Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES
July 2022, Volume 16, Number 3

Research Paper

Phyllanthus Emblica Extract Protects the Rat Liver Cells Against the Toxicity of Monosodium Glutamate: Experimental Evidence

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How to cite this paper Babu Thangachi S, Sriram Mokhasi V, Kanna Sathyamoorthy S, Bharat Kumar Pinnelli V, Chiruthanur S. *Phyllanthus Emblica* Extract Protects the Rat Liver Cells Against the Toxicity of Monosodium Glutamate: Experimental Evidence. Iranian Journal of Toxicology. 2022; 16(3):203-210. http://dx.doi.org/10.32598/IJT.16.3.923.1

http://dx.doi.org/10.32598/IJT.16.3.923.1

Article info:
Received: 10 Dec 2021
Accepted: 01 Feb 2022
Online Published: 01 Jul 2022

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ABSTRACT

Background: Monosodium Glutamate (MSG), used widely in the food industry, is a threat to the public health. We investigated whether the MSG administration depletes non-enzymatic antioxidants, i.e., vitamins C and E in the liver of Wistar albino rats. We also examined the restorative effect of the ethanolic extract of *Phyllanthus emblica* (*P. emblica*).

Methods: Wistar albino rats (n=42) were adapted and then randomly divided into seven groups of: 1) control, 2, 3, 4) MSG treatment, and 5, 6, 7) combined MSG and *P. emblica* extract treatment. All rat groups were treated daily for 120 days. They were orally administered either MSG alone or MSG plus the extract combined. The rats were then sacrificed and the liver was harvested from each group, and homogenized to examine the levels of vitamins C and E in the liver, using RP-HPLC method.

Results: The vitamins C and E levels significantly declined ($P<0.05$) in the liver of MSG treated groups compared to those of the control rats. The combined treatment (extract + MSG) at low and moderate doses restored the vitamin C levels but it restored vitamin E only at the low dose ($P<0.05$).

Conclusion: This study clearly demonstrated the deterioration of non-enzymatic antioxidants, i.e., vitamins C and E in the rats' liver after chronic exposure to MSG. The findings support the toxic effect and oxidative stress due to MSG exposure to the liver and the beneficial effect of the extract of *P. emblica* that inhibits the MSG's harmful effect on the liver.

Keywords: Antioxidants, Monosodium glutamate, Oxidative stress, *Phyllanthus emblica*, Vitamin C, Vitamin E

Introduction

The worldwide rise in the use of food additives is alarming as there are progressive increases in the consumption of packaged and restaurant-based foods by the public. Professor Kikunae Ikeda from Japan was the first person to identify and extract monosodium glutamate from seaweed [1]. The term "Ajinomoto" is popularly proclaimed for Monosodium Glutamate (MSG) globally, which contains the sodium salt of L-glutamic acid ($C_5 H_8 NO_4 Na$) [2]. The most celebrated snacks, such as chips, jelly, pastry, candy, biscuit, chocolate, french-fries, and pizza attract more consumers since they offer savory tastes, which is due to the addition of MSG to them [3]. The reason why MSG boosts the fla-

vor of foods is because it stimulates taste receptors in humans [3, 4]. Both the production and consumption of MSG are increasing daily in developing countries, which is proportional to the growth of packaged foods [4]. Although the U.S. Food and Drug Administration (FDA) recognizes MSG as being safe for human foods, scientists worldwide still believe that it is harmful to the human health [5].

The cellular metabolism produces free radicals, generating highly reactive, unpaired electrons, which cause damage to cellular membranes and organelles, and to biological molecules in cells, resulting in oxidative stress [6]. Superoxide, hydrogen peroxide, singlet oxygen and hydroxyl groups are free radicals called Reactive Oxygen Species (ROS) [7]. The disproportionate rise in ROS and the limited body's antioxidant defense against oxidative stressors are the current major concerns about human health. These factors lead to disruption in the signal transduction systems, DNA damages, and peroxidation of the cellular lipid-rich membranes [8]. Liver is the major gastrointestinal gland, performing a large number of biochemical functions in the body, notably on carbohydrate, protein and lipid metabolisms; however, it is the most affected organ by oxidative stressors [9].

The oxidative stress quenches both enzymatic and non-enzymatic antioxidants in the liver [10, 11]. The consumption of MSG in animal studies has been shown to be hepatotoxic by altering redox state. This process results in abnormal levels of vital liver enzymes, such as Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), albumin, total protein and total bilirubin in the blood [12]. Vitamin C, chemically known as L-ascorbic acid, is a water-soluble compound, which is essential for multiple biological functions. Also, it acts as a non-enzymatic antioxidant and aids the tissues in scavenging free radicals in biological systems, such as hydrogen peroxide, hydroxyl radicals and oxygen singlets [13].

Further, vitamin E, or α-tocopherol, is a lipid soluble vitamin known for its antioxidant activity in protecting biological membranes, proteins and DNA strands, ensuring the health of human cells [14]. *Phyllanthus emblica* (Amla = *P. emblica*), commonly known as gooseberry in India, has multiple protective properties against cancers, ulcers, fever and microbial pathogens [15]. It is also an antilipidemic compound, enhances the immune system, and protects us against liver and heart diseases, and diabetes [15]. Further, *P. emblica* is rich in vitamin C, carbohydrates, proteins, fiber, minerals, potent polyphenols, like gallic acid [16]. Most earlier studies were not conclusive in proposing the dose for MSG as well as mode of administration based on human consumption, which was taken into consideration in the current study [17].

Aim of the study: This research was planned to explore whether a single dose of *P. emblica* was enough to quench the oxidative stress caused by three different doses of MSG. For this purpose, we used a standard dose of *P. emblica* extract (75mg/kg) to see whether it would quench MSG at low, mid or high doses in a Wistar rat model. We have obtained exciting results, which are presented in this article.

Materials and Methods

Animals: Forty-two adult Wistar albino rats of either sex were acquired and cared for based on the guidelines of the Institutional Animal Ethics Committee of Vydehi Institute of Medical Sciences and Research Center in Bengaluru, Karnataka, India (Registration # VIMS/IAEC/2016/03). The rats were kept properly in well maintained polypropylene cages at 23 ± 1°C under 12hr of alternating light and dark cycles. They were fed with standard laboratory food pellets and hydrated with clean water ad libitum. Also, they were adapted to the laboratory environment before commencing the study. The study protocol and animal handling followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals at the same university.

Experimental design: The rats were randomly assigned to seven groups of six each in the current study. Both the food grade MSG and the ethanolic extract of *P. emblica* were administered to the rats daily via oral gavage for a consecutive period 120 days. The animal groups were as follows:

- Group 1 (control) rats were fed with distilled water.
- Group 2 (low dose) rats received 180 mg/kg MSG.
- Group 3 (mid dose) rats received 360 mg/kg MSG.
- Group 4 (high dose) rats received 720 mg/kg MSG.
- Group 5 (low dose + treatment) rats received 180 mg/kg MSG+75 mg/kg extract.
- Group 6 (mid dose + treatment) rats received 360 mg/kg MSG+75 mg/kg extract.

• Group 7 (high dose+reatment) rats received 720 mg/kg MSG+75 mg/kg extract.

Vitamins C & E analyses: At the end of the study period, each animal was euthanized and sacrificed by cervical dislocation, and the abdomen was dissected to harvest the liver. One portion of the liver from each rat was homogenized, centrifuged at 12000 rpm at 2°C for 10 minutes, and the supernatants were collected for the estimation of vitamins C and E contents. The vitamins' analyses were performed using an RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) method [18].

Statistical analyses: The data were statistically analyzed as the Mean±SD for each rat group. GraphPad Prism software, v. 9.1.2, was used for the data analysis. For this purpose, we used one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. The statistical differences were considered as significant if the P was equal to or less than 5%. The P in the current study are illustrated as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

Results

The present study found significant differences (P<0.05) in the vitamin C levels of the rats' liver homogenate comparing the control with the groups that received MSG. The chronic administration of MSG significantly (P<0.05) reduced the liver vitamin C levels to 29.13%, 44.28% and 59.94%, respectively, in groups that received low, mid and high doses of MSG compared to those of the control group (Figure 1).

Treating the rat groups that had received the low and mid doses of MSG with a standard dose of the *P. emblica* extract significantly raised the liver vitamin C levels by 46.97% and 35.69%, respectively (Figure 2).

The group that was treated with a high dose of MSG failed to make a significant difference in the liver vitamin C levels, compared to the rats that received the extract at a high dose. The vitamin E levels exhibited significant decreases by 23.52%, 48.28% and 53.60%, respectively, in the groups that received low, mid and high doses of MSG compared to those of the control group (P<0.05; Figure 3).

The low dose MSG given concurrently with a single dose of the extract caused a significant rise (P<0.05) in the liver vitamin E levels compared to those of the group that did not receive the extract with the same low dose MSG (Figure 4). The liver vitamin E levels in the groups that received the mid or high doses of the extract did not exhibit a significant difference compared to the corresponding groups that were exposed only to the mid and high doses of MSG.

Discussion

Briefly, this study demonstrated that MSG at three different doses caused significant oxidative stress in adult Wistar rats by depleting vitamins C and E in the liver. However, a single dose of the *P. emblica* extract protected the liver effectively.

Suggestions from previous studies related to the deleterious effects of MSG on various organ systems found in animal models are still incomplete [4]. This is because the MSG doses administered to animals via various routes are not comparable to those consumed by humans, particularly for fast foods [17]. The current study investigated the levels of vitamins C and E in the liver of Wistar albino rats with a focus on the chronic consumption of MSG at comparable dosage per kilogram body weight of humans in developing and developed nations. The use of the *P. emblica* extract as an alternative treatment for MSG-treated rats was hypothesized because of the well-known performance of the extract in treating hepatotoxicity in animals [19-21].

The abnormally reduced liver vitamin C levels at the three doses of MSG given to the rats may suggest that this compound indeed has its role in causing oxidative stress in the rats' liver and other organs [22]. Naturally, vitamin C acts as a reducing agent to quench free radicals, such as oxygen singlets and hydrogen peroxide, and prevents lipid peroxidation in cells [23]. Previous studies have clearly pointed out to significant reductions in endogenous antioxidants, such as Glutathione Peroxidase (GPX), Superoxide Dismutase (SOD), Catalase (CAT) in MSG-treated rats, most likely due to a rise in the oxidative stress at cellular levels [24].

Our findings of 29.13%, 44.28% and 59.94% decreases in the liver vitamin C levels in the rat groups treated with low, mid and high doses of MSG, provide ample evidence that MSG at the doses used in this study definitely demonstrates its ability to cause oxidative stress in the rats' liver. The damaging effect of MSG, as shown in the present study is highly likely to be via the increased ROS activity around the rats' liver cells. This in turn might be the main reason for the decline in the vitamin C levels, implying that the liver cells tried actively and

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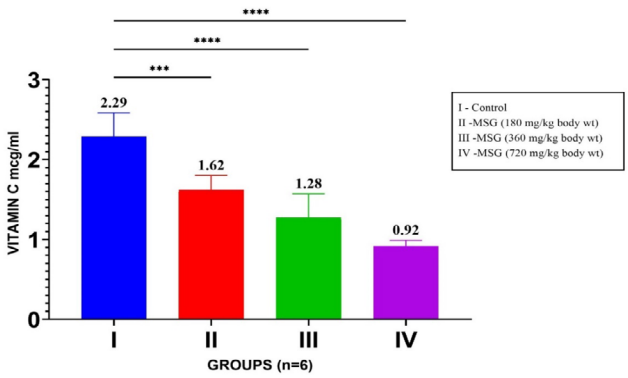

Figure 1. Vitamin C levels in the liver homogenates of monosodium glutamate induced groups.

Values are displayed as Mean±SD. *Denotes significantly different from the control group at P<0.05. **Indicates significantly distinct from the control group at P<0.01. ***Specifies significantly peculiar from the control group at P<0.001. ****Implies significantly different from the control group at P<0.0001.

utilized the vitamin to combat the oxidative stress imposed by MSG [10].

Vitamin E, another potent non-enzymatic antioxidant, quenches free radicals by donating its phenolic hydrogen to free radicals, thus preventing lipid peroxidation in living cells [25]. Our study data indicate that the vitamin E levels in the rats' liver decreased by 23.52%, 48.28% and 53.60%, respectively, versus low, mid and high doses of MSG given to the animal groups. The findings may indicate the effective utilization of α-tocopherol to inhibit or minimize the high levels of oxidative stress in the rats' liver cells. The current study's effective treatment of the oxidative stress damages that MSG induced in the liver cells by using the *P. emblica* extract may be attributed to the preservation of the bioavailability of vitamins C and E, gallic acid and other effective phytochemicals in the rats' liver [15]. The increase in the liver's vitamin C levels at low and mid extract doses in the treatment groups compared to the corresponding MSG groups without the extract provides strong evidence that the extract increased the levels of liver vitamin C to fight effectively against the oxidative stress imposed by MSG. The group that received the high treatment dose did not show a significant change in the liver vitamin C level. This finding may be convincing in that the standard dose of the extract might have not been sufficient to treat the MSG toxicity at the high dose.

The vitamin C concentration in *P. emblica* are several times more than those found in other citrus fruits, thus making it a potent antioxidant fruit [26]. There have been numerous studies to show the *P. emblica*'s well-known phytochemical activity against hepatotoxicity caused by ethanol consumption [27]. The fact that the vitamin E levels showed significant rises in the low dose extract treatment group compared to that in low dose MSG group may confirm that the treatment in the low dose

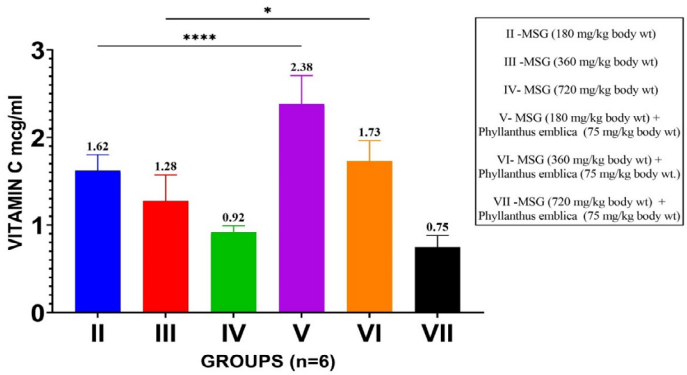

Figure 2. Vitamin C levels in the liver homogenates of monosodium glutamate induced groups treated with the ethanolic extract of *Phyllanthus emblica* versus monosodium glutamate induced groups. Values are displayed as the Mean±SD.

*Expresses significantly different from the control group at P<0.05. **Indicates significantly distinct from the control group at P<0.01. ***Specifies significantly peculiar from the control group at P<0.001. ****Implies significantly different from the control group at P<0.0001.

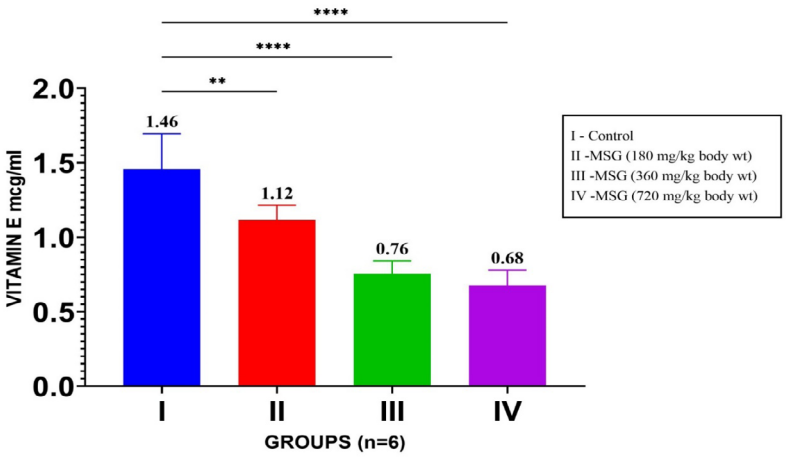

Figure 3. Vitamin E levels in the liver homogenates of monosodium glutamate induced groups.

Values are shown as the Mean±SD. *Expresses significantly different from the control group at P<0.05. **Indicates significantly distinct from the control group at P<0.01. ***Specifies significantly distinct from the control group at P<0.001. ****Implies significantly different from the control group at P<0.0001.

MSG group positively protected the liver cells against the oxidative stress. The vitamin C present in the extract may be advantageous over vitamin E, since the latter vitamin gets oxidized by free radicals in the process of ROS elimination [13]. The oxidation of vitamin E generated tocopheryl radicals, which recruit electron donation from vitamin C to become vitamin E. The mid and high doses treatment groups failed to demonstrate significant changes compared to the corresponding MSG groups. This may imply that the insufficient dose of the *P. emblica* extract to combat the oxidative stress provoked by the high MSG dose.

Conclusion

The current study findings suggest that oxidative stress alters the redox state linked to the non-enzymatic antioxidants in liver cells may be due to the involvement of MSG in various cellular metabolic processes that generate large amounts of ROS. The *P. emblica* extract, well-known for its antioxidant property, effectively inhibited the oxidative stress induced by MSG but the limitation of using a single dose of the extract for curbing the effects of three different doses of MSG confirmed that the single extract dose may not be sufficient against MSG toxicity at multiple doses. The competition among food industries in the global market invariably increases the use of MSG to retain their market profitability and posi-

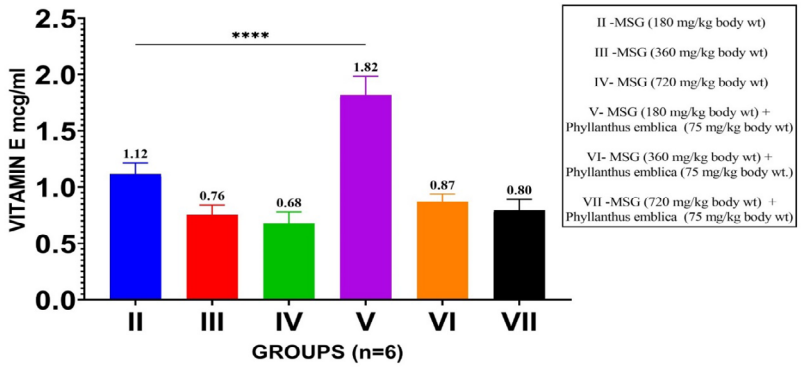

Figure 4. Vitamin E levels in the liver homogenates of monosodium glutamate induced groups treated with the ethanolic extract of *Phyllanthus emblica* versus monosodium glutamate induced groups. Values are displayed as the Mean±SD.

*Expresses significantly different from the control group at P<0.05. **Indicates significantly distinct from the control group at P<0.01. ***Specifies significantly different from the control group at P<0.001. ****Implies significantly different from the control group at P<0.0001

tion in the world. The main purpose of using *P. emblica* extract in the current study was not only to prove its efficacy, but also to encourage the public to consume Indian gooseberry regularly in support of maintaining a healthy lifestyle when it is not practical to fight the presence of food additives, such as MSG, in everyday foods in the market.

Limitations of the study: The current study findings on the chronic exposure to MSG in rats cannot be directly extrapolated to humans, as this requires proper clinical trials to confirm the MSG toxicity in humans.

Recommendations for future studies: The current study recommends that future research should concentrate on genotoxicity induced by MSG, primarily in vital organ systems. Still, there should be further studies confirming whether MSG exaggerates the preexisting liver pathologies such as cirrhosis, hepatitis, alcoholic and non-alcoholic fatty liver diseases and other drug-induced liver conditions.

Ethical Considerations

Compliance with ethical guidelines

The entire study protocol and animal handling were preceded based on the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethical Committee (IAEC) permission with Registration No: VIMS/IAEC/2016/03 was acquired before the initiation of the study.

Funding

Funding for this work was provided solely by the authors.

Authors' contributions

Conceptualization & supervision: Varsha Sriram Mokhasi and Venkata Bharat Kumar Pinnelli; Design: Varsha Sriram Mokhasi & Surendra Babu Thangachi; Data collection and analysis: Surendra Babu Thangachi. Drafting of the manuscript: Surendra Babu Thangachi & Venkata Bharat Kumar Pinnelli; Review and editing: All authors. Read and approved the final version of the manuscript: All authors.

Conflict of interest

The authors declare no conflict of interests with any internal or external entities in conducting this study.

Acknowledgements

The authors wish to acknowledge the Vydehi Institute of Medical Sciences & Research Center, in Bengaluru, India, for their support and provision of laboratory spaces toward the conduction of this research project.

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