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Mitigating Potential of Ascorbic Acid against Monosodium Glutamate-induced Liver Fibrosis and Oxidative Stress in Wistar Rats

VC Ezeuko[a], M. Omorose[a], S.M. Nweke[a], J.E. Aig-Unuigbe[a] and E.O. Imafidon[a]

[a]Department of Anatomy,
School of Basic Medical Sciences,
College of Medical Sciences,
University of Benin,
Benin City,
Edo State, Nigeria.

Email: chukwuma.ezeuko@uniben.edu

Abstract

Hepatotoxicity is a major health concern globally, with the liver being particularly vulnerable to damage from various substances, including monosodium glutamate (MSG), a common food additive. While MSG is generally recognized as safe, excessive consumption has been linked to liver damage through oxidative stress, inflammation, and cell damage. This study aimed to explore the mitigating potential of ascorbic acid against MSG-induced liver fibrosis and oxidative stress in adult Wistar rats. Wistar rats were randomly divided into four groups: Group A served as control; Group B received 200 mg/kg body weight of MSG; Group C received 200 mg/kg body weight of MSG and 100 mg/kg body weight of ascorbic acid; Group D received 100 mg/kg body weight of ascorbic acid. After 28 days, liver function, oxidative stress markers, and histology were assessed. Results showed that there were significant reductions ($p<0.05$) in albumin, total protein and total bilirubin levels, glutathione peroxidase, catalase, superoxide dismutase, with significantly increased ($p<0.05$) alanine aminotransaminase and malondialdehyde levels in the MSG-only group when compared to control. However, co-administration of MSG and ascorbic acid significantly improved these parameters. Histological findings showed that MSG caused zonal necrosis and mild Kupffer cell activation, infiltrates of inflammatory cells, and increased collagen deposit in the liver. However, on co-administration of ascorbic with MSG, there was significant improvement in liver histology, as evidenced by mild inflammatory cells and collagen deposition in the MSG and ascorbic acid treated group. In conclusion, findings from this study presented compelling evidence of MSG-induced hepatic damage and the protective potential of ascorbic acid protects against MSG-induced liver histological alterations, fibrosis and oxidative stress in Wistar rats.

Keywords: Monosodium glutamate, Hepatotoxicity, ascorbic acid, Wistar rats.

INTRODUCTION

Hepatotoxicity is a significant health concern worldwide, with various factors contributing to its development (Fernández-Lázaro *et al.*, 2020). The liver plays a crucial role in detoxification, metabolism, and nutrient processing, making it susceptible to damage from harmful substances (Mahadevan *et al.*, 2020). Despite its remarkable regenerative capacity, the liver is

Author for Correspondence

susceptible to damage from various assaults, including environmental toxins, medications, and dietary factors. One such dietary factor is the consumption of monosodium glutamate (MSG), a common food additive that is widely used to enhance the flavor of food.

MSG is the sodium salt of the amino acid glutamic acid (Thuy *et al*., 2020). Glutamic acid or glutamate is one of the most common amino acids found in nature. It is the main component of many proteins and peptides and is present in most tissues (Halim *et al*., 2020). It is made commercially by the fermentation of molasses but exists in many products made from fermented proteins, such as soy sauce and hydrolyzed vegetable protein (Thuy *et al*., 2020).

While generally recognized as safe by regulatory authorities, excessive consumption of MSG has been associated with adverse health effects, including liver damage (Halim *et al*., 2020; Thuy *et al*., 2020). Studies have shown that high levels of MSG can lead to oxidative stress, inflammation, and cell damage in the liver, ultimately impairing its function (Halim *et al*., 2020). The danger of MSG lies in its ability to overstimulate glutamate receptors in the brain, leading to excitotoxicity and potentially damaging effects on liver cells (Abdou *et al*., 2020). Additionally, MSG has been linked to the development of non-alcoholic fatty liver disease (NAFLD), a condition characterized by the accumulation of fat in the liver (Banerjee *et al*., 2021; Shakour *et al*., 2022).

Epidemiological studies have highlighted the prevalence of MSG consumption and its potential impact on liver health. In many countries, MSG is widely used in processed foods, restaurant meals, and fast food, contributing to high levels of exposure among the population (Thuy *et al*., 2020). This has raised concerns about the long-term health effects of chronic MSG consumption, particularly its role in liver disease. Antioxidants play a crucial role in protecting the liver from such damage by neutralizing harmful free radicals and reducing oxidative stress (Engwa *et al*., 2022).

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin and a potent antioxidant (Colunga Biancatelli *et al*., 2020). It plays a crucial role in protecting cells from oxidative damage by scavenging free radicals, which are highly reactive molecules that can cause cellular damage and contribute to the development of various diseases, including liver damage (Carr and Rowe, 2020). Ascorbic acid also regenerates other antioxidants in the body, such as vitamin E, further enhancing its antioxidant properties (Doseděl *et al*., 2021). It works to enhance the antioxidant defense system in the liver, scavenges free radicals directly and enhances the ability of the liver to neutralize oxidative stress and protect against liver damage, thus, making it a potential candidate for therapeutic intervention.

Unfortunately, the mitigating potential of this strong antioxidant against MSG-induced liver toxicity has not been fully explored which makes this study imperative to investigate the mitigating potential of ascorbic acid against MSG-induced liver histological alterations, fibrosis and oxidative stress in adult Wistar rats.

MATERIALS AND METHOD

Experimental Animals: Wistar rats were procured and bred in the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The rats were kept in polypropylene cages at room temperature, with natural light and dark cycle photoperiodicity. The animals were fed with pelleted feed (manufactured by Premier Feed Milling Company Limited, 1 Eagle Flour Road, Lagos-Ibadan Expressway, Toll Point, Ibadan, Oyo State, Nigeria) and clean tap water *ad*

libitum. The animals were weighed weekly before commencement and throughout the duration of the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

Experimental Design: Twenty (20) adult Wistar rats weighing between 170g and 200g were used for this study. They were divided into four (4) groups of five (5) rats each. Group A animals served as control group and were given distilled water only; group B rats were given 200 mg/kg body weight of MSG only; group C animals were given 200 mg/kg body weight of MSG and 100 mg/kg body weight of ascorbic acid (AA); group D animals were given 100 mg/Kg body weight of AA only. Administration lasted for twenty-eight (28) days and all administrations were carried out via oral route.

At the end of the 28 days administration, the animals were fasted overnight and sacrificed under chloroform anaesthesia. The liver from each animal was excised, blotted clean of blood and fixed in 10% neutral buffered formal saline for 48 hours prior to processing.

Liver Function Assessment: Blood sample was taken for liver function assessment. The blood samples collected were centrifuged at 3000 rev/min using a centrifuge for 10 minutes. Serum alanine aminotransaminase (ALT) and total bilirubin were assayed for spectrometrical analysis using Randox diagnostic kits (Reitman and Frankel, 1957) by calorimetric method. Total protein, and Albumin were assayed for by the Biuret method.

Oxidative Stress Parameters: Portions of the harvested and weighed liver tissues were homogenized with acid-washed sand and phosphate-buffered saline (PBS) in a porcelain mortar and pestle after being washed twice in cold PBS. Centrifuging the homogenate at 10,000 rpm for 15 minutes at 4°C. The supernatants were collected to estimate the results of several endogenous antioxidant enzymes. Evaluation of antioxidant activity was carried out as previously described; glutathione peroxidase (Nyman, 1959); superoxide dismutase (Misra and Fridovich 1977); catalase (Cohen *et al*., 1970); malondialdehyde activity (Buege and Aust, 1978).

Histological Assessment: Briefly, the fixed liver tissues were processed and routinely stained using hematoxylin and eosin, according to the method previously reported by Drury and Wallington (1980). Also, Masson's trichrome staining was carried out as previously reported by Bencosme (1954).

Statistical Analysis: The data were analyzed using IBM statistical Package for Social Sciences, Version 23 (manufactured by International Business Corporations {IBM}; released in 2015). Results were presented as (mean ± SEM). The parameters for all groups were compared using analyses of variance (ANOVA). *Post hoc* analysis was done using Least Square Difference (LSD). Differences in means were considered significant at 95% *confidence level (that is when probability was less than 0.05 {P < 005}).*

RESULTS

Effect of treatment on liver function: Table 1 shows the effect of treatment on liver function. Results showed that there was a significant increase (p<0.05) in ALT and a corresponding significant decrease (p<0.05) in albumin, total bilirubin and total protein in the rats given MSG only when compared to control. However, on co-administration of ascorbic acid, there was a

significant decrease ($p<0.05$) in the ALT and a significant increase ($p<0.05$) in albumin, total bilirubin and total protein when compared to the MSG-only group. Rats given ascorbic acid only showed no significant difference ($p>0.05$) when compared to control.

Effect of treatment on antioxidant enzyme activity and lipid peroxidation: Table 2 shows the effect of treatment on antioxidant enzymes and lipid peroxidation markers across the experimental groups. There was a significant decrease ($p<0.05$) in glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activity and a corresponding increase in malondialdehyde concentration in the MSG-only group. However, on co-administration with ascorbic acid, there was a significant increase ($p<0.05$) in antioxidant enzyme (SOD, CAT, and GPx) activity, alongside a significant decrease ($p<0.05$) in lipid peroxidation (MDA) when compared to the MSG-only group. Rats given ascorbic acid only showed no significant difference ($p>0.05$) when compared to control.

Effect of treatment on liver histology: Photomicrographs of the liver tissues of all the experimental groups are shown in Plate 1. Control group, MSG and ascorbic acid co-treated group and ascorbic acid-treated group show normal histological features; radiating hepatocytes (H) with large round nuclei, sinusoids (S), portal vein (PV), and bile duct (BD) while MSG-treated group show some histological alterations; zonal necrosis, infiltrates of inflammatory cells (IC).

Plate 2 demonstrates collagen deposition in the liver tissues of all the experimental groups. Control group, MSG and ascorbic acid co-treated group and ascorbic acid-treated group show mild collagen deposition around the portal tract (encircled) while MSG-treated group show dense collagen deposition around the portal tract.

Table 1: Effect of monosodium glutamate and ascorbic acid on Serum liver function biomarkers

	Control	MSG	MSG+AA	AA
ALT (U/L)	32.13±2.13	46.70±1.47*	34.32±2.72#	33.09±2.01#
Albumin (g/L)	31.31±2.74	20.48±4.21*	28.53±3.43#	30.93±1.73#
Total Bilirubin (mg/dl)	0.68±0.03	0.55±0.02*	0.59±0.04#	0.68±0.01#
Total Protein (g/dl)	6.51±0.13	5.83±0.22*	6.43±0.10#	6.49±0.20#

*Values are given as mean ±SEM. * p<0.05 (significantly different) compared with the control group; # p<0.05 (significantly different) compared to the MSG-only group. MSG = Monosodium glutamate; AA = ascorbic acid*

Table 2: Effect of monosodium glutamate and ascorbic acid on liver oxidative stress parameters

	Control	MSG	MSG+AA	AA
GPx (mM/L)	140.18±0.10	128.73±0.26*	141.46±0.15#	140.25±0.09#
CAT (U/ml)	231.99±0.73	217.01 ±0.99*	228.01±0.66#	230.81±0.66#
SOD (U/ml)	8.51±0.03	3.71±0.08*	8.85±0.04#	8.58±0.13#
MDA (µmol/L)	20.78±0.34	37.81±0.49*	28.29±0.45#	21.04 ±0.11#

*Values are given as mean ±SEM. * p<0.05 (significantly different) compared with the control group; # p<0.05 (significantly different) compared to the Arsenic trioxide-only group. MSG = Monosodium glutamate; AA = ascorbic acid*

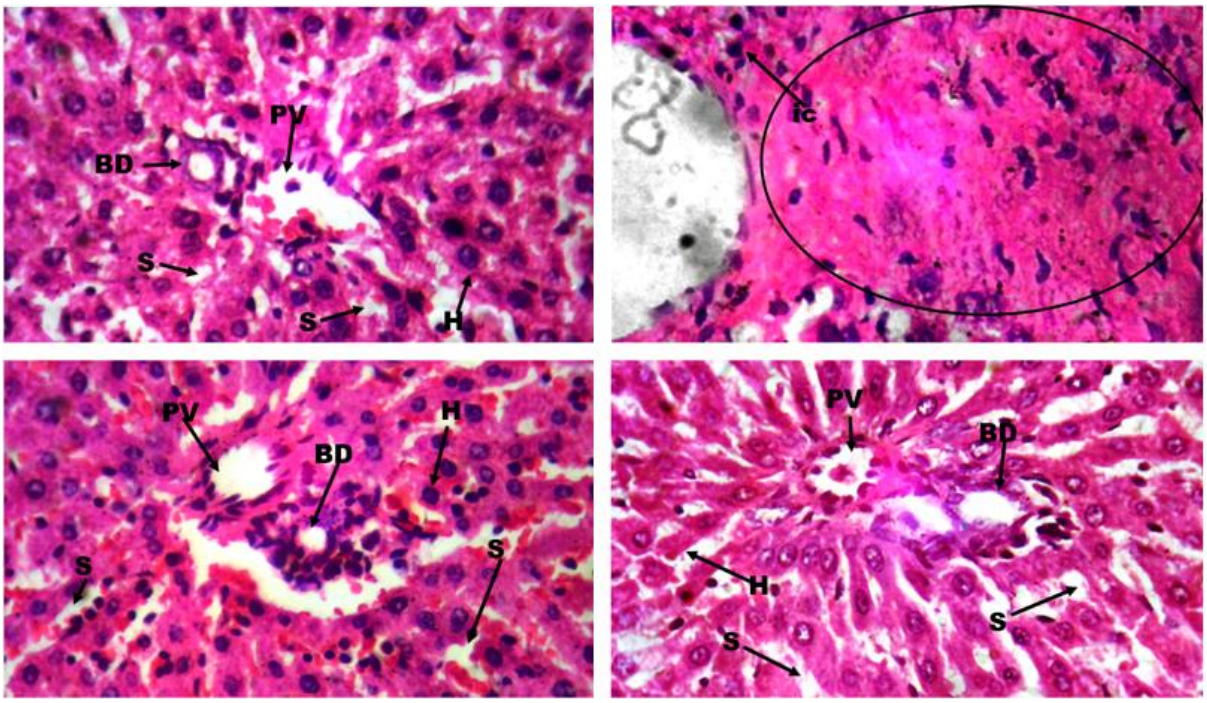

Plate 1: Representative photomicrographs of the liver tissues of all the Experimental groups: upper left: control group, upper right: MSG-treated group, lower left: MSG + AA- treated group, lower right: AA-treated group. Control group, MSG+AA-treated group and AA-treated group show normal histological features; radiating hepatocytes (H) with large round nuclei, sinusoids (S), portal vein (PV), and bile duct (BD) while MSG-treated group show some histological alterations; zonal necrosis, infiltrates of inflammatory cells (IC) (H&E; 400×)

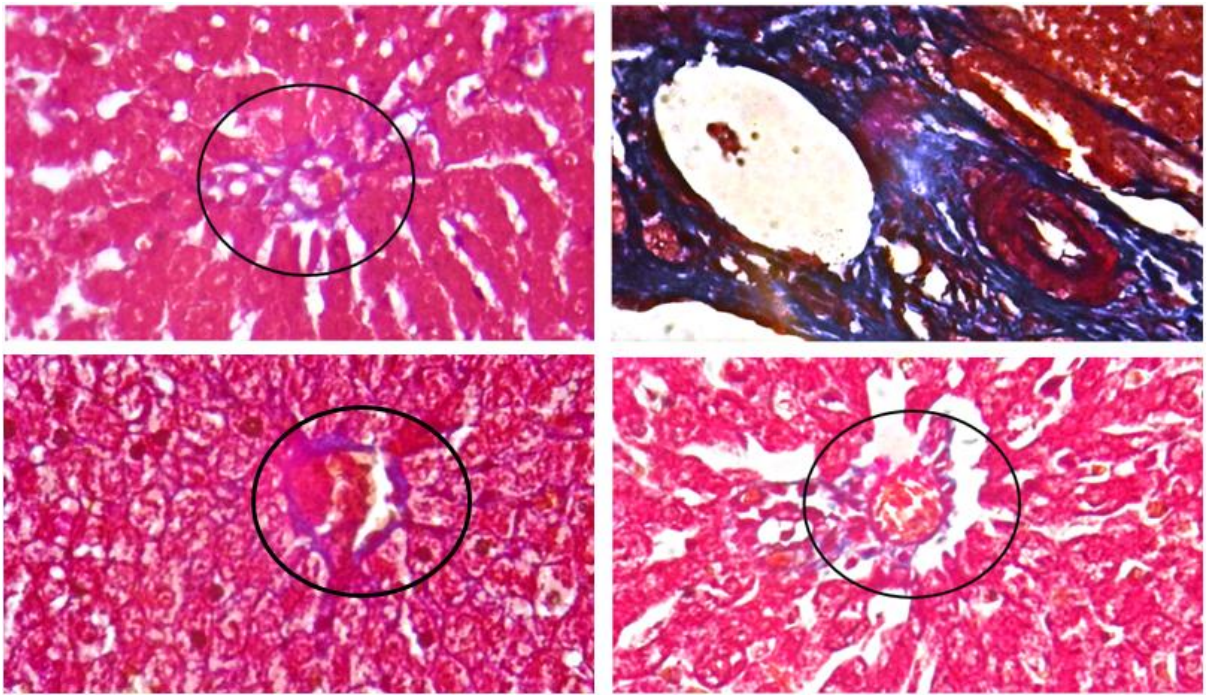

Plate 2: Representative photomicrographs demonstrating collagen deposition in the liver tissues of all the experimental groups: upper left: control group, upper right: MSG-treated group, lower left: MSG + AA-treated group, lower right: AA-treated group. Control group, MSG + AA-treated group and AA-treated group show mild collagen deposition around the portal tract (encircled) while MSG-treated group show dense collagen deposition around the portal tract (Masson's trichrome; 400×).

DISCUSSION

The liver plays a crucial role in maintaining homeostasis and detoxifying harmful substances, including food additives like MSG (Kazmi *et al.,* 2017). Ascorbic acid, a well-established antioxidant with hepatoprotective effects, acts by scavenging free radicals and reducing oxidative stress. This mechanism might enhance its potential to mitigate MSG-induced liver damage, positioning it as a promising candidate for therapeutic intervention.

Liver enzymes play a crucial role in maintaining overall health by participating in various metabolic processes essential for the body's proper functioning. Their activity levels serve as critical indicators of liver function. MSG is reported to induce hepatotoxicity by disrupting liver function, affecting key biomarkers such as ALT, albumin, total bilirubin, and total protein (Tawfek *et al.*, 2015; Ahmed *et al.*, 2019; Mohamed *et al.*, 2021). Findings from this study showed that MSG caused an increase in serum ALT activity. ALT, which is specific to the liver and mediates the conversion of alanine to pyruvate and glutamate, is a helpful biomarker of hepatic injury (McGill, 2016). Increased levels of these enzymes are indicative of cell invasion and disruption of liver cell membrane function (Watkins, 2013). This increase could be as a result of free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage. Also, the concentration of proteins, bilirubin and albumin in the serum are important indicators of the state of the liver. Results from this study showed a significant reduction in albumin and total bilirubin concentrations in serum arising from MSG uptake. This decrease may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissue spaces. However, on co-administration of vitamin C, there were significant improvements in liver function markers, as was evidenced by a significant decrease in ALT and an increase in albumin, total protein, and total bilirubin levels in rats treated with Vitamin C and MSG when compared to the MSG-only group.

Previous studies have reported that MSG induces liver damage via modulation of oxidative stress (Al-Harbi *et al.,* 2014; Rosa *et al.,* 2018; Hazza *et al.,* 2020). Oxidative stress occurs when the body's production of harmful free radicals overwhelms its natural defenses (Birben *et al.,* 2012). These free radicals can damage cells and tissues. An imbalance between the production of reactive oxygen species (ROS) and the cell's ability to detoxify and repair this damage is the root cause of oxidative stress (Birben *et al.,* 2012). This imbalance can arise from two main factors: increased generation of ROS or decreased activity of antioxidant defences. Antioxidants, such as enzymes like superoxide dismutase (SOD) and glutathione (GSH), play a critical role in neutralizing free radicals and protecting cells from oxidative damage (Ifeanyi, 2018). Measuring the levels of malondialdehyde (MDA), a stable marker of lipid peroxidation, allows for the assessment of the extent of oxidative stress. In the present study, the increase in liver MDA level (a by-product of lipid peroxidation) is accompanied by the decrease in GPx, CAT and SOD activities, in the liver tissues of the MSG-treated group. El Agouza *et al.* (2010) reported that administration of MSG induced oxidative stress leading to an increase in the intracellular concentration of Ca^{+2}; the increased Ca^{+2} levels could theoretically act either to enhance lipid peroxidation or to stimulate degeneration of phospholipids. However, there was a significant improvement in antioxidant defense system on co-administration of ascorbic acid and MSG. This was evidenced in the improvement in CAT, SOD and GPx activities as well as a significant reduction in MDA levels. This improvement in the antioxidant defense system could be attributed to the antioxidant property of ascorbic acid and its ability to scavenge free radicals and inhibit oxidative stress, thereby fortifying the body's natural defense mechanisms against oxidative damage.

Findings from this study showed that the MSG-treated group displayed histological alterations including zonal necrosis and infiltrates of inflammatory cells. Zonal necrosis refers to the death of liver cells in specific zones within the liver lobule. This disrupts the liver's ability to perform its vital functions. The presence of inflammatory cell infiltrates suggests an ongoing inflammatory response to MSG exposure. These findings align with previous studies demonstrating that MSG can induce hepatotoxicity (liver damage) (Waiz *et al.,* 2015; Ibrahim *et al.,* 2019; Ahmed *et al.,* 2019). Oxidative stress and inflammation have been reported to be the mechanism of MSG toxicity. MSG can potentially overwhelm the glutamate-glutamine cycle in liver cells, leading to excitotoxicity and oxidative stress (Abdou *et al.,* 2020; Al-Otaibi *et al.,* 2022). This oxidative stress can damage cellular components and trigger inflammatory pathways, ultimately leading to cell death (zonal necrosis). In contrast, co-administration of MSG and ascorbic acid resulted in a remarkable improvement in liver histology as was evidenced by relatively normal histological features. This suggests that ascorbic acid may offer protection against MSG-induced liver damage. These results are supported by previous studies demonstrating the antioxidant and anti-inflammatory properties of ascorbic (Suliburska *et al*., 2014; Gawron-Skarbek *et al.,* 2023). Ascorbic acid is believed to scavenge free radicals generated by oxidative stress, and modulate inflammatory pathways, potentially mitigating the inflammatory response triggered by MSG, thereby protecting liver cells from damage. Masson's trichrome is a histological staining technique used to differentiate collagen fibres from other tissues in histological sections. It is particularly useful for visualizing connective tissues, such as collagen, in various organs, including the liver (Van De Vlekkert *et al.,* 2020). Collagen deposition around the portal tract serves as an indicator of liver fibrosis. Masson's trichrome staining revealed distinct differences in collagen deposition between the control group and the MSG-treated group. Results showed that the liver tissue from the control group exhibited mild collagen deposits around the portal tract, indicating normal liver histology. This is consistent with the expected findings in healthy liver tissue (Karsdal *et al*., 2020). However, in the MSG-treated group, there was a notable increase in collagen fiber density around the portal tract, indicative of liver fibrosis. This finding suggests that MSG possesses detrimental effects on liver health, potentially leading to the development of liver fibrosis. This is consistent with previous studies by Heil *et al*. (2020), Hazzaa *et al*. (2020) and Zazula *et al.* (2023). Results from the co-administration of MSG and ascorbic acid showed a reduction in collagen deposition when compared to the MSG-treated group. Vitamin C is known for its antioxidant properties (Pehlivan, 2017), which may help mitigate the oxidative stress and inflammation associated with MSG-induced liver damage. This finding suggests that vitamin C may have a protective effect against MSG-induced liver fibrosis.

CONCLUSION

In conclusion, findings from this study presented compelling evidence of MSG-induced hepatic damage and the protective potential of ascorbic acid protects against MSG-induced liver histological alterations, fibrosis and oxidative stress in Wistar rats.

REFERENCES

Abdou, H.M., Hassan, E.H. and Aly, R.G. (2020). Monosodium glutamate (MSG): promoter of neurotoxicity, testicular impairment, inflammation and apoptosis in male rats. *Swedish Journal of BioScience Research*, 1(2), 78-90.

Ahmed, R.R., Abdul-Hamid, M., Galaly, S.R. and Hamdalla, H.M. (2019). Monosodium glutamate-induced liver microscopic and biochemical changes in male rats, and the possible amendment of quercetin. *Egyptian Journal of Zoology*, 71(71), 44-55.

Al-Harbi, M.S., El-Shenawy, N.S. and Al-Weail, N.O. (2014). Effect of monosodium glutamate on oxidative damage in the male mice: modulatory role of vitamin C. *Advanced Food Science*, 36(4), 167-176.

Al-Otaibi, A.M., Emam, N.M., Elabd, H.K. and Esmail, N.I. (2022). Toxicity of monosodium glutamate intake on different tissues induced oxidative stress: A Review. *Journal of Medical and Life Science*, 4(4), 68-81.

Banerjee, A., Mukherjee, S. and Maji, B.K. (2021). Worldwide flavor enhancer monosodium glutamate combined with high lipid diet provokes metabolic alterations and systemic anomalies: An overview. *Toxicology Reports*, 8, 938-961.

Bencosme, S.A. (1954). A trichrome staining method for routine use. *American Journal of Clinical Pathology*, 24(11), 1324-1328.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. and Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World allergy organization journal*, 5, 9-19.

Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310). Academic press.

Carr, A.C. and Rowe, S. (2020). Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients*, 12(7), 1963.

Cohen, G., Dembiec, D. and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical biochemistry*, 34(1), 30-38.

Colunga Biancatelli, R.M.L., Berrill, M. and Marik, P.E. (2020). The antiviral properties of vitamin C. *Expert review of anti-infective therapy*, *18*(2), 99-101.

Doseděl, M., Jirkovský, E., Macáková, K., Krčmová, L.K., Javorská, L. *et al.* (2021). Vitamin C-sources, physiological role, kinetics, deficiency, use, toxicity, and determination. *Nutrients*, *13*(2), 615.

Drury, R.A.B. and Wallington, E.A. (1980). General Staining Procedures. In R.A.B. Drury & E.A. Wallington (Eds.), Carleton's Histological Techniques, 5th edition; Oxford University Press, 125-150.

El Agouza, I.M., El Nashar, D.E. and Eissa, S.S. (2010). The possible ultra-structural ameliorative effect of taurine in rat's liver treated with monosodium glutamate (MSG). *The Open Hepatology Journal*, *2*(1), 1-9.

Engwa, G.A., Nweke, F.N. and Nkeh-Chungag, B.N. (2022). Free radicals, oxidative stress-related diseases and antioxidant supplementation. *Alternative Therapies in Health & Medicine*, *28*(1), 114-128.

Fernández-Lázaro, D., Fernandez-Lazaro, C.I., Mielgo-Ayuso, J., Navascués, L.J., Córdova Martínez, A. *et al.* (2020). The role of selenium mineral trace element in exercise: antioxidant defense system, muscle performance, hormone response, and athletic performance. A systematic review. *Nutrients*, *12*(6), 1790.

Gawron-Skarbek, A., Guligowska, A., Prymont-Przymińska, A., Nowak, D. and Kostka, T. (2023). The anti-inflammatory and antioxidant impact of dietary fatty acids in cardiovascular protection in older adults may be related to vitamin C intake. *Antioxidants*, *12*(2), 267.

Halim, J., Bouzari, A., Felder, D. and Guinard, J.X. (2020). The Salt Flip: Sensory mitigation of salt (and sodium) reduction with monosodium glutamate (MSG) in "Better-for-You" foods. *Journal of food science*, *85*(9), 2902-2914.

Hazzaa, S.M., El-Roghy, E.S., Abd Eldaim, M.A. and Elgarawany, G.E. (2020). Monosodium glutamate induces cardiac toxicity via oxidative stress, fibrosis, and P53 proapoptotic protein expression in rats. *Environmental Science and Pollution Research*, *27*, 20014-20024.

Heil, E.M., Al-Bazi, W. and AL-Aameli, M.H. (2020). The Histological Alteration of Proximal Part of Aorta Exposed to (MSG) and Protective Effect of an a Lipoic Acid (ALA) in Male Rabbits. *Indian Journal of Forensic Medicine & Toxicology*, *14*(3), 942-948.

Ibrahim, M., Khalifa, A., Saleh, A. and Tammam, H. (2019). Histopathological and Histochemical Assessment of Monosodium Glutamate-Induced Hepatic Toxicity and the Amelioration with Propolis. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*, *33*(2), 24-36.

Ifeanyi, O.E. (2018). A review on free radicals and antioxidants. *International Journal of Current Research in Medical Sciences*, *4*(2), 123-133.

Karsdal, M.A., Daniels, S.J., Holm Nielsen, S., Bager, C., Rasmussen, D.G. *et al.* (2020). Collagen biology and non-invasive biomarkers of liver fibrosis. *Liver International*, *40*(4), 736-750.

Kazmi, Z., Fatima, I., Perveen, S. and Malik, S.S. (2017). Monosodium glutamate: Review on clinical reports. *International Journal of food properties*, *20*(sup2), 1807-1815.

Mahadevan, V. (2020). Anatomy of the liver. *Surgery (Oxford)*, *38*(8), 427-431.

McGill, M.R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. *Experimental and Clinical Sciences, International Online Journal for Advances in Sciences*, *15*, 817.

Misra, H.P. and Fridovich, I. (1977). Superoxide dismutase: a photochemical augmentation assay. *Archives of Biochemistry and Biophysics*, *181*(1), 308-312.

Mohamed, P., Radwan, R., Mohamed, S.A. and Mohamed, S. (2021). Toxicity of monosodium glutamate on liver and body weight with the protective effect of tannic acid in adult male rats. *Mansoura Journal of Forensic Medicine and Clinical Toxicology*, *29*(2), 23-32.

Nyman, N. (1959). Determination of glutathione peroxidase in tissue. *Analytical Biochemistry*, *28*, 481.

Pehlivan, F.E. (2017). Vitamin C: An antioxidant agent. *Vitamin C*, *2*, 23-35.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

Rosa, S.G., Chagas, P.M., Pesarico, A.P. and Nogueira, C.W. (2018). Monosodium glutamate induced nociception and oxidative stress dependent on time of administration, age of rats and susceptibility of spinal cord and brain regions. *Toxicology and applied pharmacology*, *351*, 64-73.

Shakour, S.Y., Barhoma, R.A.E., Tahoon, N.M.E.F. and ELshrbiny, R.A. (2022). Study on the effect of vitamin D on obesity and fatty liver induced by monosodium glutamate in male albino rats. *Tanta Medical Journal*, *50*(2), 125-131.

Sulibursk a, J., Bogdanski, P., Krejpcio, Z., Pupek-Musialik, D. and Jablecka, A. (2014). The effects of L-arginine, alone and combined with vitamin C, on mineral status in relation to its antidiabetic, anti-inflammatory, and antioxidant properties in male rats on a high-fat diet. *Biological trace element research*, *157*, 67-74.

Tawfek, N., Amin, H., Abdalla, A. and Fargali, S. (2015). Adverse effects of some food additives in adult male albino rats. *Current Science International*, *4*(4), 525-537.

Thuy, L.N., Salanta, L., Tofana, M., Socaci, S.A., Fărcaş, A.C. *et al.* (2020). A mini review about monosodium glutamate. *Bulletin UASVM Food Science and Technology*, *77*(1), 1-12.

Van De Vlekkert, D., Machado, E. and d'Azzo, A. (2020). Analysis of generalized fibrosis in mouse tissue sections with Masson's trichrome staining. *Bio-protocol*, *10*(10), e3629-e3629.

Waiz, S.A., Raies-ul-Haq, M., Waiz, H.A., Gupta, S. and Pathak, A.K. (2015). Preliminary study on the protective effect of vitamin C on monosodium glutamate-induced hepatotoxicity in rats. *Comparative Clinical Pathology*, *24*, 1063-1068.

Watkins, P.B. (2013). Biomarkers for drug-induced liver injury. In *Drug-Induced Liver Disease,* Academic Press, 275-286.

Zazula, M.F., Saraiva, D.F., Theodoro, J.L., Maciel, M., Sepulveda, E.V.D.S. *et al.* (2023). An Early and Sustained Inflammatory State Induces Muscle Changes and Establishes Obesogenic Characteristics in Wistar Rats Exposed to the MSG-Induced Obesity Model. *International journal of molecular sciences*, *24*(5), 4730.