Protective Roles of Green Extract Zinc Oxide Nanoparticles on Monosodium Glutamate-Induced Hepatotoxicity in Adult Female Rat

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Abstract

Monosodium glutamate (MSG), chemically known as AJI-NO-MOTO (at the origin of flavor) is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids. MSG is entering our bodies with absolutely no limits in hundreds of food items daily. The reports have indicated that MSG is toxic to human and experimental animals. Therefore, MSG has become a controversial food additive, and the scientific reality still remains obscure. The objectives of this study are to assess the histological effects of MSG on liver tissue of Wistar Albino Rats. In the present study, 16 rats were divided into four groups. Each group contained 4 rats. Group one was control and received standard diet with 1 ml distilled water orally by gavage, Group tow were received 4 mg/kg body weight MSG dissolved in 2 ml distilled water orally by gavage, Group three will received 1 mg/g body weight green extract zinc oxide nanoparticles dissolved in 2 ml distilled water intraperitoneally, and Group four will received the combination of the MSG and ZnO-NP for 14 days. At the end of the experiment, liver specimens were processed for histopathological studies. Histological examination of liver tissues showed that MSG caused a variety of histological changes, including hepatic cell degeneration, inflammatory cells infiltration close to the portal vein, fatty changes in the hepatocytes and congestion of blood vessels with blood cells. The earlier histological findings caused by MSG alone were improved in the group treated with ZnO-NPs/MSG combination.

I. Introduction

Monosodium glutamate, the sodium salt of the amino acid glutamic acid, is considered one of the most common food additives/flavor enhancers that enhance

the meaty flavor of foods (Bera et al., 2017). Glutamic acid (glutamic acid) is found in many types of foods such as meat, seafood (fish), milk, cheese, poultry, and some vegetables (Jinap and Hajeb, 2010) and is commercially produced. While it's been around, it's also present in most organizations. present in fermentation of molasses and fermented proteins such as soy sauce and hydrolyzed vegetable proteins (Kurihara, 2009) and is now produced in commercial quantities by bacterial fermentation (Leung, 1980) monosodium glutamate is considered to be the most commonly used food. In many countries it is added under the trade name of Ajinomoto, Chinese salt. It is commonly used in many foods, including pasta, flavored potato chips, many snack foods, canned sauces and soups, marinated meats, frozen foods, stuffed and cooked poultry (Musselman, 1996, Eweka et al., 2011)

Moreover, it is widely used not only in fast food and restaurant kitchens, but also in the home and food industry (Eweka et al., 2011, Bojanić et al., 2009). Many effects of MSG consumption on the brain, obesity, sex organs, and metabolism have been demonstrated (Husarova and Ostatnikova, 2013). MSG is known as a non-essential amino acid found as the sodium salt. It has been shown to improve the flavor of foods as previously described (Z Hamza et al., 2018).

Glutamate is a naturally occurring amino acid found in many foods in varying concentrations, but you should be aware that it is the free glutamate molecule that is toxic. Conjugated glutamate, which is naturally present in foods, is less dangerous because it is slowly broken down and absorbed in the intestine, making it available to tissues, especially muscles, before toxic levels build up (Blaylock, 1999) Glutamate supplements are free glutamate, which is not completely bound to other proteins, is easily and rapidly absorbed, and causes elevated blood glutamate

levels. No, but free glutamic acid is dangerous. This is because the body does not need to break down the free form of glutamate (Henry, 2013).

Despite improved gustatory stimulation and appetite stimulation, different types of detrimental effects on different organs have been reported in experimental animals. (Ismail, 2012) MSG induced changes in the metabolic rate of glucose utilization and reduced antioxidant defenses. Generation of reactive oxygen species in various somatic cells damages polyunsaturated fatty acids in cell membranes, causing damage to DNA, lipids, and proteins in cell membranes, and lipid peroxidation, leading to cell death by apoptosis. It is known that (Diniz et al., 2004) The mechanism of MSG damage involves the generation of free radicals that alter mitochondrial activity and genetic information (Singh and Ahluwalia, 2003). Metabolized in the liver and excreted by the kidneys (Schwerin et al., 1950).

Zinc oxide nanoparticles (ZnO NPs) have been applied as important components in various sectors of manufacturing as medicine and textile, as well as automotive markets. Several chemical and physical techniques have been used in the preparation of ZnO NPs, and biomaterials applied using various substrates (e.g. bacteria, microorganisms, plant extracts, and enzymes) are theoretically guided as ecofriendly alternatives compared with chemical ones (Mirzaei and Darroudi, 2017). Zinc oxide nanoparticles (ZnO NPs) are growingly used in personal cosmetic products and sunscreen because it has strong UV absorption characters (Kołodziejczak-Radzimska and Jesionowski, 2014). ZnO NPs are a cheap and less toxic products with excellent biomedical capabilities, such as anticancer, drug discovery, antibacterial, and diabetes treatment; anti inflammatory medicine; wound healing; and bioimaging (Xiong, 2013, Kim et al., 2017).

In a study by Al-Salmi et al. Oxidative stress and inflammation were induced by his MSG in the liver after 28 days of exposure. A histological study was

performed and liver parenchymal metastases were observed. GTE/ZnO NPs exerted a partial hepatoprotective effect against MSG in 0.001 M aqueous solution (Al-Salmi et al., 2019).

MSG is dose-dependently induced by all antioxidants [lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD), xanthine oxidase (XO), myeloperoxidase (MPO), and glutathione (GSH)] affected by It also affects inflammatory markers such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α). Such parameters are considered indicators of oxidative stress. In addition, insufficient molecular mechanisms of antioxidants can lead to liver damage. MSG induces oxidative stress by generating oxygen radicals and hydrogen peroxide, causing oxidative DNA damage and cell membrane peroxidation and cell death (Hazzaa et al., 2020). Husarova and Ostatnikova (2013) observed oxidative stress in the rat liver after her 10-day oral administration of MSG at a dose of 0.6 mg/g body weight. Human (Husarova and Ostatnikova, 2013) and animal tissues (Eweka et al., 2011, Yoneda et al., 2011), therefore, the aim of this study was to clarify the antioxidant effects of ZnO-NP administration in alleviating MSG-induced hepatotoxicity in female rats.

II. Material and Methods

A. Experimental Animals

In the current study, 16 mature female albino rats were used, all of the rats were healthy, and weighed between 200 and 220g. The animals were raised and housed in groups of four rats per cage in a room with a controlled temperature of 24 to 30°C in animal house belong to Biology department College of Science/Salahaddin University - Hawler Kurdistan. Animals were fed a typical rat diet.

B. Experimental Design:

The rats were divided randomly into four groups consisted of four rats per cage as shown:-

1. **First Group**

Control group, this group was given just standard chow and normal saline by intraperitoneal injection daily for 14 days.

2. **Second group**

The rats of this group were given standard chow and 4mg/kg body weight (B.W) of MSG by intraperitoneal injection daily for 14 days.

3. **Third group**

Rats given standard chow and 10mg/kg B.W ZnO-NPs by gavage daily for 14 days.

4. **Fourth group**

Rats given standard chow and 4mg/kg B.W of MSG by intraperitoneal injection and 10mg/kg B.W ZnO-NPs by gavage daily for 14 days.

C. Body Weight Recording:

The body weight of rats in all groups was recorded twice; at the beginning and at the end of the experiment, then the B.W gain was calculated as follow: Body weight gain (in gram) = B.W at the end – B.W at the beginning. Such B.W gain was recorded for each rat individually.

D. Anesthesia Dissecting and liver Removal:

The rats were given intraperitoneal injections of ketamine hydrochloride 80 mg/kg (Trittau, Germany) and xylazine 12 mg/kg (Interchem, Halland). The rats were then sacrificed, and their livers were taken out, after that they were fixed in the 10% buffered formalin fixative.

E. Histological Examination:

Liver sections were immediately fixed in 10% buffered formalin. The fixed specimens were dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated and embedded in paraffin at 60ºC. Using a rotary micrtome (bright, MIC), 4-6 μm thicknesses of the liver were cut and stained routinely with haematoxylin and eosin for investigation of the histological studies. Under a light microscope, the specimens were examined and photographed (digital binocular compouned microscoft 40x- 2000x- built in 3MP Us 3 camera) (Suvarna *et al*., 2018).

F. Statistical Analysis

Statistical analysis was performed with SPSS computer program (SPSS version 22 software for windows and graph pad prism) data were analyzed using one way analysis of variance (ANOVA). Results are reported as mean values ± SE and difference were considered significant at $p<0.05$.

III. Results and Discussion

1. Body weight gain

In the current study, MSG caused a statistically significant increase ($p<0.01$) in the body weight (BW) gain when compared to the control group, while no significant difference in B.W gain was observed between the control, ZnO and MSG /ZnO groups (Figure3.1).

(Mohammed et al., 2022) reported that MSG induced a significant increase in final body weight and weight gain in adult rats. Similar results were observed in albino mice by Muslim, (2020). (He et al., 2011) showed that the MSG consumption was significantly associated with human body weight.

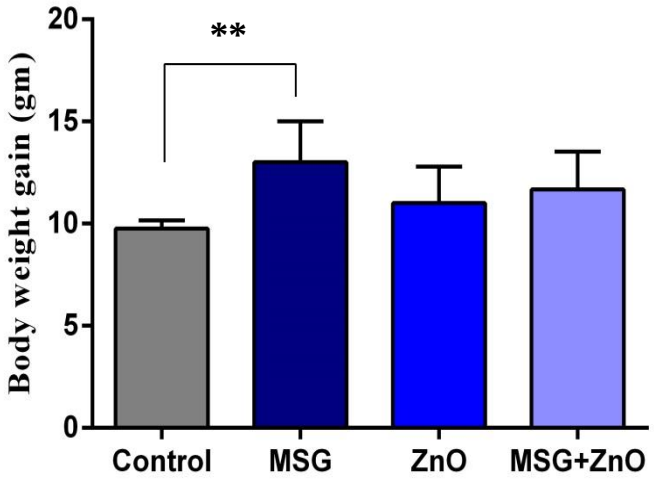

Figure3.1 Effect of MSG, ZnO and MSG/ZnO-NPs on body weight gain of rats. ** indicates that the value is significant compared to control at $P<0.01$

2.Histological Analysis of the Liver

In this investigation, MSG applied to the rats lead to histological structure of the liver which included hepatic cell degeneration, inflammatory cells infiltration close to the portal vein, fatty changes in the hepatocytes and congestion of blood vessels with blood cells compared to the control (Figure 3.2 and 3.3).

(Hazar, 2012), found that oxidative stress plays a vital role in the hepatic degeneration and fibrosis development. The presence of the inflammatory cells in the hepatic tissues indicated that MSG is the primary cause of the production of many inflammatory diseases. This result is confirmed by increasing the levels of TNF-α and IL-6. MSG can induce oxidative stress by producing oxygen radicals and hydrogen peroxide, causing oxidative DNA damage and cell membrane peroxidation as well as cell death (Okwudiri et al., 2012, Hazzaa et al., 2020). Husarova and Ostatnikova (2013) observed oxidative stress in the liver of rats after oral administration of MSG at a dose of 0.6 mg/g body weight for 10 days. There

have been several studies reporting the toxic effects of MSG on humans (Husarova and Ostatnikova, 2013) and animal tissues (Eweka et al., 2011, Yoneda et al., 2011).

Pretreatment with zinc oxide nanoparticles extract preserved the general architecture of the liver but a mild fatty change in the hepatocytes was still exist (Figure 3.5). In the present study, the mechanisms assigned to the ZnO NPs/GTE complex hepatoprotective effect against MSG which could be contributed to catechins present in GTE and are considered to suppress the Fenton-like reaction (Srichairatanakool et al., 2012). Those catechins act as a potent radical scavenger, so reactive (OH·) radicals were inhibited, and this could prevent LPO. The catechins induce the low oxidative stress levels which could lead to more expression of endogenous intracellular antioxidants (Lambert and Elias, 2010). ZnO NPs/GTE complex shows a hepatoprotective effect on the liver cells architecture against MSG.

IV. Conclusion

The prolonged use of MSG may induce oxidative stress, inflammation, histopathological and ultrastructural changes in the liver depending on the dose of MSG and based on ROS generation. Based on our results, we propose that the green synthesis of metal nanoparticles (ZnO NPs) could provide a protective benefit against MSG induced hepatotoxicity through its potent antioxidant properties.

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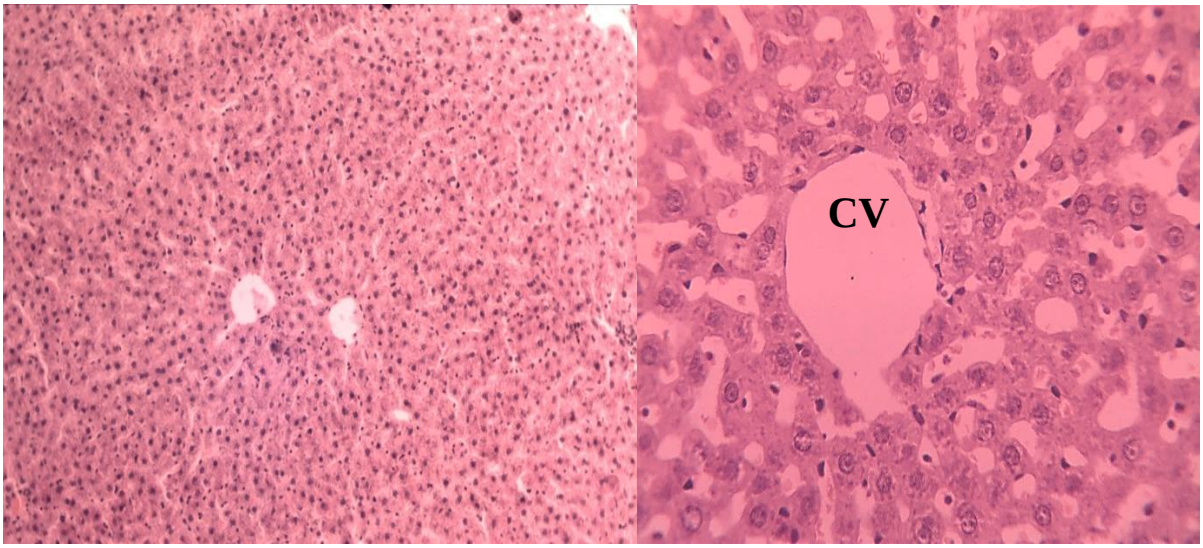

Figure 3.2: Histological section through the liver of control rats showing normal histological architecture: CV:central vein, S:sinusoids, H:hepatocytes, arrows:central veins, H&E.(A):100X,(B):400X.

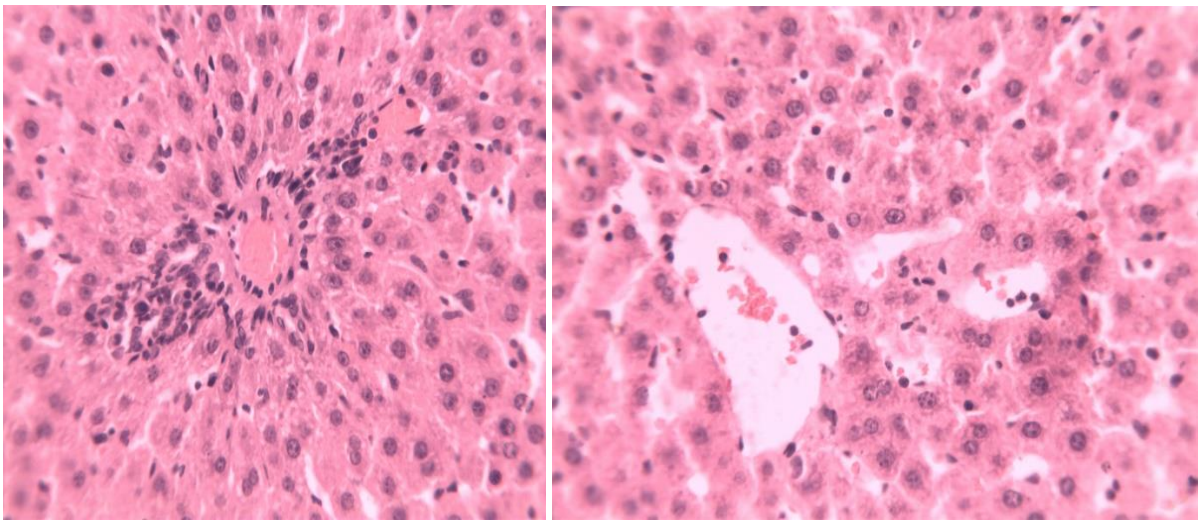

Figure 3.3: Histological sections through the liver of MSG treated rats showing various alterations.(A) Hepatocytes (H) degeneration and dilation in blood sinusoids(S).(B) Inflammatory infiltrated leucocytes close to the portal vein and the vein seen congested with blood cells. Fatty changes in the hepatocytes (H) are also seen, H&E. Both are 400X.

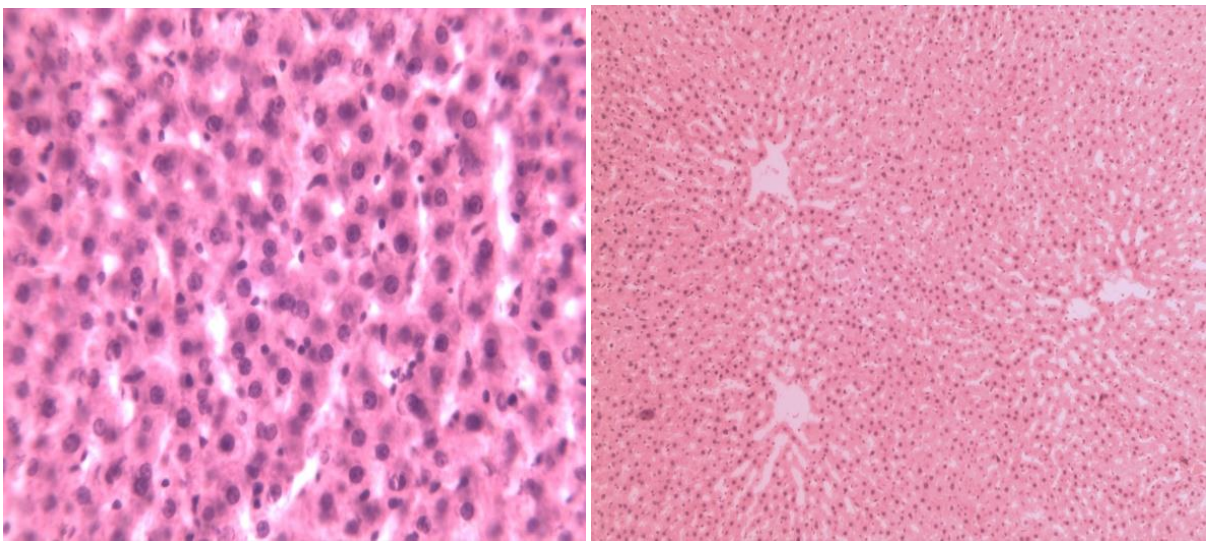

Figure 3.4: Histological sections through the liver of rats treated with ZnO NPs extract showing approximately normal histological architecture, mild fatty changes are still exist: CV:central vein, H:hepatocytes, arrows:central veins, H&E.(A):100X,(B):400X.

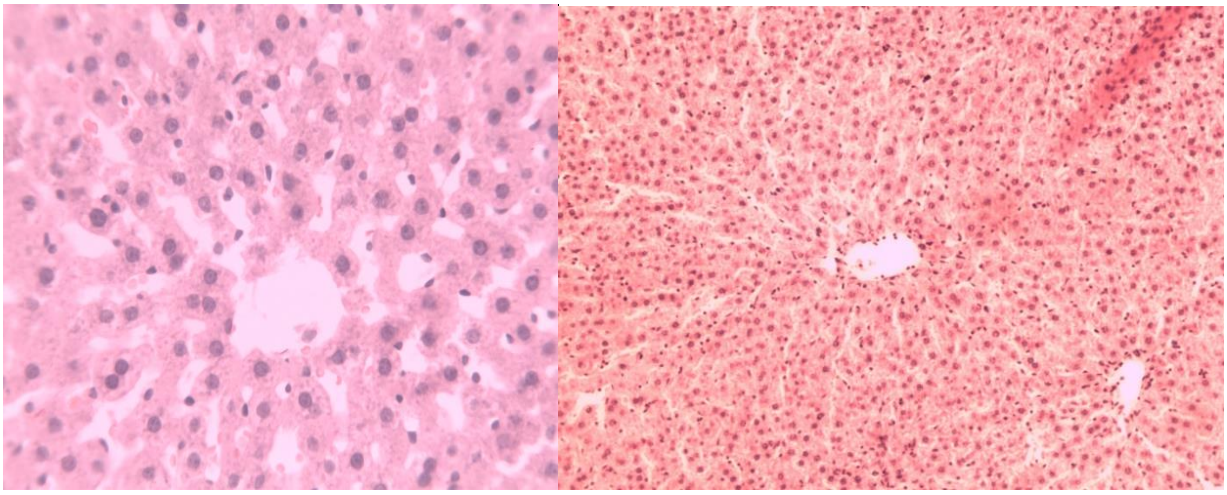

Figure 3.5: Histological sections through the liver of rats treated with MSG and ZnO NPs extract showing approximately normal histological architecture, mild fatty changes are still exist: CV:central vein, H:hepatocytes, arrows:central veins, H&E.(A):100X,(B):400X.