostatnikova, 2013; Henry-Unaeze, 2017).

The liver plays a major role in metabolic activities which involve synthesis, biotransformation, and storage of numerous substances, so it is one of the most affected organs by toxic substances such as MSG (Kazmi et al., 2017).

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Tannic acid (TA) is a potent antioxidant that increases the antioxidant enzymes and it plays a protective role against oxidative stress through having highly effective ferric reducing and scavenging free radical power and it acts as electron donors (Babby et al., 2019).

The present study aimed to evaluate the toxicity of MSG on rat liver with the possible protective role of TA.

Materials & Methods:

Chemicals

1- Monosodium glutamate (MSG) powder and tannic acid (TA) powder were purchased from Sigma Aldrich Company, Germany.

2- Kits of alanine aminotransferase (ALT) and kits of aspartate aminotransferase (AST) were purchased from Beckman Coulter Inc. Company, USA.

Animals

Forty healthy adult white albino male rats were used. They were 7 weeks old and weighed (200 ± 20 g). Animals were purchased from the Animal Facility Center of the Faculty of Medicine Helwan University. The animals were housed in the animal house, under ambient temperature and they were kept under fixed appropriate humidity and light conditions. They were acclimatized to the laboratory condition for one week before starting the treatment protocol. Animals were fed with standard pellet food and water. The protocol was approved by the local ethical committee of the Faculty of Medicine, Sohag University.

Experimental design

At the end of the adaptation period, the rats were weighed and divided randomly into 4 groups, 10 rats each.

Group I (control): The animals received water and diet orally for 4weeks.
Group II (TA): The animals received daily100 mg/kg of TA by oral gavage for 4weeks (Hassan et al., 2011).

Group III (MSG): The animals received daily 2g/kg of MSG which represents 1/7 of oral LD_{50} by oral gavage for 4weeks (Calis et al., 2016; Sayed et al., 2016).

Group IV (MSG +TA): The animals received both TA (100 mg/kg) and MSG (2 g/kg) by oral gavage for 4 weeks.

After completing the study period, the experimental rats were weighed then blood and tissue samples were collected immediately after they were sacrificed.

Methods

Body weights and Relative Liver Weights

The body weight of each animal was determined before treatments and before sacrifice. The liver of each rat was washed with normal saline, dried between blotting paper, and then weighed. The value of each was assumed as 100% relative liver weight (RLW) was calculated according to the formula previously described by (Ashafa et al., 2012).

$$RLW = \frac{\text{liver weight}}{\text{Body weight}} \times 100$$

Sample collection and storage

The blood samples (3ml) were taken from retro-orbital blood vessels, collected in centrifuge tubes, centrifuged at 4000 rpm for 15 min then serum was immediately stored at -20°C until their use for assessment of liver enzymes. Then the animals were dissected to

expose the liver organ for histopathological examination.

Biochemical analysis

The liver enzymes: Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were estimated by using enzyme-linked immunosorbent assay by spectrophotometry apparatus (Beckman Coulter AU480).

Histopathological analysis

Slices from the liver tissues were fixed in 10% neutral formalin. Liver tissues were processed and prepared for serial paraffin section of 5µm thickness examination for histopathological examination by Light microscopy.

Statistical analysis

Statistical Package for Social Science (IBM-SPSS), version 24 (May 2016); IBM, Chicago, USA was used for statistical data analysis. The data were expressed as mean± standard deviation (SD), number, and percentage. Mean and standard deviation was used as a descriptive value for quantitative data. The Student's t-test was used to compare the means between two groups, and a One - Way analysis of variants (ANOVA) test was used to compare means of more than two groups.

Results:

Regarding the body weights, table (1) showed a significant difference in both initial body weight **(**IBW) and final body weight (FBW**)** with weight gain (WG) in the studied groups as compared to the control group with $p<0.001$.

The animals treated with MSG (Group III) showed a very highly significant statistical increase in the FBW where the WG was 32.70% as compared to the control group (Group I) where the WG was 10.7% With $p<0.001$. While there was a highly significant statistical reduction in FBW in group IV that was treated with TA where the WG was 10.52% as compared to group III where the WG was 32.70% with $p<0.01$.

Table (1): Mean value and ±SD of body weight (initial, final and weight gain ratio) in the studied groups (n=40).

Variables	Mean (± SD)				p-value				
					t-test				ANOVA
	I	II	III	IV	II versus I	III versus I	IV versus I	III versus IV	
IBW(g)	193.30 ± 9.60	217 ± 11.59	204 ± 5.16	209 ± 11.0	0.000*	0.018*	0.001*	0.255	0.000*
FBW(g)	214 ± 13.49	238 ± 13.16	270.5 ± 20.33	231 ± 23.89	0.006*	0.000*	0.045*	0.000*	0.000*
WG %	10.7	9.67	32.70	10.52	0.811	0.000*	0.910	0.000*	0.000*

*** p < 0.05:** Significant**, SD:** Standard deviation**, %:** Percentage**, IBW**: Initial body weight, **FBW:** Final body weight, **WG:** Weight gain, N; Number, **Group I:** Control group**, Group II:** Rats treated by TA, **Group III**: Rats treated by MSG, **Group IV**: Rats treated by (TA+MSG)**.**

As regards, the relative liver weight (RLW), table (2) shows no significant difference in studied groups as compared to the control group with p> 0.05 and MSG had a non-significant effect on the liver weight in group III when compared to group I with p> 0.05.

Table (2): Mean value and ±SD of relative liver weight in the studied groups (n=40)

Variables	Mean (± SD)				p value				ANOVA
					t-test				
	I	II	III	IV	II versus I	III versus I	IV versus I	III Versus IV	
RLW	3.76 ± 0.81	3.44 ± 0.42	4.15 ± .032	3.70 ± 0.68	0.237	0.152	0.823	0.1	0.082

SD: Standard deviation**, RLW:** Relative liver weight**, (ANOVA):** One-Way Analysis of Variance**, group I:** Control group**, group II:** Rats treated by TA, **group III**: Rats treated by MSG, **group IV**: Rats treated by (TA+MSG).

The biomarkers of liver function (AST and ALT) in table (3) showed a significant rise in their serum level in the studied groups with p< 0.001. Comparing MSG treated group to the control group, revealed a very highly significant statistical increase in both liver enzymes (ALT and AST) level which returned to normal levels when they were treated with the anti-oxidant TA (Group IV) rather than those treated with MSG only with p< 0.001.

Table (3): Mean value (±SD) of liver function tests between the studied groups (n=40).

Variables	Mean (± SD)				p-value				
					t-test				ANOVA
	I	II	III	IV	II versus I	III versus I	IV versus I	III Versus IV	
Serum ALT (U/L)	39.27 ± 3.64	38.03 ± 4.1	62.1 ± 10.33	48.8 ± 5.77	0.540	0.000*	0.003*	0.008*	0.000*
Serum AST (U/L)	145.4 ± 24.4	163.3 ± 26.4	254.7 ± 32.5	213.7 ± 14.59	0.187	0.000*	0.000*	0.007*	0.000*

*Significant**, ALT**: Alanine aminotransferase**, AST:** Aspartate aminotransferase, **group I:** Control group**, group II:** Rats treated by TA, **group III**: Rats treated by MSG, **group IV**: Rats treated by (TA+MSG)

Histopathological results

The histopathological examination of group II (Figure 1B) didn't show significant difference with group I (Figure 1A). While the effect of MSG was markedly declared on group III (Figure 1C) where there was severe damage in the hepatic tissues such as congestion and dilatation of blood vessels with different cellular change including; necrosis, apoptosis, polymorphism and prominent Kupffer cells as compared to groups I and II (Figure 1A & 1B). Meanwhile, administration of TA in rats showed improvement in the hepatic architecture with less or normal of its cellular components (Figure 1D) as compared to MSG treated group (Figure 1C).

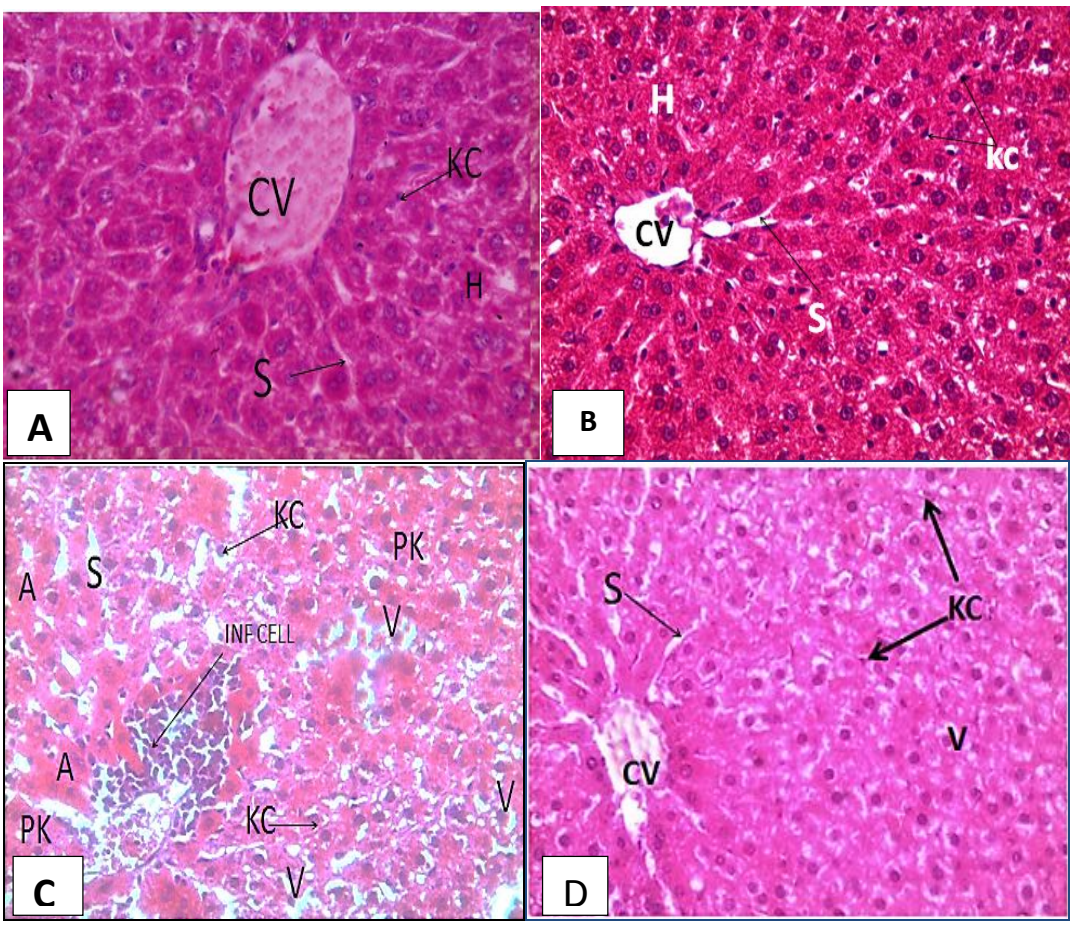

Fig.(1): A photomicrograph of a liver section(A) and (B) with H&E stained from the control group (I) and TA treated group (II) respectively showing normal hepatic architecture with the normal central vein (CV), Normal hepatocytes (H) and kupffer cells (KC) with normal blood sinusoids (s) between the hepatic plates. Liver section **(C)** from MSG treated group (III) showing complete disturbance in hepatic architecture with different cellular changes; vacuolated cytoplasm (V) and highly acidophilic cytoplasm (A), pyknosis (PK), Prominent Kupffer (KC) cells and dilated blood sinusoids (S). A Photomicrograph of liver section **(D)** from TA and MSG treated group (IV) showing improvement in hepatic architecture. The hepatocytes are more or less normal with milder vacuolated cytoplasm (V) and less prominent kupffer cells (KC) with normal blood sinusoids (S) in between.

Discussion:

Nowadays, there is a great tendency towards the usage of processed food containing many food additives such as MSG which is considered a double-edged sword on human health (Henry-Unaeze, 2017).

The present study revealed that MSG affects the body weight in a positive manner where the weights of rats were significantly heavier in group III when compared to group I.

The mechanism of MSG-induced obesity is not quite clear. MSG might be neurotoxic through induction of neuronal necrosis via damage in the hypothalamic arcuate nucleus (ARC) or by an increase in energy intake (Bautista et al., 2019).

The current study was in harmony with (Kumbhare et al., 2015) and Ahmed (2016) who revealed a significant increase in body weight in MSG- treated rats as compared to non -treated group.

MSG didn't induce any significant difference in the mean value of relative liver weight (RLW) when compared to control group which was supported by the Ibegbulem et al. (2016).

On contrary, Nagata et al.(2006); Sasaki et al. (2009) and Tawfik and Al-Badr (2012) reported that RLW significantly increased in MSG treated group compared to the control group.

The present results revealed that TA could control the body weight in group IV as compared to group III while no significant difference was observed in the liver weight which was in agreement with Barszc et al. (2018).

The present results showed that the serum levels of AST and ALT enzymes were significantly elevated in the MSG treated group as compared to the control group.

The degree of hepatic toxicity induced by MSG could be detected by measuring the serum level of hepatic enzymes (AST & ALT) which are released into the circulation from the necrotic membrane of hepatocytes (Ahmed et al, 2019).

This could be explained by the oxidative stress and free radicals production that reacts with polyunsaturated fatty acids of the cell membrane leading to disturbance in the mitochondrial and plasma membranes function resulting in enzymatic release (Okediran et al., 2014; Ahmed et al., 2019).

The current study was following many researchers many researchers who recorded elevation in the AST and ALT enzyme levels as a result of MSG administration. Some investigators used single high doses as Ortiz et al. (2006), Soliman (2011) and Okediran et al. (2014). While other investigators as Onyema et al. (2006); Egbuonu et al. (2009) and Abd-Ella et al. (2016) used small doses.

Moreover, combined administration of TA with MSG in the current study was effective in improving liver function through significant reduction in the levels of ALT and AST enzymes which might reflect the inhibitory effect of TA on MSG induced-hepatotoxicity.

The present study was in agreement with many researchers who showed the ameliorative effect of TA on different toxins induced liver damage which resulted in significant recovery of hepatic amino-transferase activities such as Omar et al. (2003) who used TA to improve the aluminium - induced hepatic toxicity also, Sehrawat et al. (2006) studied the preventive effect of TA on acetyl amino fluorine (2-AAF) similarly, El-Sayed et al. (2006) studied the protective role of TA on lead poisoning.

The current results showed severe destruction in the hepatic architecture in MSG treated group such as vacuolar degeneration, pyknosis and necrosis in the hepatocytes in addition to congestion and dilatation in the blood sinusoid when compared to the control group.

These findings were in accordance to Eweka et al. (2011) and AL-Mosaibih (2013) who revealed disruption in the liver architecture with different cellular necrotic changes in MSG treated group compared to the control group while administration of TA with MSG restored the alterations in the degree of liver toxicity towards the normal levels.

The TA was found to be an effective natural antioxidant that has different antioxidant activities when compared to other antioxidants such as BHA, BHT, a-tocopherol and Trolox as references antioxidant (Glüçin et al., 2010).

The current results were in agreement with Omar et al. (2003); Jianping et al. (2017); Basu et al. (2018) and Alechinsky et al. (2020) who used TA to protect the liver against different toxins.

On contrary to the current results; Calis et al. (2016) reported that combined TA with MSG administration in group IV didn't cause significant histological changes in the liver due to short duration (7days).

Conclusion

Oral consumption of MSG with large dosage resulted in varying degrees of liver injury and TA played an important role in the protection and improvement of liver against (MSG) toxicity.

Acknowledgement

The authors would like to express their deepest thanks and gratitude to all staff members of the Forensic Medicine and Clinical Toxicology and Histology Departments, Sohag University for their contribution in this study.

Conflict of interest:

The authors had no conflict of interest to be cleared.

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سمية الجلوتاميت احادي الصوديوم علي الكبد ووزن الجسم مع التاثير الوقائي لحمض التانيك علي ذكور الجرذان البالغة

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يعد الجلوتاميت أحادي الصوديوم من احد مكسبات الطعم التي تستخدم على نطاق واسع في صناعة المواد الغذائية والذي له خصائص محسنة للنكهة. وتعتبر مادة الجلوتاميت أحادي الصوديوم سيفً ذو حدين على صحة الإنسان. حمض التانيك هو أحد مضادات الأكسدة الطبيعية التي تعمل ضد العديد من المواد السامة وقد هدفت الدراسة الي دراسة التأثير السام لمادة الجلوتاميت احادي الصوديوم علي الكبد والتاثير الوقائي لحمض التانيك علي ذكور الجرذان البيضاء البالغة وقد تم اجراء البحث على عدد ٤٠ من ذكور الجرذان البيضاء البالغة متوسطة الوزن (٢٠٠ ± ٢٠ جم) حيث تم تقسيم الحيوانات عشوائيا إلى ٤ مجموعات متساوية كل مجموعة تضم ١٠ جرذان. المجموعة الاولي: كانت المجموعة الضابطة وتم تغذيتها بالطعام والماء فقط. المجموعة الثانية: اعطيت حمض التانيك فقط بجرعة ١٠٠ مجم/ كجم يوميا. المجموعة الثالثة: (المجموعة المعالجة) تم اعطاءها مادة الجلوتاميت احادي الصوديوم بجرعة ٢جم/ كجم يوميا والمجموعة الرابعة: تم اعطاءها حمض التانيك بجرعة ١٠٠ مجم/كجم مع مادة الجلوتاميت بجرعة ٢ جم /كجم لمدة ٤اسابيع. وفي نهاية الدراسة تم اجراء التحليل البيوكيميائي والفحص النسيجي كما تم قياس وزن الجرذان وقياس الوزن النسبي للكبد .وقد تبين من النتائج ان الجلوتاميت احادي الصوديوم قد ادي الي زيادة ذات دلالة احصائية في الوزن الكلي للجسم وفي مستوي انزيمات الكبد في الدم مقارنة بالمجموعة الضابطة بينما لم يكن هناك اختلاف ذو دلالة احصائية في الوزن النسبي للكبد وبالفحص النسيجي تبين ان الجلوتاميت احادي الصوديوم قد احدث العديد من التغيرات الخلوية مثل الخلايا النخرية والخلايا ذات الموت المبرمج بالاضافة الي احتقان وتمدد الأوعية الدموية مقارنة بالمجموعة الضابطة وقد تم تحسين معظم هذه التأثيرات الي حد كبير عند اعطاء حمض التانيك في المجموعة الرابعة.