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# Histological Effects of Aqueous *Allium sativum* Bulb Extract on Monosodium Glutamate-induced Hepatotoxicity in Guinea Pigs

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## ABSTRACT

Monosodium glutamate (MSG) is among the most common food additives in the developing world and is commonly used as a flavor enhancer. It contains glutamic acid, one of the naturally occurring amino acids that the body transforms into glutamate. *Allium sativum* (Garlic) has tremendous pharmacological effects due to its biologically active constituents, which are vital in nutraceutical applications. The present study aimed to evaluate the effects of aqueous *Allium sativum* bulb extract on MSG-induced hepatotoxicity in Guinea pigs. Five groups (N = 5) were named and treated as follows; Group A (control) received normal saline. Group B received MSG (150 mg/kg body weight). Groups C, D, and E received 150 mg/kg of MSG with 500 mg/kg, 1000 mg/kg, and 1500 mg/kg of aqueous *Allium sativum* extract respectively. The administration lasted for 21 days. Animals were sacrificed humanely; tissue samples were collected, and fixed in 10% formal saline for histological studies. Histological findings revealed changes in form of dilatation of the central vein, which contained lysed RBC, edematous of liver parenchyma, and atrophic and degenerative changes on the hepatic tissues of Guinea pigs administered with MSG. However, co-administration with various doses of *Allium sativum* produced a positive effect on evading the harmful effects of MSG in hepatic tissue. In conclusion, this study showed that excessive MSG intake may have some harmful effects on the liver and by extension could affect its functions. Moreover, supplementations with *Allium sativum* reduced MSG toxicity and restore the pathological changes of the hepatic tissue.

**Keywords;** Monosodium glutamate, *Allium sativum*, hepatotoxicity, Guinea pigs, glutamic acid.

## INTRODUCTION

Some chemicals found in the environment, including food additives and industrial pollutants, have been linked with detrimental consequences [1]. The majority of food additives serve as either palatability enhancers or preservatives. One such food additive is monosodium glutamate (MSG), marketed by West African Seasoning Company Limited and sold as "Ajinomoto" in most open market stalls and stores in Nigeria. MSG is a food additive that is referred to as "hydrolyzed vegetable protein" or "flavoring" on food labels. MSG stimulates the orosensory receptors, which increases food palatability and favorably affects hunger, leading to weight gain [2]. Reports suggest that excessive MSG consumption is hazardous to humans and experimental animals, even though it stimulates taste and improves hunger [2, 3]. Even though glutamate is an amino acid that occurs naturally and may be found in various amounts in a wide variety of meals, the hazardous part of the molecule is the free form. Naturally occurring in food, bound glutamate is less harmful since the stomach absorbs it gradually and uses it for muscle and other tissues before lethal quantities accumulate [4]. The body's typical reactions to aberrant environmental stimuli might lead to some diseases such as malignancies [5]. Diseases are caused by harmful external causes such as pathogenic microbes, trauma, inadequate nutrition, and inherited variables that may operate alone or in complicated interactions with environmental factors [6].

The liver is the largest internal organ in mammals, weighing about 1.4 to 1.5 kg [7]. Furthermore, to be vital to metabolism, it also performs an assortment of other functions in the body, notably storing glycogen, synthesizing plasma proteins, and synthesizing bile, an

alkaline material that aids in digestion and most chemical detoxification. Due to its involvement in carrying out these many tasks, the liver is vulnerable to damage from poisonous chemicals. Liver diseases are common problems worldwide and are induced by some compounds or metabolites in a dose-dependent fashion [7].

The use of herbal medicine is becoming increasingly commonplace as breakthroughs in clinical research, analysis, and quality control have demonstrated the benefits of herbal medicine in the treatment and prevention of a wide range of illnesses [9]. *Allium sativum* (Garlic) is a common culinary spice that has long been used medicinally. One of the first recognized therapeutic herbs is garlic, in which its bulbs, or cloves, were utilized in ancient Egypt as a panacea [10, 11].

## MATERIALS AND METHODS

### Procurement of Animals

Twenty-five (25) adult guinea pigs, with a weight range of 360 –558 g, were procured from the animal house of the Department of Biological Science, Bayero University Kano. The experimental animals were given two weeks to acclimatize at the Department of Anatomy, Bayero University Kano. They were kept in conventional transparent plastic cages with wood shavings as bedding before and during the experiment, with unlimited access to food (Chikun grower mash Olam product, Kaduna, and cabbages) and water *ad-libitum*. Animals were treated under the Animal Care and Use for Research Ethics Committee (ACUREC).

### Plant Materials

*Allium sativum* (bulbs) were purchased at Kwari Market in Fagge LGA, Kano State. They were eventually taken to the herbarium unit in the Department of Plant Biology, Bayero University Kano, for identification and authenticity, in which a voucher specimen number BUKHAN 0297 was issued. The bulbs were peeled and pulverized into coarse pieces before grinding into fine pieces using mortar and pestle. The particles were measured using an electronic weighing balance (Flintec, UK) in which 684 g was soaked in 2 liters of distilled water for 48 hours in a standard volumetric flask. The extract was filtered off using Whitman's filter paper into pre-weighed evaporating dishes, while the residues were discarded. The filtrate was evaporated using a rotatory extractor set at 40 C (10). A total of 250g was obtained as sticky brown gel, which was pooled together into an air-tight container and stored at room temperature until required for use.

### Chemical

MSG under the brand of AJI-NO-MOTO (containing 99%) commercially packaged and marketed by Ajinomoto Co., Inc., Tokyo, Japan was purchased from Sabon Gari Market, Kano state.

### Experimental Design

Twenty-five (25) experimental animals weighing between 360-558g were divided randomly into five groups A, B, C, D, and E. They were treated as follows;

**Group A** (negative control) received 1 ml of normal saline.

**Group B** (positive control) received 150 mg/kg bwt of MSG

**Group C** received 150 mg/kg bwt of MSG + 500 mg/kg of aqueous *A. sativum* extract

**Group D** received 150 mg/kg bwt of MSG + 1000 mg/kg of aqueous *A. sativum* extract

**Group E** received 150 mg/kg of MSG + 1500 mg/kg of aqueous *A. sativum* extract.

All administrations were via oral route and lasted for twenty-one (21) days. After the last administration, all rats were fasted for 12 hours and sacrificed after mild anesthesia with chloroform, soaked in cotton wool placed in an anesthetic box, and covered with a lid.

### Biochemical Analysis

Blood samples were collected in sterilized EDTA sample bottles via cardiac puncture, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels were determined using enzymatic kits (CYPRESS Diagnostic, Langdrop, Belgium) according to the manufacturer's instructions.

### Histological Studies

The liver tissues of the experimental guinea pigs were harvested by making a median incision through the abdominal cavity, the harvested liver tissues were fixed in a 10% formalin saline. The tissues were later dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene, and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinized sections were stained with Hematoxylin and eosin reagents. The stained sections were examined using a light microscope according to Bancroft JD and Gamble M [12].

### Statistical Analysis

Data obtained were expressed as mean SEM. One-way analysis of variance (ANOVA) was employed to compare the means between and within the groups, and a P value of less than 0.05 was considered significant. A post-hoc test (Bonferonni) was applied to assess significant differences between the groups. Statistical analysis was done using SPSS version 20

## RESULTS

In the study, the histological features of the studied experimental Guinea pigs from the different groups were evaluated after careful processing of the hepatic tissue. The histological sections of the hepatic tissues obtained from the studied Guinea pigs in the different groups in the current study are presented in Figure 1.

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Photomicrograph sections of Guinea pigs from the control group revealed normal histoarchitecture with radiating cords of hepatocytes, each consisting of cords of a sinusoidal network and a central vein located in the center of the lobule as shown in the plate above. Hepatic tissue sections of Guinea pigs in the group administered with MSG revealed vacuolar cytoplasmic degeneration with binucleated and pyknotic hepatocytes. Edematous of liver parenchyma, with massive exudate of lymphocytes and inflammatory cells around the portal area, sinusoidal dilatations are obvious, with an enlarged central vein as shown in Figure 1. Co-administration of both MSG and varying doses of aqueous *Allium sativum* bulb extract revealed a dose-dependent hepatoprotective effect with the normal architecture of hepatic parenchyma with, the cord of hepatocytes well preserved, no fatty degeneration and change, moreover, the sinusoids arranged in radial position with the central vein

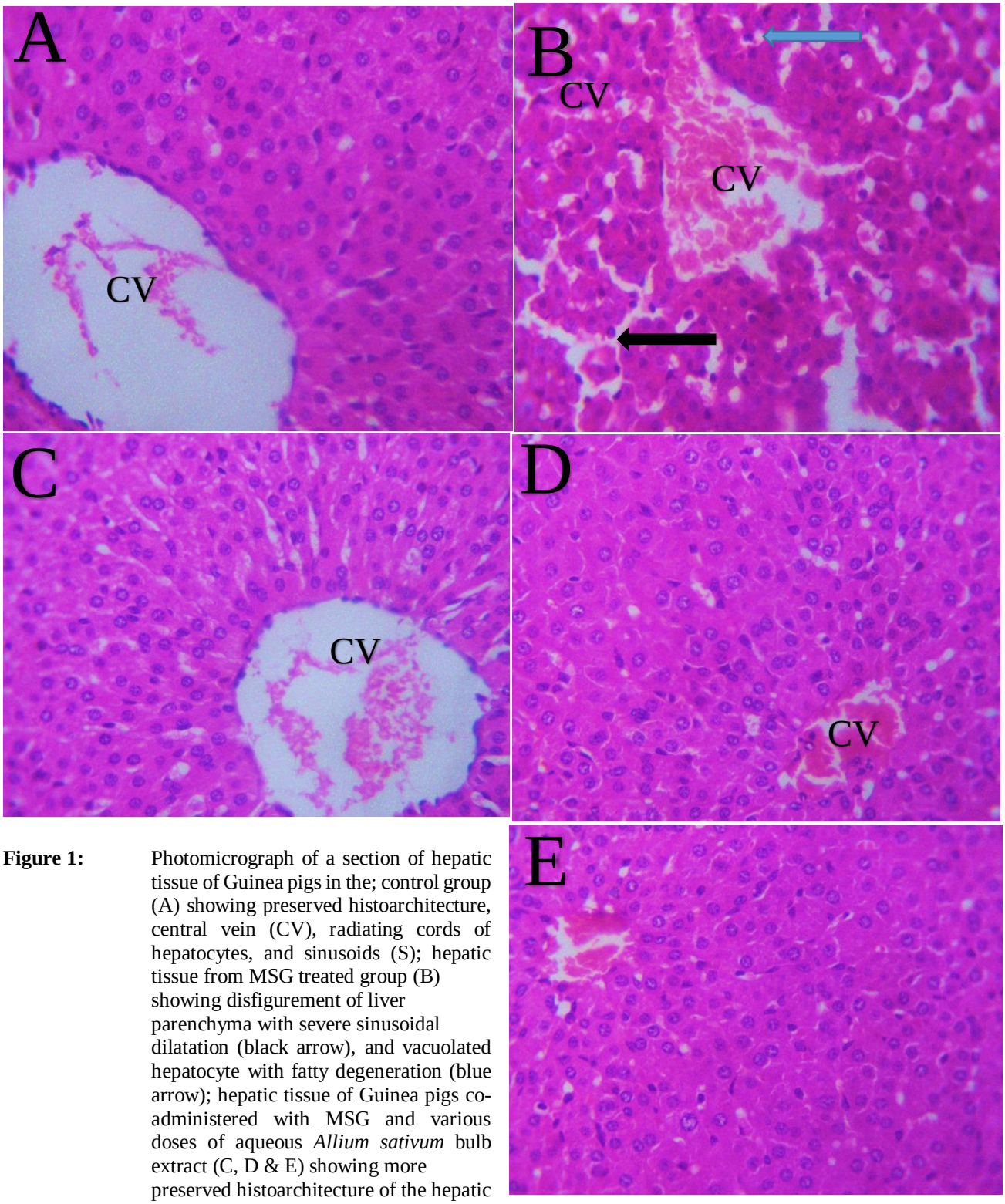


**Figure 1:** Photomicrograph of a section of hepatic tissue of Guinea pigs in the; control group (A) showing preserved histoarchitecture, central vein (CV), radiating cords of hepatocytes, and sinusoids (S); hepatic tissue from MSG treated group (B) showing disfigurement of liver parenchyma with severe sinusoidal dilatation (black arrow), and vacuolated hepatocyte with fatty degeneration (blue arrow); hepatic tissue of Guinea pigs co-administered with MSG and various doses of aqueous *Allium sativum* bulb extract (C, D & E) showing more preserved histoarchitecture of the hepatic tissues in a dose-dependent manner. The best restoration was observed at higher doses of *Allium sativum*. (H & E X 400)

The results of the serum levels of liver function-related enzymes were also evaluated for presentation to each of the study groups. The level of the circulating liver enzymes is usually assessable as part of a liver function test. In the current study, the levels of the liver enzymes were assessed with the individual values from each group. The entire individual values from the group were computed together to obtain a representative average value of the group. The same procedure was employed to obtain the average representative values for all the other groups, including their measure of dispersion are presented in Figures 2 and 3.

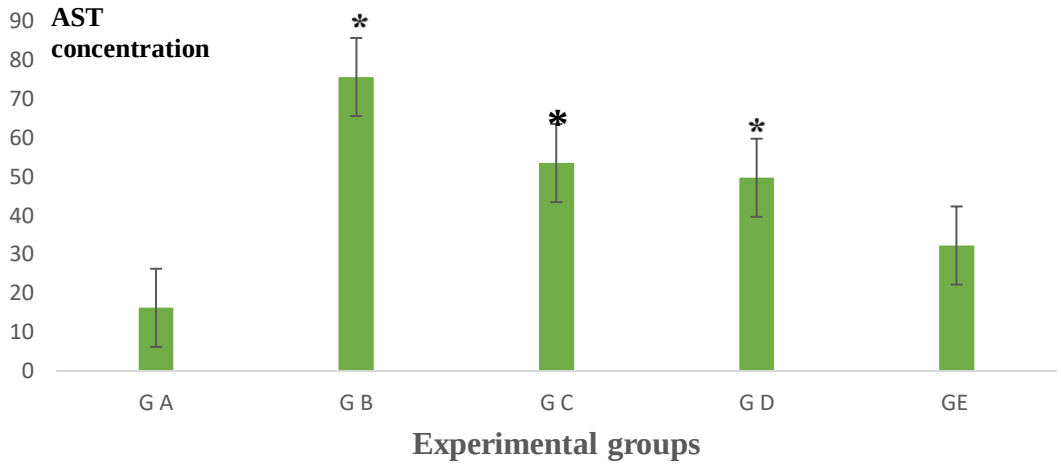


**Figure 2**: The mean concentration of expressing the level of liver aspartate aminotransferase AST (UI/L). GB (group B), G C (group C), and GD differed significantly from GA (group A) (P<0.05) when assessed using the Benferonni posthoc test.

***** Statistically significant

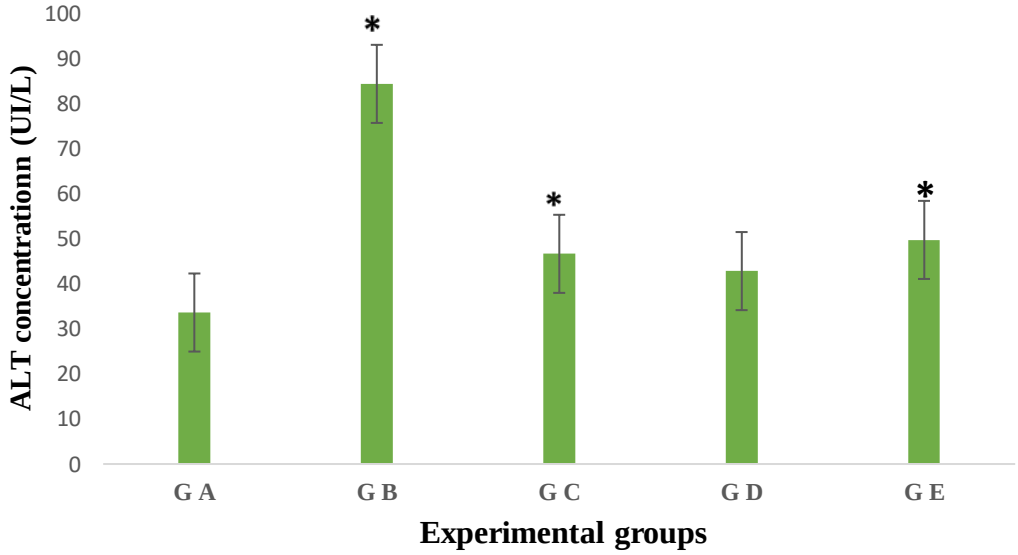


**Figure 3**: The activity of liver alanine aminotransferase ALT. GB (group B), G C (group C), and GE differed significantly from GA (group A) (*P*<0.05) when assessed using the Benferonni posthoc test. ***** Statistically significant

## DISCUSSION

Due to its widespread usage of MSG as a flavor enhancer, it has become one of the food additives most often utilized in modern nutrition worldwide. MSG finds its way into body systems daily through hundreds of food items (frozen dinners, canned soups, potato chips, fast food, etc.). Due to several factors, processed meals and restaurant food products are becoming increasingly popular daily. MSG is found in most foods. Due to its affordability and accessibility, this flavor enhancer is also being used in domestic cooking [13, 14, 15].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals that are produced from the products of normal cellular metabolism. They can take electrons from other compounds to become stable because of their high reactivity. As a result, the attacked molecule loses an electron and turns into a free radical, starting a series of events that eventually damage the living cell [16]. The ROS/RNS function as both poisonous and helpful substances for the living system. ROS/RNS have favorable effects at moderate or low levels and are involved in multiple physiological activities, including cellular signaling pathways, immunological function, mitogenic response, and redox regulation [17, 18]. However, at greater concentrations, RNS and ROS produce nitrosative and oxidative stress, respectively, which could damage biomolecules. When there is excess ROS/RNS generation on one side and an imbalance of either enzymatic or non-enzymatic antioxidants on the other, oxidative stress and nitrosative stress arise. Most importantly, the excess ROS can damage the integrity of various biomolecules including lipids, proteins, and DNA leading to increased oxidative stress in various human diseases [19, 20].

The pathological observations from this study are in line with results from earlier studies that evaluated the effects of MSG on the hepatic tissue of albino Wistar rats [2, 14, 16] which reported moderate MSG administration distorts the histology of the hepatic and renal tissues, body weights, and liver function enzymes. Moreover, another study reported hepatotoxic effects following MSG administration [17]. These pathological alterations could be due to the high concentrations of RNS and ROS that result in nitrosative and oxidative stress, respectively, which could damage biomolecules of the hepatic tissues. This preceding description may explain the ease with which MSG toxicity can develop with its propensity for hepatotoxicity.

Allicin is the most well-known and extensively researched component of garlic. Allicin is created quickly when fresh garlic cloves are diced or crushed, or when carefully dried garlic powder is introduced to water. Allicin can decompose into a broad range of compounds, including S-allyl mercapto cysteine, allyl mercaptan, diallyl disulfide, and allyl methyl disulfide, which are reported to have a strong affinity towards scavenging free radicals [18].

The effect of aqueous *Allium sativum* bulb extract on MSG-induced hepatotoxicity observed in this study is in line with the finding in the literature by Boric [19], that reported significant pharmacological effects of aqueous extract of *Allium sativum* of its biologically active constituent, allicin, and its derivatives, which are organosulfur compounds. The plant's chemical composition indicates that the most significant constituents are these compounds, which include allicin, diallyl disulfide, S-allyl cysteine, and diallyl trisulfide. These compounds play a crucial role in the plant's nutraceutical applications. Photomicrograph sections of the hepatic tissues that receive co-administered both MSG and various doses of aqueous *Allium sativum* extract revealed dose-dependent hepatoprotective activity with low doses showing little improvement with best restoration observed at the higher doses. The protective ability ascertained by the extract reported in this study could be a result of the allicin and other numerous antioxidants possessed by the extract as reported by [20].

One sensitive indicator of liver injury is the ALT enzyme [21]. Consequently, a rise in serum ALT activity could indicate liver injury. MSG had no trouble dissociating to release glutamate and set it free. When glutamate is diluted, ammonium ions are produced, which may be hazardous if the urea cycle processes do not eliminate them. Therefore, the liver may be harmed by the potential ammonium ions overload that results from an increase in glutamate after consuming MSG, which could then release the ALT enzyme and cause the rise that has been reported. The formation of free radicals, which react with the polyunsaturated fatty acids in cell membranes to cause damage to the mitochondrial and plasma membranes, could also account for this rise in enzyme leakage [22, 23]. The observed significant increase in serum AST and ALT in this study supports the histological alterations of the hepatic tissues in MSG-treated groups. This finding supports an earlier study conducted by Airaodion [24, 25] which reported a marked and significant increase in serum liver enzymes particularly AST and ALP in Wistar rats exposed to MSG.

## CONCLUSION

The present study suggests that orally administered aqueous *Allium sativum* bulb extract has a dose-dependent hepatoprotective effect on liver toxicity induced by orally administered monosodium glutamate (MSG).

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