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RESEACH ARTICLE

ALLIUM SATIVUM BULB AQUEOUS EXTRACT: A NATURAL PROTECTOR AGAINST MONOSODIUM GLUTAMATE-INDUCED RENAL TOXICITY IN GUINEA PIGS

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Abstract

Background: Monosodium Glutamate (MSG) is one of the most widely used food additives. MSG sold under the brand name AJI-NO-MOTO is a sodium salt of naturally occurring (non-essential) L-form of glutamic acid routed into the body with absolutely no limits in hundreds of food items daily. Therefore, its toxicological effects, are a major public health challenge. This research investigated the effects of aqueous extract of *Allium sativum* bulb (AEASB) (Garlic) on MSG-induced renal toxicity in Guinea pigs. **Methods:** In the present study, 25 Guinea pigs were divided into 5 groups, named and treated as follows; Group A (Control) received normal saline. Group B received MSG (150 mg/kg bwt). Groups C, D, and E received 150 mg/kg of MSG with 500 mg/kg, 1000 mg/kg, and 1500 mg/kg of (AEASB) respectively. All administration was via oral route and lasted for 21 days. Twenty-four hours (24) after the last administration experimental animals were sacrificed via chloroform inhalation, kidneys were harvested for histological studies, and blood was collected for biochemical studies. **Results:** In MSG-treated Guinea pigs, examined sections revealed degenerated lumen, and shrunken glomeruli with the widening of capsular spaces, tubular dilations, and shortening of tubular epithelium. Additionally, MSG caused a significant increase in urea and creatinine levels. Notably, these changes were reduced by (AEASB). **Conclusion:** The study showed MSG caused distortions in renal tissue, which can impair renal functions as corroborated by urea and creatinine levels. However, (AEASB) (Garlic) is protected against MSG-induced renal toxicity by its antioxidant properties.

Keywords; Monosodium glutamate, Renal Toxicity, *Allium sativum* (garlic), Guinea pigs, Nephroprotective.

Introduction

Monosodium glutamate (MSG) is a widely used flavor enhancer in the food industry, especially in West Africa, where it is marketed by companies like the West African Seasoning Company Limited under the brand name “Ajinomoto.” MSG, commonly labeled as "hydrolyzed vegetable protein" or "flavoring" on food packaging, is composed mainly of glutamate (78%), which activates the umami taste receptors on the tongue and enhances the savory flavor of foods. The remaining 21% consists of salt (Thongsepee *et al.*, 2024). While moderate consumption of MSG is generally considered safe, increasing evidence from animal studies suggests that excessive intake may induce harmful effects, particularly in organs such as the kidneys. Studies have linked high doses of MSG to oxidative stress,

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inflammation, and cellular damage (Thongsepee *et al.*, 2022). The kidneys, being crucial for waste elimination and maintaining fluid and electrolyte balance, are especially vulnerable to such toxins. The mechanism of MSG-induced renal toxicity is believed to involve glutamate excitotoxicity, mitochondrial dysfunction, and oxidative stress, leading to the production of free radicals and resultant tissue damage (Carmen Contin *et al.*, 2017).

In light of the potential harms posed by MSG and other environmental toxins, there is growing interest in natural products that may help mitigate organ damage. Garlic (*Allium sativum*), a widely consumed herb with a long history of medicinal use, has gained attention for its therapeutic properties, including its anti-inflammatory, immune-boosting, and cardiovascular benefits (Tesfaye, 2021). Garlic's bioactive compounds, particularly sulfur-containing molecules like allicin, have been shown to possess potent antioxidant, anti-inflammatory, and anti-apoptotic effects (Talib *et al.*, 2024). These properties make garlic a promising candidate for protecting against MSG-induced kidney toxicity.

Despite the well-documented health benefits of garlic, its specific nephroprotective effects, particularly in counteracting MSG-induced renal damage, remain underexplored. Previous research has primarily focused on garlic's general antioxidant and anti-inflammatory actions, but there is a need to investigate its role in ameliorating kidney damage induced by MSG. Additionally, while various forms of garlic have been studied, aqueous extracts, which are thought to retain a high concentration of bioactive compounds, have not been thoroughly examined for their renal protective effects. This study aims to investigate the effects of aqueous *Allium sativum* bulb extract on MSG-induced renal toxicity in Guinea pigs. Specifically, on the histological and biochemical alterations in kidney tissues. Moreover, this seeks to provide new insights into the nephroprotective potential of aqueous *Allium sativum* bulb and its underlying mechanisms of action, which may offer a novel approach to managing toxin-induced renal damage.

This study aims to investigate the effects of aqueous Allium sativum bulb extract on MSG-induced renal toxicity in guinea pigs, focusing on the histological and biochemical alterations in kidney tissues. Specifically, the study will explore the mechanisms through which garlic's bioactive compounds, such as allicin, may protect against oxidative stress and inflammation in the kidneys. By filling the gap in the literature on the nephroprotective effects of garlic against MSG toxicity, this research aims to provide new insights into the potential use of garlic as a natural remedy for toxin-induced kidney damage.

The novelty of this study lies in its dual focus: evaluating the protective effects of aqueous Allium sativum extracts specifically against MSG-induced renal damage and elucidating the underlying mechanisms that may contribute to its nephroprotective potential. This research could have significant implications not only for public health, by offering a natural solution for MSG toxicity, but also for clinical practices, where garlic-based interventions could serve as adjunctive treatments for kidney protection in toxin-related diseases.

MATERIALS AND METHODS

Experimental animals

Twenty-five (25) adult Guinea pigs (*Cavia porcellus*), weighing between 360 and 558 g, were procured from the Animal House of the Department of Biological Science, Bayero University Kano. Guinea pigs were selected for this study due to their renal physiology, which shares similarities with humans in terms of glomerular filtration and response to nephrotoxic substances. This makes Guinea pigs an appropriate model for studying MSG-induced renal toxicity and the nephroprotective effects of *Allium sativum* (garlic). The animals were housed in the Department of Anatomy Animal House under a 12-hour light/dark cycle at normal room temperature. They were provided unlimited access to food (Chikun grower mash, Olam Products, Kaduna) and water *ad libitum*. The experimental animals were acclimatized for 7 days before the commencement of the experiment. All experimental procedures adhered to the guidelines of the Animal Care and Use for Research Ethics Committee (ACUREC) to minimize animal suffering during the study.

Collection and preparation of *Allium sativum* extract

Fresh *Allium sativum* (garlic) bulbs were purchased from Kwari Market in Fagge LGA, Kano State, Nigeria.

The bulbs were identified and authenticated by the Herbarium Unit in the Department of Plant Biology, Bayero University Kano, with voucher specimen number BUKHAN 0297 issued. The fresh garlic bulbs were peeled, thoroughly washed, and pulverized into coarse pieces before grinding them into a fine paste using a mortar and pestle. A total of 684 g of garlic paste was soaked in 2 liters of distilled water for 48 hours in a standard volumetric flask (Flintec, UK). The extract was then filtered through Whitman filter paper into pre-weighed evaporating dishes, and the residue was discarded. The filtrate was concentrated using a rotary evaporator set at 40°C (Revathy, Elumalai, & Antony, 2011). yielding 250 g of a sticky brown gel. This gel was stored in an air-tight container at room temperature until required for use. A fraction of each batch was subjected to High-Performance Liquid Chromatography (HPLC) analysis for allicin content to guarantee the extract's uniformity and purity. This guaranteed the garlic extract's bioactive components, which are essential to its nephroprotective properties.

Chemical

Monosodium glutamate (MSG), branded as AJI-NO-MOTO, containing 99% MSG, was purchased from Ajinomoto Co., Inc., Tokyo, Japan, and obtained from Sabon Gari Market, Kano State. The MSG was used to induce renal toxicity in the experimental animals.

Experimental Design

The twenty-five (25) Guinea pigs were randomly divided into five groups (n = 5 per group) 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 (AEASB)

Group D received 150 mg/kg bwt of MSG + 1000 mg/kg of (AEASB)

Group E received 150 mg/kg of MSG + 1500 mg/kg of (AEASB)

All treatments were administered via oral gavage for 21 days. The MSG dosage of 150 mg/kg bwt was selected based on previous toxicological studies indicating significant renal damage at this level in similar models (Carmen Contin *et al.,* 2017). The garlic extract doses were based on prior literature and preliminary trials showing effective doses in reducing renal damage. After the 21-day treatment period, the guinea pigs were euthanized by chloroform inhalation. Blood samples were collected by cardiac puncture and stored at 4°C until biochemical analysis. The kidneys were carefully excised, washed in saline, and preserved in 10% formalin for histopathological evaluation.

Histological Studies

Kidney tissue samples were processed using standard histological techniques. The tissues were embedded in paraffin, and sections of 5 µm thickness were cut using a microtome. These sections were stained with Hematoxylin and Eosin (H&E) to assess general renal morphology. Histopathological changes, including tubular degeneration, glomerular damage, interstitial inflammation, and necrosis, were assessed by pathologists blinded to group assignments.

Biochemical Analysis

The serum levels of urea and creatinine were measured using commercially available kits (Biovision, USA) to assess renal function. The assays were performed according to the manufacturer's instructions, and results were expressed in mg/dL.

Serum urea and creatinine levels were measured using commercially available kits (Biovision, USA), following the manufacturer's instructions. These biochemical markers indicate renal function and were assessed to determine the extent of kidney damage. The results were expressed in mg/dL.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value of ≤ 0.05 was considered statistically significant. The sample size of 25 animals (5 per group) was determined based on power analysis, which indicated

that this sample size would provide adequate statistical power (80%) to detect significant differences in renal function and histopathological scores. All statistical analyses were conducted using SPSS version 22.0 (SPSS Inc., Chicago, IL).

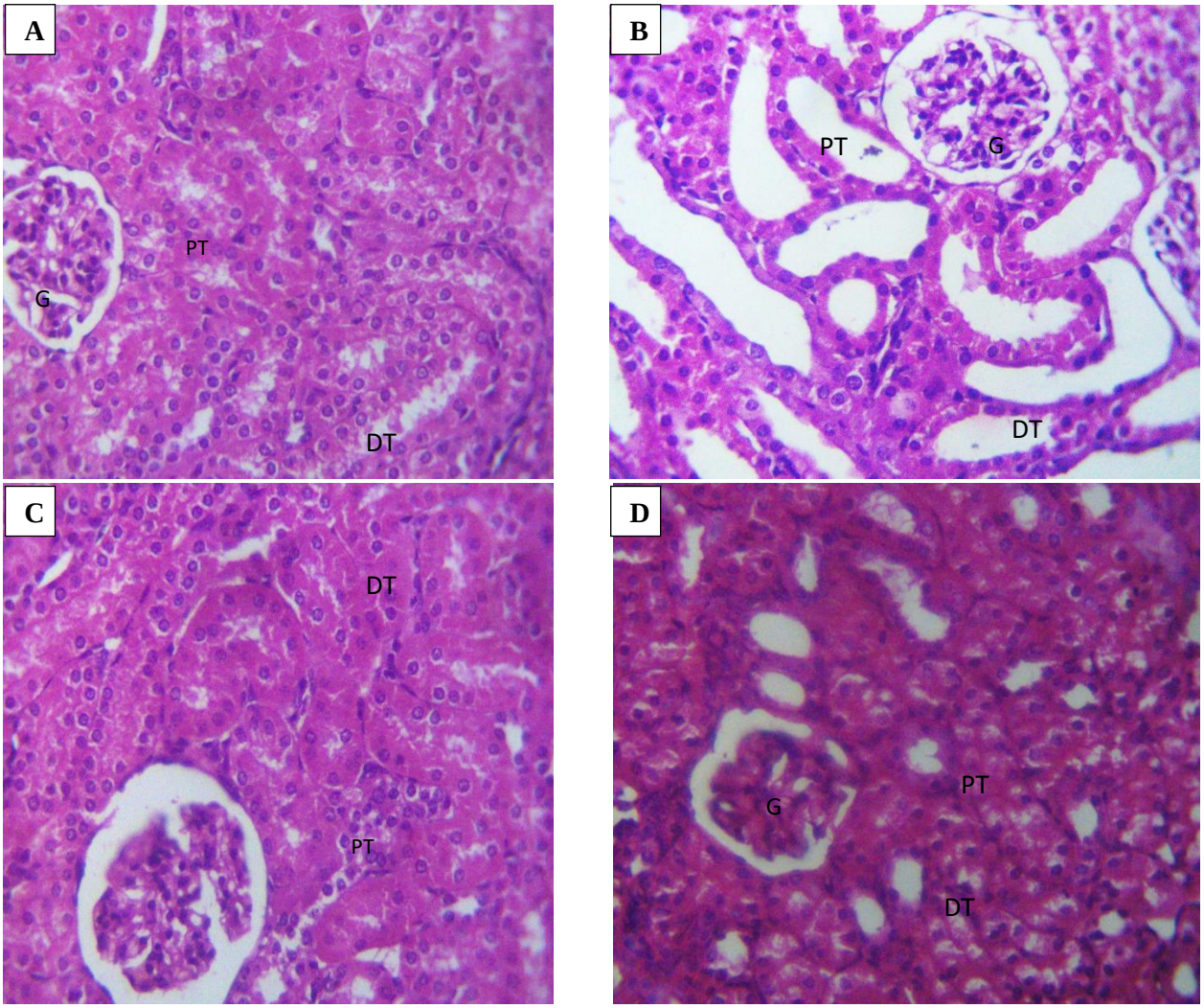
RESULTS

Histological Findings

Plate I shows the renal histology of all experimental groups (H & E, X 100). **Normal renal morphology** was observed in **Group A (Negative Control)** Guinea pigs, which consisted of the glomerulus, proximal convoluted tubule, and distal convoluted tubule.

The glomerulus (G) was intact, surrounded by the capsule of Bowman, with normal-sized proximal (PT) and distal tubules (DT). **MSG-treated Group B** showed marked histological distortion in the form of shrunken and irregularly shaped glomeruli (G), wide Bowman's space (S), and wide tubular enlargement. In the groups that received MSG alongside various doses of (AEASB), a noticeable improvement in the renal tissue histomorphology was observed.

The glomeruli (G) appeared more normal and well-formed compared to the MSG-only group, and the extent of tubular degeneration was significantly reduced. While some areas showed mild vacuolar changes, there were fewer tubular dilations compared to the MSG-only group.



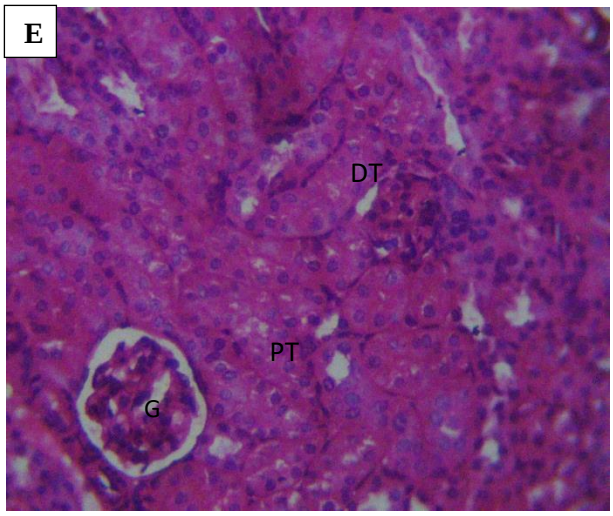


Plate I shows the renal histology of all experimental groups. In the control group (A), normal renal morphology was observed with intact glomeruli and well-formed proximal and distal tubules. The MSG-treated group (B) exhibited significant histological distortions, including shrunken glomeruli, wide Bowman's space, and tubular enlargement. In the groups treated with MSG and AEASB (C, D & E) a noticeable improvement in renal tissue architecture was observed, with better-formed glomeruli and reduced tubular degeneration compared to the MSG-only group. Mild vacuolar changes and fewer tubular dilations were noted in these groups. (H & E, x100).

Biochemical findings

Figure 1 illustrates the mean concentration levels of creatinine in the serum of all experimental groups, expressed as mean ± SEM. Creatinine is a commonly used biomarker for evaluating renal function, as it reflects the kidneys' ability to filter waste products. In the study, a significant reduction in serum creatinine levels was observed in the groups treated with AEASB, particularly in the higher-dose groups (500 mg/kg, 1000 mg/kg, and 1500 mg/kg). These reductions indicate an improvement in kidney function and suggest that AEASB has a nephroprotective effect, reversing or mitigating the renal damage induced by monosodium glutamate (MSG). The statistical analysis shows that the reduction in creatinine levels in the AEASB-treated groups is significant ($p \leq 0.05$), highlighting the protective potential of garlic extract in preserving kidney function.

Figure 2 depicts the mean concentration levels of blood urea nitrogen (BUN) in all experimental groups, with values expressed as mean ± SEM. BUN is another important marker of kidney function, as it reflects the kidneys' ability to eliminate nitrogenous waste products from the bloodstream. Similar to the creatinine results, significant improvements in BUN levels were observed in the groups receiving AEASB, particularly at higher doses (500 mg/kg, 1000 mg/kg, and 1500 mg/kg). This decrease in BUN levels indicates a reduction in renal impairment and supports the hypothesis that AEASB has protective effects against MSG-induced renal toxicity. Statistical analysis of BUN levels also shows significant differences between the treated and MSG-only groups ($p \leq 0.05$), further confirming the beneficial impact of garlic extract on kidney function.

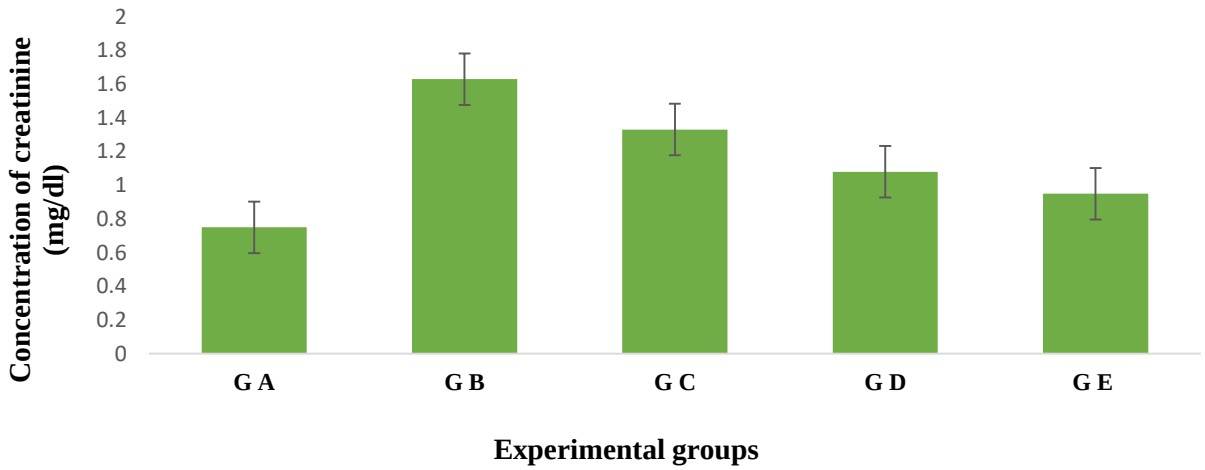


Figure 1. Bar chart showing Mean concentration levels of creatinine (mg/dL) in all experimental groups. Values are mean ± SEM of data $p \leq 0.05$.

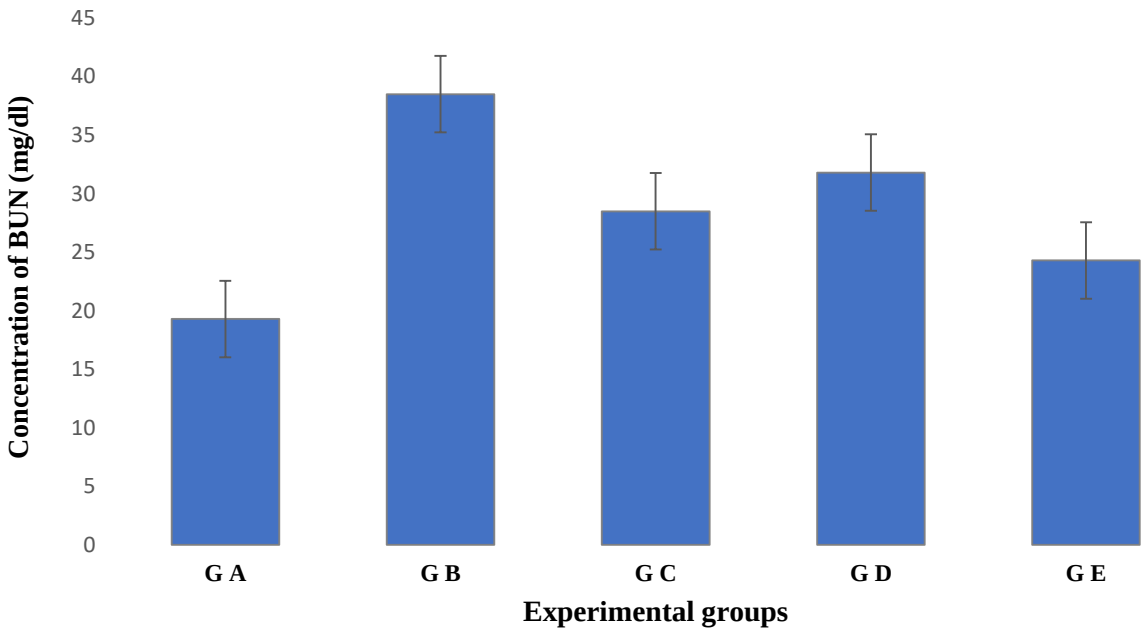


Figure 2. Bar chart showing Mean concentration levels of blood urea nitrogen (mg/dL) in all experimental groups. Values are mean ± SEM of data $P \leq 0.05$

Discussions

Monosodium glutamate is consumed in considerable amounts in almost all forms of foods in Nigeria. The histological sections of renal tissues from Guinea pigs treated with monosodium glutamate (MSG) revealed significant renal pathologies. These pathological findings align with previous studies reporting that MSG induces nephrotoxicity through mechanisms such as oxidative stress and glutamate toxicity, leading to renal dysfunction and cellular damage (Kassab *et al.,* 2022; Onyesife *et al.,* 2023). These histological distortions are indicative of renal injury and suggest that MSG adversely affects kidney tissue integrity.

Moreover, Guinea pigs were treated with varying dosages of AEASB, and a substantial reduction in the severity of these histological changes was observed. This histological improvement highlights the protective potential of *AEASB* against MSG-induced renal toxicity. Similar findings have been reported in other studies, where garlic was shown to exhibit notable antioxidant and anti-inflammatory properties, contributing to renal protection in various animal models (Tesfaye *et al.,* 2015; Abdel-Daim *et al.,* 2020; El-Saber *et al.,* 2020). The observed restoration of

kidney tissue integrity in the *AEASB-treated* groups can be attributed to the bioactive compounds, particularly **allicin**, present in the extract. Allicin has been extensively studied for its antioxidant, anti-inflammatory, and anti-apoptotic activities, which may play a pivotal role in mitigating oxidative stress and inflammatory damage in renal tissues (Abdel-Daim *et al.,* 2020). By neutralizing free radicals generated during MSG metabolism, allicin and other compounds in *AEASB* may prevent oxidative damage to renal cells, thereby preserving kidney function. In addition to oxidative stress, inflammation is a crucial factor in MSG-induced kidney damage (Sharma, 2015). The anti-inflammatory properties of the AEASB may aid in lowering the inflammatory response in kidney tissues. In particular, it has been demonstrated that garlic prevents nuclear factor kappa B (NF-κB) from activating (Shi *et al.,* 2017). This transcription factor is in charge of upregulating pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 (Akhtar *et al.,* 2020). AEASB lessens the synthesis of these inflammatory

mediators by inhibiting NF-κB activation, which lessens renal inflammation and shields renal tissues from more harm.

Biochemical investigations of renal Function biomarkers corroborate the protective effects of AEASB. Blood urea and creatinine levels were observed to be much higher in the MSG-treated group, suggesting compromised kidney function. Because they demonstrate the kidney's capacity to filter and remove waste, these biochemical markers are frequently employed to evaluate renal impairment (Cabral *et al.,* 2021; Liu *et al.,* 2022). Renal failure in MSG-induced nephrotoxicity has been closely associated with elevated urea and creatinine levels, which are suggestive of renal dysfunction. Perhaps as a result of garlic's antioxidant properties, the decrease in these biochemical markers suggests an improvement in renal filtration and excretory function. This observed improvement in renal function is probably due in part to garlic's capacity to lower oxidative stress and increase the activity of kidney antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) (Albrakati, 2021).

AEASB has multifactorial protective properties. Its antioxidant qualities lessen oxidative damage to renal tissues by scavenging free radicals. While its anti-apoptotic properties shield renal cells from programmed cell death, its anti-inflammatory properties prevent the activation of inflammatory cytokines and pathways like NF-κB. When combined, these pathways aid in maintaining the integrity and functionality of renal tissue when MSG-induced nephrotoxicity occurs. The results of this study are in line with previous investigations showing that *Allium sativum* has therapeutic potential in a range of organ toxicity models, such as those involving the kidney, liver, and heart (Fowotade *et al.,* 2017; Dorrigiv *et al.,* 2020; Tudu *et al.,* 2022). These findings add to the increasing amount of data that supports the use of natural products such as garlic as adjuvants in the management and avoidance of kidney damage brought on by toxins.

While this study shows that Allium sativum has promising nephroprotective properties, some things need to be investigated further. It is necessary to further describe the precise molecular pathways that underlie the preventive properties of allicin and other active chemicals found in garlic. Future research should concentrate on clarifying the precise signaling mechanisms at play and investigating the possible synergistic benefits of garlic in combination with other medicinal substances. Additionally, clinical research is required to evaluate Allium sativum's safety and effectiveness in human populations, especially in those who have renal impairments or have been exposed to nephrotoxic chemicals. Translating these findings into therapeutic applications will require an understanding of the clinical significance of garlic's protective benefits in human beings.

Conclusion

This study offers compelling evidence of the nephroprotective benefits of garlic, or *Allium sativum,* against kidney damage brought on by MSG. Garlic's anti-inflammatory, anti-apoptotic, and antioxidant qualities provide a multimodal strategy for reducing kidney damage. According to these results, garlic may be a viable natural supplement for preventing kidney damage brought on by pharmaceuticals or environmental pollutants. The molecular mechanisms of action and clinical verification of garlic's potential as a treatment for renal disorders should be the main topics of future research.

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Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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Authors Contribution

ASA contributed to the manuscript write-up, while ASK and NAS were responsible for conducting the

practical experiment and procuring research equipment. AG performed the statistical analysis, whereas RB provided the skeletal framework of the research and secured funding. MA carried out the extraction of the extract, while MMM and IAT were responsible for interpreting the histological slides. RIF reviewed and proofread the manuscript, and ABU revalidated the histological interpretations and findings, ensuring accuracy and consistency in the results.

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