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The Effects of Monosodium Glutamate and Tannic Acid on Adult Rats

Ibrahim Ugur Calis,[1] Didem Turgut Cosan,[1,*] Faruk Saydam,[2] Umut Kerem Kolac,[1] Ahu Soyocak,[3] Hulyam Kurt,[1] Hasan Veysi Gunes,[1] Varol Sahinturk,[4] Fezan Sahin Mutlu,[5] Zeynep Ozdemir Koroglu,[6] and Irfan Degirmenci[1]

[1]Department of Medical Biology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[2]Department of Medical Biology, Recep Tayyip Erdogan University, Faculty of Medicine, Rize, Turkey
[3]Department of Medical Biology, Istanbul Aydin University, Faculty of Medicine, Istanbul, Turkey
[4]Department of Histology and Embryology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[5]Department of Biostatistics, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[6]Vocational School of Health Services, Eskisehir Osmangazi University, Eskisehir, Turkey

[*]*Corresponding author*: Didem Turgut Cosan, Department of Medical Biology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey. Tel: +90-2222392979, Fax: +90-2222392986, E-mail: dcosan@gmail.com

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Abstract

Background: Monosodium glutamate (MSG) is a widely-used flavor enhancer and stabilizer in ready-made or packaged foods. The excessive use of MSG has been shown to increase oxidative stress in different organ systems and causes glucose metabolism disorders, obesity, and coronary diseases.
Objectives: In this study, the antioxidant activity of tannic acid was investigated experimentally with respect to its protective effects against overdosed MSG-induced oxidative stress in rats. The study took place in Turkey in August 2013.
Methods: Four groups (n = 7) of three- to four-month-old Sprague-Dawley female rats were used in this study. The first group was the control, who were administered saline. The second group received tannic acid (50 mg/kg, 3 days) intraperitoneally (i.p.). The third group received MSG (2 g/kg, 7 days) i.p., and the fourth group received both tannic acid (50 mg/kg, 3 days, pretreatment) and MSG (2 g/kg, 7 days) i.p. The animals were euthanized ten days later. Blood was collected for determining the hematological values and blood glucose levels. Superoxide dismutase (SOD) and malondialdehyde (MDA) levels were determined in the brain, liver, and kidney homogenates, and in the erythrocyte hemolysate. Histopathological examination of the brain, liver, and kidneys was conducted through hematoxylin-eosin staining.
Results: The data showed that the tannic acid treatment statistically decreased the MDA levels in the brain tissues of the group administered MSG and tannic acid ($P < 0.001$) when compared to the corresponding values of the control group. The SOD activities in the blood hemolysates of the MSG and tannic acid group increased when compared to the corresponding values for the MSG group ($P < 0.01$). Additionally, we found that pretreatment with tannic acid reduced blood glucose levels in comparison to the levels of the MSG group ($P = 0.029$). The results of our study show that tannic acid pretreatment in adult rats decreased blood glucose levels and oxidative stress.
Conclusions: In the literature, it was observed that short-term MSG exposure does not cause significant histological changes in the kidneys, liver, or brain cortex. These findings should be re-evaluated in additional long-term studies.

Keywords: Monosodium Glutamate, Oxidative Stress, Antioxidant, Tannic Acid

1. Background

Oxygen is very important for life. However, some reactive oxygen species have the potential of damaging cells and tissues (1). Reactive oxygen species (ROS) formed as a result of metabolic processes are more chemically active when compared to molecular oxygen (2). ROS are capable of causing damage to cellular components, and base damage is mostly caused by ROS. ROS can also disrupt the structure of DNA (3).

Monosodium glutamate (MSG) is a sodium salt of glutamic acid (4). MSG is widely used as a flavor enhancer in the marinades of red meat, fish, chicken, vegetables, sauces, soups, and pre-packaged foods (5). MSG has been shown to have negative effects on glucose metabolism. More specifically, overdoses of MSG have been shown to cause Type 2 diabetes in several test models (6). It is known that high doses of MSG in fetal and small rodents increases body weight and fat mass (7). Thus, MSG may affect human body weight as well. Additionally, overdoses can also dam-

age organs. MSG specifically impairs the brain's physical functions and causes oxidative damage (8). Furthermore, excess MSG may cause sudden neuron death (9). The in vivo neurotoxic effects of MSG are related to acute degenerative changes and chronic neurodegenerative defects, such as in vivo hypoglycemia, ischemia, trauma, multisystem atrophy, Huntington's disease, and both dementia and amyotropic Alzheimer's disease (10). Furthermore, it has been shown that MSG overdose during the neonatal period causes disorders in learning and memory mechanisms, and can impair the neural system, retinas, and kidneys. Moreover, in older subjects, MSG overdose has been shown to cause neurodegenerative diseases, obesity, infertility, growth retardation, Parkinson's disease, and epilepsy (11).

The antioxidant vitamins C and E and the polyphenolic compound quercetin have been shown to be effective against MSG-induced damage in rat livers, kidneys, and brains (12). Tannins have several pharmacological properties, such as anticancer, antioxidant, and antimicrobial activity. Tannins are generally divided into two groups: hydrolysable and nonhydrolysable. Hydrolysable tannins are gallotannins and ellagitannins (13). Tannic acid is rich with tannins, and is widely found in plants and plant-based products such as tea, coffee, grapes, and nuts. Tannic acid functions to defend the plant against predators (14-16). Furthermore, the antioxidant effects of tannic acid have been reported to remove free radicals such as Fe and Cu metals from the environment of human lymphocyte DNA damage mediated by H_2O_2 (17). In addition, tannic acid has also been shown to regulate blood glucose levels (13).

2. Objectives

In this study, we aimed to evaluate the following parameters in the blood, kidney, liver, and brain tissues of rats in order to determine the biological effects of MSG overdose and the antioxidant tannic acid: hematological evaluation, histological analysis, blood glucose level, superoxide dismutase (SOD) level, and malondialdehyde (MDA) level.

3. Methods

3.1. Animals

In this experimental study, 28 three- to four-month-old Sprague-Dawley female rats weighting 250 to 300 g were used, which were obtained from the medical and surgical experimental research center in Eskisehir, Turkey. The experiment was performed after a stabilization period in the medical faculty laboratory at the department of medical biology, Eskisehir Osmangazi University for seven days. This study was approved by the local animal ethics committee of Eskisehir Osmangazi university in Turkey (Approval number: 2013/62-363). All animals were housed in polycarbonate cages in a room with a controlled temperature (22 $\pm$ 2°C) and humidity level (50 $\pm$ 5%). The animals had a 12-hour light and dark cycle. The animals were fed laboratory pellet chow and water ad libitum, and were placed into four groups consisting of seven rats per group.

3.2. Experimental Design

The groups were treated with normal saline as a control (n = 7), MSG (n = 7), tannic acid (n = 7), or MSG and tannic acid (n = 7). The MSG group was given MSG (2 g/kg), and the tannic acid group was given tannic acid (50 mg/kg) for seven days. The combination MSG and tannic acid group was given only tannic acid for three days in advance for protection, and then tannic acid (50 mg/kg) and MSG (2 g/kg) were injected intraperitoneally (i.p.) for seven days.

3.3. Biochemical Analyses

All of the animals were euthanized under anesthesia at the end of the experiment. The brains, livers, and kidneys were harvested and cleared of peripheral tissues. The blood glucose levels were measured using an IME-DC device (IME-DC GmbH, Hof, Germany). The hematologic evaluations were performed on blood samples using a Coulter LH 750 device (Beckman Coulter Inc., Florida, USA). The tissue samples were washed in 0.9% NaCl solution (isotonic) containing 0.16 mg/mL heparin to prepare the homogenates. Then, isotonic saline solution was added to the tissue. The tubes were homogenized at 8,000 cycles for 10 seconds on ice (UltraTurrax T25, Janke and Kunkel IKA, Staufen, Germany). The homogenates were centrifuged at 3,000 g for 10 minutes at 4°C. The supernatants were transferred to vials and kept frozen at -80°C until the measurement period. The erythrocyte hemolysates were prepared using the method reported by Sun et al. (18). The SOD activity was measured spectrophotometrically in tissue homogenates and erythrocyte hemolysates by a previously described method (18). Briefly, the MDA levels were measured spectrophotometrically through thiobarbituric acid reactive substance formation as a lipid peroxidation product according to the method of Mihara and Uchiyama in tissue homogenates and erythrocyte hemolysates (19).

3.4. Histopathological Evaluation

The tissue samples were fixed in 10% formaldehyde and then dehydrated in alcohol solution by a follow-up routine application method and embedded in paraffin. These tissues were used for histopathological examination. Five micrometer (μm) thick serial sections were stained with

hematoxylin-eosin and analyzed histologically using a photomicroscope (Olympus BH 2, Tokyo, Japan).

3.5. Statistical Analysis

Statistical analysis was performed using SPSS for Windows (ver. 20, SPSS Inc., Chicago, IL, USA). A Shapiro-Wilk test was used to determine the normality of variable distribution. The parametric data with a normal distribution are presented as mean ± standard deviation (SD).

According to the bootstrap method, comparison between groups by one-way analysis of variance (ANOVA) and a Tukey post-hoc test was conducted for multiple comparisons. The results that did not show a normal distribution are shown from the 25th percentile to the 75th percentile in relation to the median. A Kruskal-Wallis test was performed with the Dunn method for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

4. Results

The data indicated that the neutrophil and basophil percentages changed significantly. The neutrophil percentage increased significantly in the combined MSG and tannic acid group when compared to the control group ($P < 0.01$). In the combined MSG and tannic acid group, the basophil percentage decreased ($P < 0.01$). In the tannic acid group, the lymphocyte percentage increased, but remained within the average value limits ($P > 0.05$, Table 1).

The tannic acid treatment increased hemoglobin levels in comparison to those of the control group ($P < 0.01$). However, the values remained within the average limits. The combination of MSG and tannic acid decreased the platelet volumes in comparison to the corresponding values of the control group ($P < 0.01$, Table 2).

We also examined the blood glucose values. The blood glucose values in the combined monosodium glutamate and tannic acid group decreased when compared to the values for the other groups. The decrease of blood glucose values was especially significant compared to the corresponding levels for the tannic acid group ($P < 0.001$) and the MSG group ($P = 0.029$, Figure 1).

The SOD activities and MDA concentrations in the brain, liver, and kidney tissue homogenates and blood hemolysates were determined. The SOD activities were not different in the brain, liver, and kidney homogenates ($P = 0.135$, $P = 0.809$, and $P = 0.343$, respectively). There was a difference between the groups regarding the blood SOD activities ($P = 0.01$). The blood SOD enzyme levels in the MSG and tannic acid group increased in comparison to the levels of the MSG group ($P < 0.01$, Table 3).

The MDA concentrations in the brain homogenates were significantly different ($P < 0.001$). The MDA levels

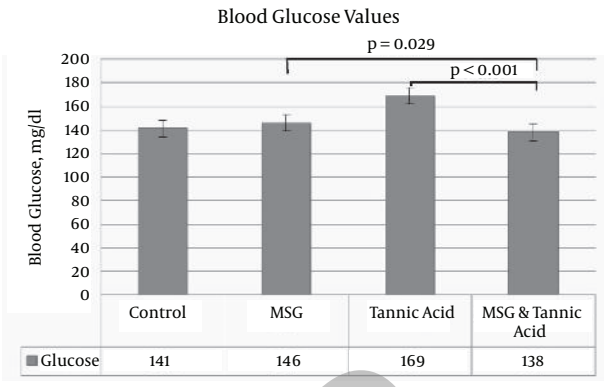


Figure 1. Blood Glucose Values

in the tannic acid and the combined tannic acid and MSG group significantly decreased in comparison to the values of the control group ($P < 0.001$ and $P < 0.001$, respectively). The MDA levels in the brain homogenates of the tannic acid group and the combined tannic acid and MSG group significantly decreased when compared to the levels for the monosodium glutamate group ($P < 0.001$ and $P < 0.001$, respectively). There were no statistical differences between the MDA levels present in the liver, kidney, and blood homogenates ($P = 0.079$, $P = 0.522$, and $P = 0.265$, respectively) (Table 4).

The histological analysis indicated that the kidney cortex and medulla were similar to the control group (Figure 2). There were no pathologies such as inflammatory cell increase, bleeding, fluid retention, or tubular cell necrosis observed. The liver tissues were analyzed for inflammatory cells, fluid retention, bleeding, congestion, and necrosis. However, there were no histopathological findings (Figure 3). The pyramidal and granular neurons were examined in the brain cortex, and no significant neuron degeneration was observed (Figure 4).

5. Discussion

Previous studies have shown MSG causes oxidative stress and damage in the brain, kidneys, and liver (20-22). MSG has been shown to impair brain functions and cause oxidative damage (20). In this study, we observed that tannic acid significantly reduced the levels of MDA induced by MSG in the brain, and increased the blood SOD enzyme levels in the MSG and tannic acid group. These results show that tannic acid as an antioxidant protects against the oxidative stress caused by MSG.

Previous studies on obese rats reported that MSG alters glucose metabolism and causes insulin resistance (23). The

Table 1. Neutrophil, Monocyte, Eosinophil, Basophil, and Lymphocyte Values in the Experimental Groups

Groups	Neutrophil		Monocyte		Eosinophil		Basophil		Lymphocyte	
	%[a]	x10^3/μL[b]	%[a]	x10^3/μL[a]	%[a]	x10^3/μL[a]	%[a]	x10^3/μL[a]	%[b]	x10^3/μL[b]
Control (1)	5.4 (2 - 5.9)	0.24 ± 0.16	0.8 (0.6 - 6)	0.1 (0 - 1.1)	1.5 (0.7 - 4.8)	0.1 (0 - 0.1)	16.18 (16.18 - 19.4)	0.3 (0.1 - 0.9)	64.06 ± 9.02	4.47 ± 2.80
Tannic Acid (2)	7.7 (3.2 - 14.5)	0.3 ± 0.2	0.7 (0.3 - 4.8)	0 (0 - 0.2)	2.5 (1.3 - 8.1)	0.1 (0 - 0.3)	9.2 (0.2 - 3.1)	0.3 (0 - 0.4)	79.06 ± 7.11	2.94 ± 0.76
MSG (3)	5.58 (5.2 - 5.7)	0.3 ± 0.25	2.15 (0.6 - 2.7)	0 (0 - 0.1)	0.8 (0 - 4.7)	0.05 (0 - 0.1)	3.5 (0.3 - 1.4)	0 (0 - 0.78)	73.52 ± 17.16	2.4 ± 1.35
MSG and Tannic Acid (4)	32.4 (24.9 - 38.2)	1.5 ± 1.3	9.3 (0.2 - 8.2)	0.1 (0 - 0.9)	1.5 (0.3 - 3.77)	0.1 (0 - 0.22)	0.7 (0.2 - 3.1)	0 (0 - 0.12)	50.87 ± 9.59	2.73 ± 1.5
P Value	0.001	0.006	0.609	0.361	0.691	0.412	0.008	0.377	0.001	0.159
Multiple Comparisons	1 - 4: 0.001, 2 - 4: 0.001, 3 - 2: 0.001	1 - 4: 0.013, 3 - 4: 0.019	-	-	-	-	1 - 4: 0.005	-	2-4: 0.005, 3 - 2: 0.001	-

[a] Kruskal-Wallis test, data are presented as median (25th percentile to 75th percentile).
[b] One-way ANOVA; data are presented as mean ± standard deviation.

Table 2. The Results of Blood Parameters in the Experimental Groups

Groups	% Mean Corpuscular Hemoglobin Concentration (MCHC), g/dL[a]	Mean Platelet Volume (MPV), fL[a]	White Blood Cells (WBC), 10^3/μL[a]	Red Blood Cells (RBC), 10^6/μL[a]	Hemoglobin (HGB), g/dL[a]	Mean Corpuscular Volume (MCV), fL[a]	Hematokrit (HCT), %[a]	Mean Corpuscular Hemoglobin (MCH), pg[a]	Platelet (PLT), 10^3/μL[a]	Red Blood Cell Distribution Width (RDW), %[b]	Platelet Distrubition Width (PDW), %[b]
Control (1)	32.6 ± 0.86	6.11 ± 0.54	4.7 ± 2.02	6.6 ± 0.73	12.2 ± 1.9	56.3 ± 5.1	37.4 ± 5.6	18.4 ± 2	733 ± 171	15.7 (12.6 - 17.7)	16.9 (15.8 - 17.6)
Tannic Acid (2)	33.7 ± 0.34	5.42 ± 0.28	3.7 ± 0.75	6.9 ± 0.54	13.2 ± 0.7	56.6 ± 2.3	39.2 ± 1.8	19 ± 0.9	884 ± 165	13.8 (13.5 - 15.1)	16 (15.8 - 16.1)
MSG (3)	33.23 ± 0.34	5.32 ± 0.13	3.6 ± 2.50	6.8 ± 0.19	12.9 ± 0.7	56.8 ± 2.1	38.7 ± 2.1	18.9 ± 0.6	793 ± 105	14.3 (13.3 - 15.4)	16.22 (16.2 - 16.3)
MSG and Tannic Acid (4)	33.5 ± 0.54	5.35 ± 0.42	5.5 ± 2.83	7.1 ± 0.45	13 ± 1.3	56.2 ± 3	40 ± 2.9	18.8 ± 0.8	861 ± 179	14.58 (11.5 - 16.7)	16.1 (15.8 - 16.5)
p valuea	0.008	0.001	0.359	0.342	0.493	0.988	0.545	0.692	0.291	0.737	0.106
Multiple Comparisons	1 - 4:0.035, 1 - 3: 0.007	1 - 3: 0.011, 1 - 2: 0.003, 1 - 4: 0.004									

[a] One-way ANOVA; data are presented as mean ± standard deviation.
[b] Kruskal-Wallis test; data are presented as median (25th percentile to 75th percentile).

Table 3. SOD Levels of Brain, Liver, and Kidney Tissue Homogenates and Blood Hemolysates

Groups	Brain SOD, % Inhibition[a]	Liver SOD, % Inhibition[b]	Kidney SOD, % Inhibition[b]	Blood SOD, % Inhibition[a]
Control (1)	39.5 (39.3 - 39.6)	38.9 ± 0.2	30.5014 ± 2.6	36.3 (34.5 - 37.1)
Tannic Acid (2)	39.2 (38.9 - 39.3)	38.9 ± 0.3	29.3857 ± 2.6	37.4 (36.1 - 38.2)
MSG (3)	39.4 (39.2 - 39.5)	39.1 ± 0.2	31.1886 ± 3.1	36.6 (35.2 - 37.9)
MSG and Tannic Acid (4)	39.3 (39.1 - 39.3)	38.9 ± 0.2	31.8143 ± 1.7	38.5 (38.1 - 38.9)
P value	0.132	0.752	0.343	0.01
Multiple Comparisons	-	-	-	1 - 4: 0.008

[a] Kruskal-Wallis test; data are presented as median (25th percentile to 75th percentile).
[b] One-way ANOVA; data are presented as mean ± standard deviation.

catechins are phenolic compounds in tannic acid that prevent Type 2 diabetes (24). Tannic acid reduces the blood glucose in adipose tissue by activating glucose transportation and the insulin signaling pathway. We observed that MSG increases blood glucose values. Although the blood sugar level was increased in the MSG-treated group, it appears as though the pretreatment with tannic acid ultimately reduced blood glucose levels.

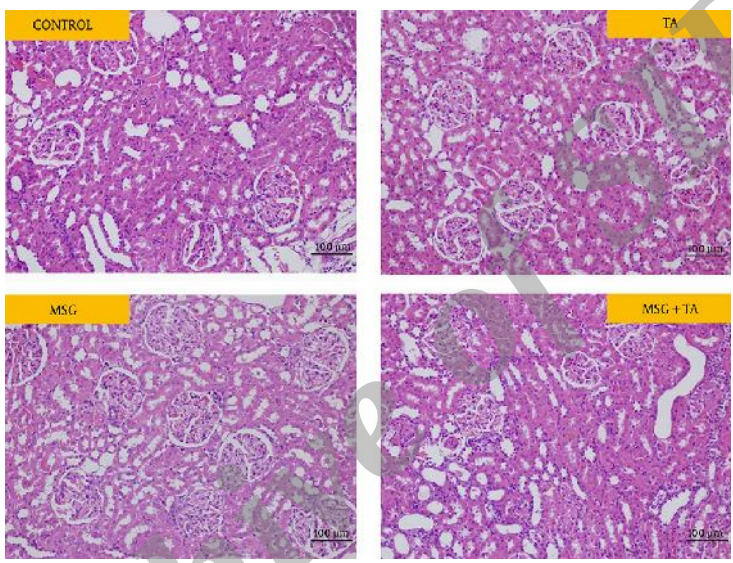
A previous study reported that MSG injected i.p. at a dose of 4 mg/g of animal bodyweight increased MDA levels in the liver, kidney, and brain. Additionally, oxidative damages in rat livers, kidneys, and brains have been shown to be reduced by using vitamin C, E, and quercetin, which are all known antioxidants. Dietary polyphenols such as tannic acid and quercetin have highly effective ferric reducing and scavenging free radical power, and act as electron donors. Along these lines, Pulido et al. indicated that tannic acid showed the highest antioxidant efficiency be-

Table 4. MDA Enzyme Levels of Brain, Liver, and Kidney Tissue Homogenates and Blood Hemolysates

Groups	Brain MDA, nmol/mL[a]	Liver MDA, nmol/mL[a]	Kidney MDA, nmol/mL[b]	Blood MDA, nmol/gHb[b]
Control (1)	1.6 ± 0.23	1.07 ± 0.31	1.340 (1.252 - 2.965)	1.510 (1.130 - 2.275)
Tannic Acid (2)	0.94 ± 0.16	0.97 ± 0.26	1.440 (1.303 - 0.475)	0.420 (0.420 - 1.705)
MSG (3)	1.4 ± 0.19	1.59 ± 0.68	1.390 (1.252 - 1.457)	0.770 (0.623 - 1.273)
MSG and Tannic Acid (4)	0.89 ± 0.93	1.22 ± 0.41	1.960 (1.460 - 3.418)	2.170 (0.730 - 2.920)
P Value	< 0.001	0.079	0.522	0.265
Multiple Comparisons	1 - 4: < 0.001, 1 - 3: < 0.001, 3 - 4: 0.001			

[a] One-way ANOVA; data are presented as mean ± standard deviation.
[b] Kruskal-Wallis test, data are presented as median (25^{th} percentile to 75^{th} percentile).

Figure 2. Normal Histological Appearance of Renal Cortex Section Represented for all Experimental Groups (H and E)



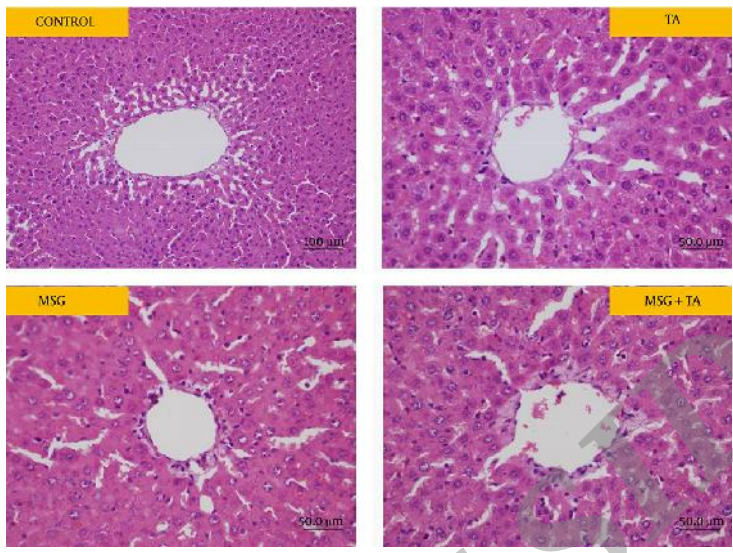
MSG, monosodium glutamate; TA, tannic acid.

tween gallic and caffeic acid in vitro (25, 26).

This study in rats also demonstrated that the MDA level was increased by MSG, but was decreased with vitamin C, vitamin E, and the polyphenolic compound quercetin. It has been also shown that quercetin in combination with vitamin C protects the brain from membrane damage (12). Another study involved the administration of MSG to rats at doses of 0.6 mg/g and 1.6 mg/g body weight for 14 days. In this study, the authors showed that low doses of MSG led to changes in both liver and kidney functions. These changes were apparent in detoxifying organs such as the liver and kidneys (23). In contrast, our findings indicate that MSG and tannic acid do not cause significant histological changes in the kidney, liver or brain cortex. Prior studies examining MSG changes also showed that there were increased MDA levels in the brain and kidney and reduced SOD levels (12).

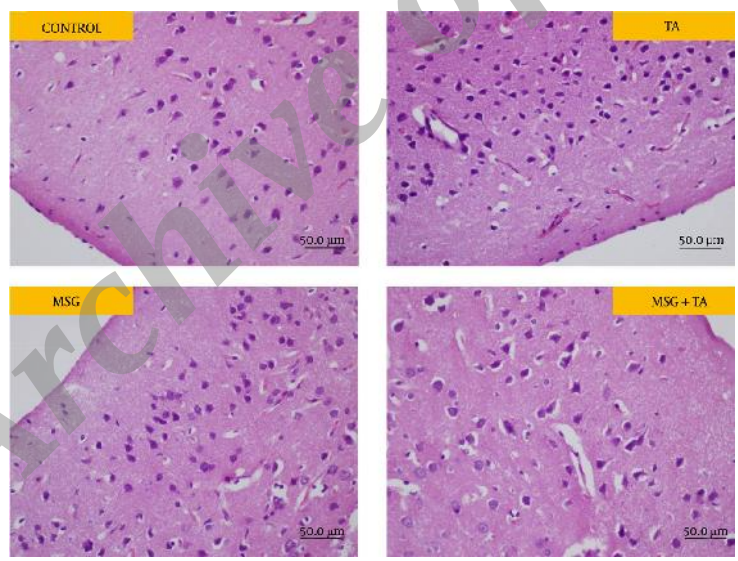
In this study, we used the antioxidant tannic acid to protect the animals before MSG treatment. Although several studies have shown that tannic acid has an effective antioxidant capacity by increasing antioxidant enzymes, the present study indicated that tannic acid administration alone did not reveal an increase in antioxidant activity of SOD compared to the control group, which is in agreement with El-Sayed's results (27). We found that tannic acid pretreatment in adult rats reduced the blood glucose and MDA increases caused by MSG. Additionally, tannic acid effectively increased the blood SOD enzyme levels. We think that tannic acid can also reduce the side effects in neonatal and non-adult rats caused by MSG. Short-term MSG use did not cause significant histological changes in kidney, liver or brain cortex. It can therefore be asserted that the histological evaluations would be more effective after longer applications.

Figure 3. Valve Liver Sections Represented for all Experimental Groups Showing Normal Hepatocytes (H and E)



MSG, monosodium glutamate; TA, tannic acid.

Figure 4. Normal Histological Appearance of the Cerebral Cortex Section Represented for all Experimental Groups (H and E)



MSG, monosodium glutamate; TA, tannic acid.

5.1. Conclusion

There has been no study on the effects of tannic acid on oxidative stress induced by MSG in the literature, although it has been shown that MSG induces oxidative stress in rats. This is the first study to our knowledge showing that tannic acid effects MSG levels. Future studies that use different doses and/or extended treatment periods are needed to further support these results.

Footnotes

Authors' Contribution: All of the authors approved the content of the manuscript, contributed significantly to the research, and were involved the writing of the manuscript.

Didem Turgut Cosan, Hulyam Kurt, Hasan Veysi Gunes, and Irfan Degirmenci were responsible for the study conception and design, preparing the draft of the manuscript, and making critical revisions to the paper for important intellectual content and English editing. Ibrahim Ugur Calis, Faruk Saydam, Umut Kerem Kolac, Ahu Soyocak, and Zeynep Ozdemir Koroglu were responsible for the data collection and laboratory analysis. Varol Sahinturk was responsible for the histological analysis. Fezan Sahin Mutlu was responsible for the statistical evaluations.

Conflict of Interest: The authors declare that they have no conflict of interest.

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