Histological Effects of Black Garlic Extract in Monosodium Glutamate-Exposed Rats

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Abstract. The study examined hepatic tissue morphology and liver weight in rats exposed to monosodium glutamate (MSG), with or without black garlic extract (BGE). Twenty-five Wistar rats were assigned at random to five groups of five animals. P0 received no experimental treatment, whereas P1 received MSG at 8 mg/kg body weight daily for 21 days. The remaining groups followed the same MSG schedule and additionally received BGE at 200 (P2), 400 (P3), or 600 (P4) mg/kg body weight for the final 16 days. Liver specimens collected after euthanasia were examined histologically. P2 showed relatively limited cell swelling and cytoplasmic vacuolation, with nuclei remaining centrally located. Lesions in P3 and P4 resembled those in P1. These observations were assessed descriptively, without inferential testing of histological differences. Liver weight varied across groups (one-way ANOVA, $p = 0.011$); Tukey comparisons identified a higher value in P1 than in P0, while comparisons between P0 and each BGE group were not significant. The tissue observations suggest a possible protective association at 200 mg/kg, but they do not establish a statistically significant histological treatment effect. Quantitative lesion assessment and biochemical measurements are needed to evaluate this preliminary finding.

Keywords: Black garlic extract, plant extracts, sodium glutamate, MSG, chemical and drug-induced liver injury, histology

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1 Introduction

Hepatic exposure to circulating compounds links the liver's metabolic functions to its vulnerability to toxic injury. In addition to processing glutamate and producing bile, the organ transforms endogenous substances and xenobiotics. Medicines, industrial substances, dietary additives, and food or beverage contaminants may therefore elicit adaptive changes or damage in hepatic tissue [1,2].

The umami flavor associated with many Asian dishes is often enhanced using monosodium glutamate (MSG), a food additive derived from the sodium salt of L-glutamic acid [3,4]. Experimental investigations have linked MSG exposure to reactive oxygen species (ROS). When antioxidant defenses are insufficient, ROS can disrupt cellular lipids, proteins, and DNA, providing a possible pathway to hepatic injury [5,6].

Interest in garlic (Allium sativum L.) as a functional food has encouraged processing approaches that preserve biological activity while reducing the strong smell and taste of fresh cloves [7,8]. Controlled heating and humidity produce black garlic through an aging process involving Maillard reactions, caramelization, and changes in sulfur-containing constituents [9]. Its chemical profile includes adenosine, uridine, DL-lactic acid, 2-acetylpyrrole, and 5-hydroxymethyl-2-furfural, alongside constituents associated with antioxidant activity [10,11]. Reports of S-allyl cysteine (SAC) concentrations five to six times those of raw garlic have also contributed to interest in its biological properties [12,13].

Antioxidant constituents and modulation of inflammatory mediators are among the proposed explanations for black garlic's hepatic effects [12,14]. Evidence from alcohol-related injury and drug-associated hepatotoxicity suggests that garlic preparations warrant evaluation in experimental liver disease [15,16]. Hepatoprotective findings have also been reported for aged black garlic in rodent models [17].

The use of MSG in experimental liver-injury studies offers an opportunity to examine whether BGE modifies hepatic lesions. Evidence for this particular exposure–extract combination remains limited. The animals analyzed here belong to the cohort used in the authors' earlier report of macroscopic hepatic observations [30]. This paper examines histopathology and liver-weight outcomes within that same research project; it does not describe a separate animal experiment. The authors investigated how MSG exposure, alone or accompanied by different BGE doses, related to hepatic morphology and liver weight. The authors' working hypothesis was that ethanol-extracted black garlic would lessen MSG-associated tissue alterations, with the extent of any change depending on dose.

2 Methods

2.1 Animals

The Pharmacology Laboratory of the Faculty of Veterinary Medicine at Universitas Syiah Kuala, Banda Aceh, Indonesia, supplied 25 male Wistar rats (Rattus norvegicus).

At enrollment, the rats were 10–12 weeks old and weighed 150–200 g. Housing consisted of polycarbonate cages with alternating 12-hour periods of light and darkness; food and water were freely available. Each animal constituted one experimental unit. Ethical authorization was granted by the faculty's Animal Medical Ethics Committee under approval 143/KEPH/V/2022.

Random allocation occurred after seven days of acclimatization, yielding five groups of five animals each. P0 remained on standard food and water without MSG or BGE. P1 received oral MSG daily at 8 mg/kg body weight throughout days 1–21. P2, P3, and P4 received that same MSG exposure plus daily oral BGE at 200, 400, and 600 mg/kg body weight, respectively. Extract dosing began on day 6 and continued through day 21, providing 16 days of co-administration and a common final treatment day for MSG and BGE.

Body weight determined the amount of each test substance administered to an individual rat. For MSG preparation, 10 mL of 0.5% carboxymethyl cellulose (CMC) was used as the solvent; the daily oral dose was 8 mg/kg over days 1–21. The extract preparation likewise used 10 mL of 0.5% CMC. Rats assigned to BGE received their designated dose orally each morning during days 6–21.

2.2 Preparation of Ethanol Extract from Black Garlic

Botanical authentication of fresh Allium sativum L. bulbs was performed by the Herbarium, Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Syiah Kuala in Banda Aceh. To prepare black garlic, aluminum-foil-wrapped bulbs were placed in a stainless-steel container and kept in an oven at approximately 60 °C for 16 days. Processing was checked at intervals of 4–5 days. Separate 100-g portions of raw garlic powder and processed black garlic underwent maceration with 70% ethanol, using 10 parts solvent per part sample (w/v). Extraction comprised seven 24-hour cycles with intermittent stirring. Filtrates were combined and reduced in a rotary evaporator operating at 50 °C; the concentrate was then weighed and stored before administration. Screening detected flavonoids, alkaloids, and triterpenoids. No quantitative standardization of SAC or other antioxidant components was undertaken. The selected BGE doses—200, 400, and 600 mg/kg body weight—were informed by earlier investigations [15,18–20].

2.3 Eligibility Criteria

The inclusion criteria consisted of comparative clinical studies involving adult patients (≥18 years) with severe traumatic brain injury that evaluated hypertonic saline and mannitol as hyperosmolar therapies. Eligible study designs included randomized controlled trials, prospective cohort studies, and retrospective comparative observational studies. Studies were required to report outcomes related to intracranial pressure, cerebral perfusion pressure, mortality, neurological outcomes, or treatment-related adverse events. Articles published between 2020 and 2026, written in English, and available as full-text publications were considered eligible. Conversely, the exclusion criteria included systematic reviews, meta-analysis, guidelines, clinical protocols, and narrative reviews,

as these sources were excluded from the final qualitative synthesis but considered as supporting evidence. Additional exclusions included studies that did not specifically address severe traumatic brain injury, pediatrics population, animal studies and laboratory-based experimental research, editorials, letters to the editor, conference abstracts, expert opinions without empirical data, articles with inaccessible full texts, and studies with poor methodological quality or unclear out-come reporting. These criteria were established to maintain topic relevance and im-prove the validity of the evidence synthesis process [14].

2.4 Histological Evaluation of Rat Liver

The terminal procedure used injectable ketamine at 100 mg/kg body weight together with xylazine at 5 mg/kg body weight for anesthesia and euthanasia, as recorded in the study protocol. Following collection, liver specimens were washed with cold saline, dried by blotting on filter paper, and weighed. Fixation used 10% phosphate-buffered formalin. Graded ethanol dehydration and xylene clearing preceded automated tissue processing and paraffin embedding. Sections measuring 4–6 μm were placed on glass slides and stained with hematoxylin and eosin (H&E). An Olympus light microscope (Tokyo, Japan) was used for examination, and micrographs were obtained at ×100. Pale cytoplasm accompanied by swelling or vacuolation was interpreted as hydropic degeneration. Necrosis was identified from disrupted cellular architecture together with pyknosis, karyorrhexis, or karyolysis. Assessment relied on descriptive/semiquantitative judgments without a validated ordinal scale. The original records did not document assessor blinding.

2.5 Statistical Analysis

Morphological findings were compared by descriptive/semiquantitative assessment of hepatocyte necrosis and hydropic degeneration. Since a validated ordinal lesion scale was not used, histological observations were not subjected to inferential analysis. Liver-weight measurements were summarized by their means and standard deviations. The Shapiro–Wilk and Levene tests assessed distributional normality and variance homogeneity, respectively. An omnibus one-way ANOVA was followed by Tukey pairwise comparisons; p-values below 0.05 were considered significant. Computations used the Windows edition of IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Liver Weight

The overall comparison of liver weight indicated variation among the five groups (ANOVA, $p = 0.011$; Table 1). Tukey testing identified P1 as having a higher liver

weight than P0. Pairwise comparisons of P0 with P2, P3, or P4 did not reach significance.

Table 1. Liver weight in the experimental groups (five rats per group)

Group	n	Liver weight (g) mean ± SD
P0 – Negative control	5	6.13 ± 0.80
P1 – MSG-exposed group	5	8.17 ± 0.63*
P2 – MSG + BGE 200 mg/kg	5	6.26 ± 0.71
P3 – MSG + BGE 400 mg/kg	5	6.52 ± 0.65
P4 – MSG + BGE 600 mg/kg	5	6.46 ± 0.62

**p < 0.05 vs. negative control (P0), Tukey's post-hoc test following one-way ANOVA.*

Microscopy identified hepatocyte injury that was not evident from gross inspection. Lesions occurred in P1 and in each of the three extract-treated groups (Table 2).

Table 2. Histological observation of liver tissue for each group.

Groups	Type of hepatocyte damage	
	Hydropic degeneration	Necrotic
Negative Control	-	-
MSG-exposed Group (8 mg/kg bw MSG)	+	+
8 mg/kg body weight MSG + 200 mg/kg body weight BGE	+ (minimal)	+ (minimal)
8 mg/kg body weight MSG + 400 mg/kg body weight BGE	+	+
8 mg/kg body weight MSG + 600 mg/kg body weight BGE	+	+

A minus sign denotes the absence of the indicated lesion, whereas a plus sign denotes an observed lesion. "+ (minimal)" describes mild, focal changes; nuclei remained centrally positioned in the minimally affected tissue. These symbols reflect visual judgments rather than a validated lesion score. P1 received MSG without extract and serves as the MSG exposure comparison group.

P0 specimens retained normal hepatic architecture. In P1, enlarged hepatocytes with pale, markedly vacuolated cytoplasm accompanied hydropic degeneration and necrosis; membrane rupture was seen in necrotic cells. P2 had less pronounced swelling and vacuolation, pale cytoplasm, and centrally positioned nuclei. Membrane disruption and associated fluid accumulation were described as minimal in this group. Degenerative and necrotic changes remained evident at the two higher extract doses, P3 and P4 (Figure 1).

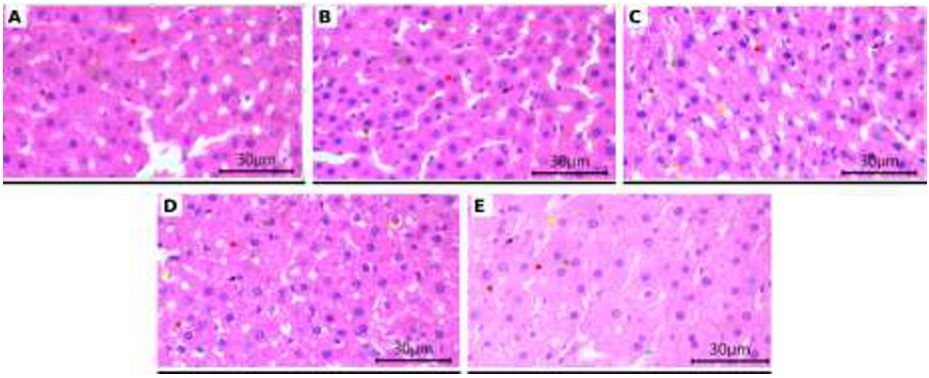


Fig. 1. Liver micrographs illustrating the five experimental groups. Panels correspond to P0 (A), P1 (B), P2 (C), P3 (D), and P4 (E). P1–P4 received 8 mg/kg body weight MSG; P2–P4 additionally received BGE at 200, 400, and 600 mg/kg body weight, respectively. Each group contained five animals, with one representative image shown per group. Green arrows identify normal hepatocytes, red arrows indicate necrosis, and brown arrows mark hydropic degeneration. These images illustrate morphology and do not quantify between-group lesion severity.

The qualitative assessments summarized in Table 2 place P1 above P0 in the extent of degeneration and necrosis. P2 appeared less affected than P1, P3, and P4. These visual comparisons describe the observed specimens; without inferential testing of lesion scores, they cannot establish significant differences among treatments.

4 Discussion

Hepatic lesions were observed after the 21-day MSG regimen at 8 mg/kg body weight. The question addressed here was whether adding BGE changed the appearance of those lesions. Earlier experimental work has also described liver-tissue alterations in MSG-exposed rats [21]. The present observations should be interpreted within this particular exposure protocol rather than as evidence of a general toxic threshold.

Liver weight and tissue morphology provided complementary observations. P1 differed significantly from P0 in liver weight, but the extract groups did not differ significantly from P0. The authors' previous publication described macroscopic outcomes in these same animals [30], so agreement between the reports is not independent replication. Separate evidence of hepatic protection by aged black garlic comes from a chronic alcohol model [15]. In the current specimens, the clearest descriptive difference concerned P2, where lesions appeared less extensive; increasing the extract dose did not yield a comparable visual improvement.

Residual necrosis and vacuolation in all three BGE groups indicate that the extract was not associated with complete preservation of hepatic tissue. P2 nevertheless retained centrally placed nuclei and displayed relatively limited swelling and cytoplasmic vacuolation. Ahmed's cyclophosphamide experiment used 200 mg/kg aged black garlic extract for two weeks and reported better-preserved hepatocyte arrangement, nuclei,

and cytoplasm, with only minor degeneration or necrosis [22]. An ethephon study likewise described reduced injury and recovery of tissue architecture following four weeks of aged garlic extract at 250 mg/kg [23]. These findings offer comparisons across experimental toxicants, although they do not establish equivalent effects under MSG exposure.

The handling of glutamate depends on the exposure route. Intestinal and hepatic metabolism are central to the disposition of orally consumed glutamate [24]. Morphological and biochemical injury has also been investigated after intraperitoneal MSG administration [25]. Because MSG was delivered orally in the present experiment, mechanisms derived from intraperitoneal exposure should not be assumed to explain these findings. The authors did not measure glutamate disposition or the metabolic pathways responsible for the observed lesions.

The visual findings at 400 and 600 mg/kg do not support an additional histological advantage at those doses. This observation remains qualitative and should not be interpreted as proof of dose equivalence or harm from higher doses. In a carbon tetrachloride model, Shin et al. reported less inflammatory-cell infiltration and necrosis with 200 mg/kg aged black garlic [17]. Alcohol-exposed rats have also shown less fatty change and lipid accumulation with a 100 mg/kg preparation [15]. Differences in toxicant and preparation prevent direct identification of an optimal dose for MSG-associated injury from these comparisons.

SAC and other sulfur-containing constituents provide a possible biochemical basis for the antioxidant activity attributed to aged garlic preparations [26,27]. Garlic constituents have also been discussed in relation to endogenous antioxidant defenses [28,29]. The authors did not assay catalase, superoxide dismutase, glutathione peroxidase, or other mechanistic endpoints. Furthermore, much of the comparative evidence concerns carbon tetrachloride, alcohol, cyclophosphamide, or ethephon. Their exposure patterns and injury mechanisms differ from those of MSG. Antioxidant-mediated protection therefore remains a hypothesis for the current model, rather than a mechanism demonstrated by the authors' measurements.

5 Limitations

Several features restrict the strength of the conclusions. Lesion assessment lacked a validated ordinal scale, and histological differences were not tested statistically. Consequently, the observations cannot establish a dose–response relationship. Neither assessor blinding nor a prospective power calculation was documented; five animals per group were used without a formal sample-size derivation. Control selection introduces further uncertainty: P0 did not receive the 0.5% CMC vehicle used for MSG and BGE, and no group received BGE in the absence of MSG. Effects attributable to the vehicle or to the extract alone therefore cannot be fully separated. In addition, the extract was not standardized for SAC or other constituents, restricting comparability across preparations and attribution to a specific compound. Subsequent work should use blinded,

validated lesion scoring, a justified sample size, vehicle-matched and extract-only controls, chemical characterization, and measurements of oxidative stress and inflammation.

6 Conclusion

Within this experiment, the 200 mg/kg BGE group displayed less pronounced hepatic lesions than the group receiving MSG alone. This is a preliminary descriptive association: the absence of validated blinded scoring and an extract-only comparator limits conclusions about protection. Further testing should combine chemically characterized BGE with quantitative histology, suitable controls, and oxidative-stress and inflammatory endpoints before a protective effect or preferred dose is established.

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