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Original Article

Black garlic exhibited hepatoprotective effect against monosodium glutamate-induced hepatotoxicity in animal model

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Abstract

Monosodium glutamate (MSG) is commonly used as a flavor-enhancing agent in foods, and studies have demonstrated its toxic effects in animal models. Black garlic is known for its antioxidant and anti-inflammatory properties; however, there is a lack of studies on the potential hepatoprotective effect of black garlic ethanol extract (BGE) against MSG-induced hepatotoxicity in rats. The aim of this study was to investigate the hepatoprotective effects of ethanol extract of black garlic against MSG-induced liver damage in animal model. Twenty-five male Wistar rats were randomly assigned to five groups (n=5): negative control, MSG only, and MSG with three different doses of BGE. The MSG only and MSG with BGE groups were orally administered with 8 mg/kg MSG daily. After MSG treatment, the MSG with BGE groups received BGE orally at daily doses of 200, 400, or 600 mg/kg body weight for 16 consecutive days. Subsequently, the levels of serum liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), interferon-gamma (IFN-γ), and cyclooxygenase-2 (COX-2) were measured. Our data indicated that the group treated with 200 mg/kg BGE had significant lower levels of AST and ALT significantly compared to the MSG-only group. The MSG-treated group had higher levels of the inflammatory markers COX-2 and IFN-γ, which were lowered by administration of 200 mg/kg BGE. In contrast, higher doses of BGE led to greater levels of COX-2 and IFN-γ compared to those in the MSG-only group. This study suggested that BGE might have hepatoprotective effects at low dose, potentially mitigating MSG-induced liver damage. However, the higher dose of black garlic extract did not alleviate inflammation, as shown by the higher levels of COX-2 and IFN-γ.

Keywords: Black garlic extract, monosodium glutamate, MSG, hepatotoxic, hepatoprotective effect

Introduction

Studies have shown that monosodium glutamate (MSG) can be toxic to humans and animal models, causing damage to lipids, proteins, and DNA by releasing free radicals and increasing reactive oxygen species (ROS) levels, leading to liver injury [1-3]. Several studies using rat models have consistently reported that MSG induces liver damage and triggers oxidative stress [4-8]. There was a correlation between pro-inflammatory reactions, ROS generation, and subsequent systemic irregularities induced by oxidative stress [9,10].

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Garlic (*Allium sativum* L.), known for its antioxidant and anti-inflammatory properties, has become a popular herbal plant [11,12]. Despite its acknowledged health benefits, the strong and undesirable taste and aroma of raw garlic make it unappealing to some individuals [13,14]. Consequently, an alternative form of garlic known as black garlic has been developed. The production of black garlic involves fermenting fresh garlic at a high temperature and humidity, a process that effectively eliminates its strong aroma while enhancing its nutrient content, bioactivity, and flavor. This process transforms volatile and labile elements of raw garlic into stable and non-odorous compounds. Black garlic is rich in organosulfur compounds and polyphenols and exhibits superoxide dismutase–like and hydrogen peroxide (H_2O_2) scavenging activity [15-18]. Recent studies have indicated that black garlic has more potent antioxidant properties than fresh garlic because fermentation increases the concentration of antioxidant compounds four-to five-fold [12,19,20]. Black garlic has traditionally been used for its health benefits, including anti-hepatotoxic, antifungal, antiviral, antibacterial, anticancer, antiseptic, and anti-inflammatory effects [13,15,18,20-23].

Several studies on black garlic have demonstrated significant antioxidant properties [12,24-26]. A study reported the hepatoprotective and antioxidant effects of aqueous aged garlic extract (150, 300, and 600 mg/kg) in diethylnitrosamine-induced hepatocarcinogenic rats [27]. Another study also revealed that 100 mg/kg of aged black garlic ethanol (BGE) was a hepatoprotective agent against ethanol-induced oxidative liver damage [17]. Aged BGE (100, 300, and 600 mg/kg) is also considered to be an antioxidant and antidiabetic agent in streptozotocin (STZ)-induced diabetic rats [28]. Furthermore, an in vitro study showed that 400 mg/kg (medium dose) and 800 mg/kg (high dose) of aged BGE were effective anticancer agents in animal models [29].

The liver can be negatively affected by toxic substances or their metabolites, such as MSG due to its involvement in metabolism and detoxification [30]. MSG can elevate the reactive free radicals and electrophiles within cells, disrupting the physiology of the body and causing cellular damage through oxidative stress. Enzymes such as alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) have been evaluated to explore the functionality of the liver [31-33]. It is expected that specific antioxidants and anti-inflammatory medicinal plants such as BGE could mitigate the harmful effects of MSG. However, the role of BGE in mitigating MSG-induced hepatotoxicity has not been investigated. Therefore, the aim of this study was to assess the potential of BGE to alleviate hepatic function impairment induced by MSG in rats.

Methods

Black garlic production and extraction process

Fresh garlic was obtained from a traditional market in Banda Aceh, Indonesia, and BGE was prepared as previously described [16,34]. Black garlic was produced and extracted at the Laboratory of Biology of the Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. Unpeeled garlic, enclosed in airtight containers, underwent a 16-day baking process at 60°C and relative humidity levels of 85–95%, without additives or additional treatments. Temperature checks were performed every four or five days to maintain consistency during fermentation. After removal of the outer layer, the garlic was crushed in a 70% ethanol solution for seven days (using 100 g of the sample in 1 L of 70% ethanol) with intermittent stirring. After filtration, the garlic mixture was evaporated in a rotary evaporator. The resulting filtrate was weighed and ready for use.

Experimental study design and groups

Wistar rats (*Rattus norvegicus*) weighing 150–200 g were obtained from the Laboratory of Experimental Animals of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Animals were maintained under controlled conditions at a room temperature of 24±5°C and a 12:12 h light-dark cycle, with access to standard food and water *ad libitum* throughout the study. The rats were acclimatized for one week before the initiation of the experiments. Using a post-test control-only group design, twenty-five male Wistar rats were randomly allocated to five groups: negative control; MSG-induced hepatotoxic model only; MSG-induced hepatotoxic model treated with BGE at doses of 200 (BGE200), 400 (BGE400), or 600

mg/kg body weight (BGE600). Five animals were used for each group. The negative control group received standard food and drinking water without any treatment. The MSG-induced hepatotoxic model was created by exposing the rats with MSG for 21 days. MSG was dissolved in 10 mL of 0.5% carboxymethyl cellulose (CMC) and administered at a dose of 8 mg/kg body weight (per oral) starting on the eighth day (after the acclimatization step). The details of the study design are illustrated in **Figure 1**.

Following 21 days of MSG induction, the animals received BGE treatment for 16 days. The BGE was mixed with 10 mL of 0.5% CMC and was administered orally once every morning, according to the dose and body weight of each group.

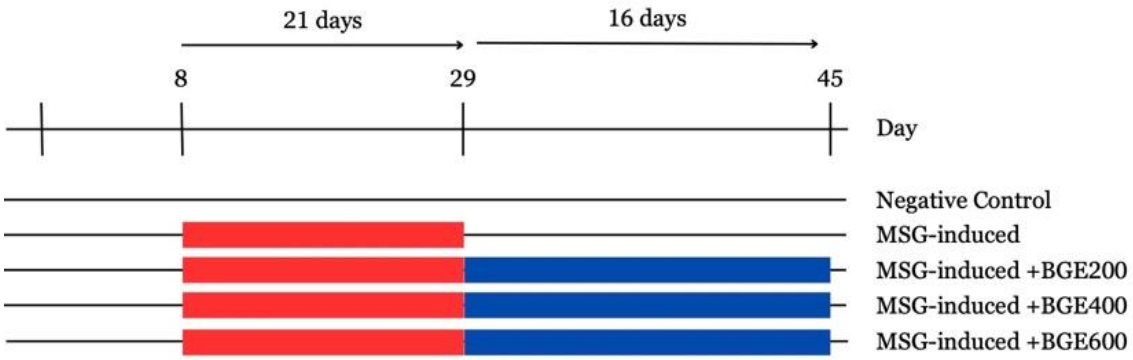

Figure 1. Experimental design and detailed intervention for each group of the study. BGE: black garlic extract; MSG: monosodium glutamate.

Blood sample collection

After completing the study, rats were anesthetized using ketamine-xylazine (100 mg/kg and 5 mg/kg, intraperitoneally) to facilitate heart puncture for blood sample extraction. Each animal yielded 5 mL of blood, which was collected in EDTA-containing tubes to prevent coagulation. The blood samples were then centrifuged at 3000 rpm for 15 min to collect the serum, which was subsequently preserved at -20°C for further biochemical analysis.

Measurement of AST and ALT levels

A kinetic method was used to measure serum levels of AST and ALT. Serum levels of liver enzymes were quantified using commercial enzymatic kits from BioMaxima (BioMaxima, Lublin, Poland). Briefly, 100 mL of rat blood serum was mixed with 800 mL of Reagent 1 and 200 mL of Reagent 2, homogenized, and subsequently analyzed using a biochemical analyzer (Yueshen Med, Guangzhou, China). The resulting values are expressed as IU/L.

Measurement of cyclooxygenase-2 (COX-2) and interferon-gamma (IFN-γ) levels

ELISA kits were used to quantify the COX-2 and IFN-γ levels (COX-2, cat no. BZ-08051920-EB, BioEnzy; IFN-γ, cat no. BZ-08051920-EB; both from BioEnzy, Jakarta, Indonesia). The procedure involved the addition of standards and samples to ELISA microwells pre-coated with COX-2 and IFN-γ antibodies, followed by incubation at 37°C. After a 90-min incubation period, the microplate was washed twice in each well using wash buffer. Biotinylated detection antibodies were added to the microwells, followed by a 60-min incubation at 37°C and three washes. Subsequently, 100 µL of HRP-streptavidin conjugate working solution was introduced to each well and incubated at 37°C for 60 min. The microplate was washed five times with wash buffer before 90 µL TMB substrate was dispensed. The stop solution was subsequently added to terminate the reaction. The optical density was measured at a wavelength of 450 nm using an enzyme immunoassay reader.

Statistical analysis

One-way analysis of variance (ANOVA), followed by Tukey's test for post-hoc analysis, was used to compare the differences in AST, ALT, COX-2, and IFN-γ levels among and between the groups. Statistical significance was determined at $p<0.05$. Data analysis was conducted using SPSS 25.0

(IBM, New York, USA) and visualized using GraphPad Prism v. 10.2.2 (GraphPad Software, Inc.; San Diego, CA, USA).

Results

Effect of BGE on AST and ALT levels

The effects of BGE on liver enzymes (AST and ALT) across all experimental groups are presented in **Table 1**. There was a significant difference in AST and ALT levels among groups (p<0.001). Our study revealed significantly higher AST (**Figure 2A**) and ALT (**Figure 2B**) levels of the MSG-treated group compared to the negative control group (p=0.03 and p<0.001, respectively). Notably, the BGE200-treated group had no statistically significant differences when compared to the negative control group (p=0.895 and p=0.548 for AST and ALT levels, respectively). Significantly, this group showed significant lower of AST (p=0.024) and ALT (p<0.001) levels than those in the MSG-treated group (**Figure 2A** and **B**). Although liver enzyme levels were higher in the BGE400 and BGE600 groups than in the negative control group, no statistically significant differences in AST and ALT levels were observed compared to the MSG-treated group (p=0.996 and p=0.968, respectively) (**Figure 2A** and **B**).

Table 1. Levels of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) among study groups

Group	Experimental detail	AST (IU/L)	*p*-value [a]	ALT (IU/L)	*p*-value [a]
		Mean±SD		Mean±SD	
Control	Negative control	127.74±23.14	<0.001	38.12±6.47	<0.001
MSG	MSG 8 mg/kg	178.50±5.44		63.10±6.98	
BGE200	Black garlic extract 200 mg/kg	138.46±10.20		44.34±6.94	
BGE400	Black garlic extract 400 mg/kg	182.80±27.09		64.30±6.15	
BGE600	Black garlic extract 600 mg/kg	186.04±19.58		65.76±5.11	

BGE: black garlic ethanol; MSG: monosodium glutamate
[a] Analyzed using one-way ANOVA

Effect of BGE on COX-2 and IFN-γ levels

The MSG-treated group had significantly higher COX-2 levels than the negative control group (p<0.001) (**Table 2**). The administration of 200 mg/kg body weight of BGE (BGE200) resulted in significantly lower COX-2 levels compared to the MSG-treated group (p=0.02). However, higher doses of BGE (400 mg/kg or 600 mg/kg) did not produce the same effect, as COX-2 levels remained higher compared to those in the MSG-treated group (**Figure 2C**). Similarly, the MSG-treated group exhibited significantly higher IFN-γ levels than the negative control group (p=0.001). Administration of a low dose of BGE (200 mg/kg) resulted in significantly lower IFN-γ levels (p=0.002). In contrast, higher doses of BGE (400 mg/kg or 600 mg/kg) resulted in higher IFN-γ levels than those observed in the MSG-treated group (**Figure 2D**).

Table 2. Levels of cyclooxygenase-2 (COX-2) and interferon-gamma (IFN-γ) among study groups

Group	Experimental detail	COX-2 (ng/mL)	*p*-value [a]	IFN-γ (ng/mL)	*p*-value [a]
		Mean±SD		Mean±SD	
Control	Negative control	2.02±0.18	<0.001	39.98±3.51	<0.001
MSG	MSG 8 mg/kg	2.85±0.16		48.29±3.96	
BGE200	Black garlic extract 200 mg/kg	2.37±0.64		39.29±2.12	
BGE400	Black garlic extract 400 mg/kg	3.36±0.22		51.07±3.73	
BGE600	Black garlic extract 600 mg/kg	3.46±0.18		52.34±1.94	

BGE: black garlic ethanol; MSG: monosodium glutamate
[a] Analyzed using One-way ANOVA

Discussion

This study investigated the potential of BGE to mitigate MSG-induced liver damage. These findings indicated that MSG led to liver injury, as evidenced by the elevated levels of liver enzymes (ALT and AST) and inflammatory markers (IFN-γ and COX-2) in rats. The antioxidant properties of BGE could mitigate the effects of MSG, and this antioxidant activity contributed to its anti-inflammatory effects.

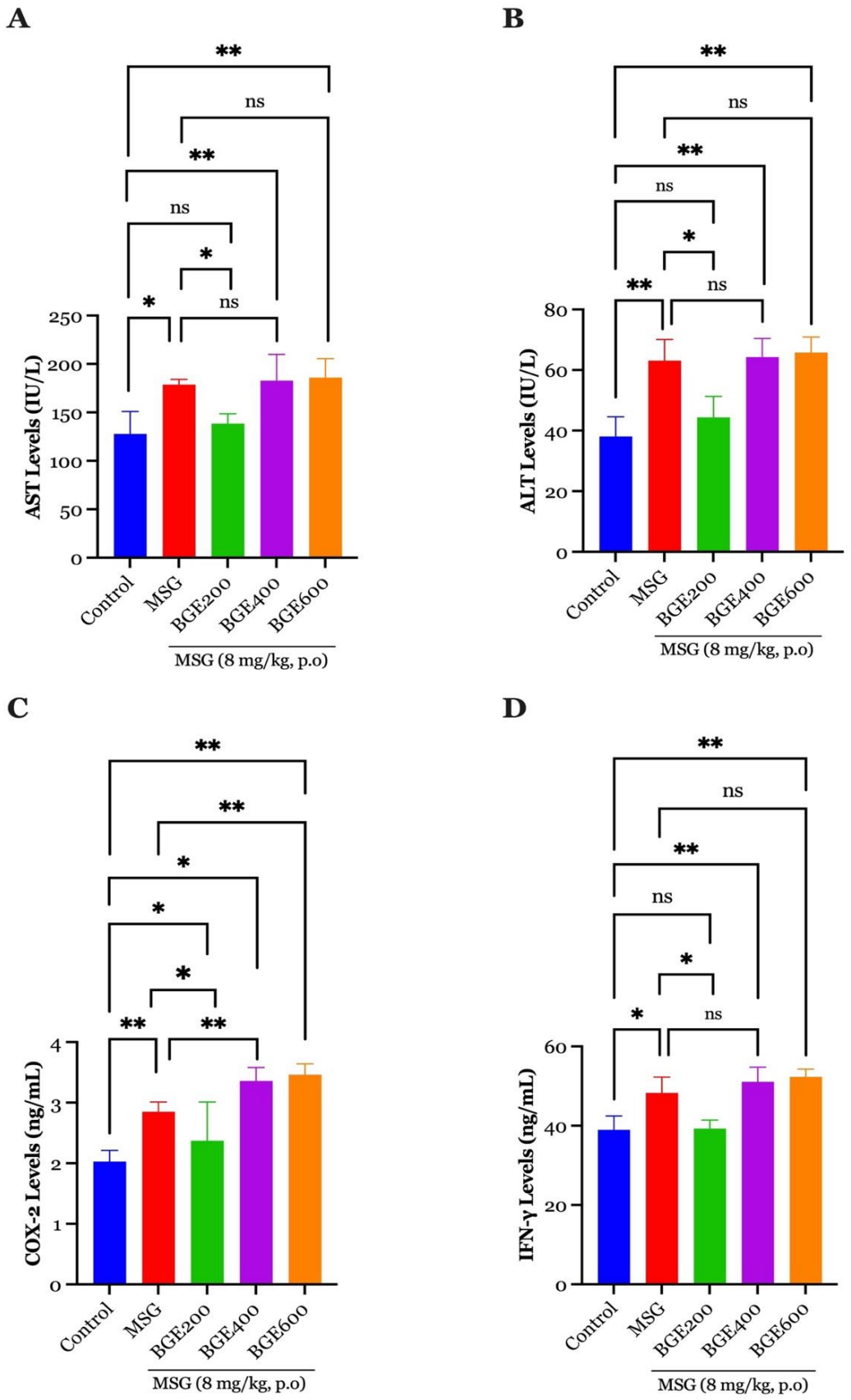

Figure 2. Effects of black garlic extract (BGE) on serum levels of (A) aspartate aminotransferase (AST); (B) alanine aminotransferase (ALT); (C) cyclooxygenase-2 (COX-2); and (D) interferon-gamma (IFN-γ) in the Wistar rat model. Significant differences between groups are indicated: ns=not significant, *significant at $p<0.05$ and ** at $p<0.001$. BGE200, BGE400 and BGE600 refer to the groups treated with BGE at 200, 400 and 600 mg/kg body weight, respectively, for 16 days.

Our findings confirmed that MSG-induced liver damage as characterized by elevated serum levels of liver transaminase enzymes (AST and ALT), consistent with previous research [5,7,8,17,18,30,35-37]. Serum levels of AST and ALT serve as markers of hepatocyte integrity, with elevated levels indicating potential damage to the cytoplasmic or mitochondrial membranes. ALT is particularly precise in monitoring hepatocellular function and is recognized as a more specific indicator of liver injury. On the other hand, AST serves as a sensitive marker of mitochondrial issues, particularly in the centrilobular regions of the liver [30,37].

Due to its cytotoxic properties, MSG significantly increases AST and ALT levels by inducing liver cell damages [35,38]. This damage leads to liver stress and the production of ROS, resulting in lipid peroxidation and degradation of liver cells, which is accompanied by an increase in liver enzyme levels [1]. Consequently, the cytotoxic effect of MSG, caused by oxidative stress, affects both liver cells and canaliculi, leading to the release of enzymes into the bloodstream [2]. Additionally, MSG stimulates the production of ammonium ions, which further contributes to liver toxicity by facilitating a reaction between ROS and polyunsaturated fatty acids [1]. This process leads to the breakdown of the plasma and mitochondrial membranes, resulting in the release of liver enzymes [1]. Overall, lipid peroxidation plays a significant role in the development of MSG-induced liver injury [1].

Our results demonstrated that the administration of BGE significantly lowered the increase in liver enzymes. BGE treatment, especially at a low dose (200 mg/kg body weight), significantly normalized serum levels of AST and ALT compared to both the MSG-only group and the groups receiving higher BGE doses (400 and 600 mg/kg body weight). These findings suggested a hepatoprotective effect, consistent with the findings of previous studies [17,18]. In line with our results, a study reported that a low dose of aged BGE (150 mg/kg) improved liver damage induced by diethylnitrosamine and carbon tetrachloride (CCl_4) in rats [27]. Furthermore, aged BGE at a dose of 200 mg/kg protected the liver from hepatic injury induced by cyclophosphamide [39] and CCl_4-induced acute hepatic injury [22].

Black garlic is rich in antioxidants in comparison to regular white garlic, particularly S-allylcysteine [14,20,21]. The antioxidant content of black garlic is hepatoprotective against liver damage and repairs liver cell damage [17,40]. The antioxidant potential of black garlic may be attributed to its various components, including pyruvate, phenolics, and flavonoids. Furthermore, black garlic contains sulfur-rich compounds such as diallyl sulfide, diallyl disulfide, and diallyl trisulfide, which collectively contribute to its higher antioxidant activity than that of fresh raw garlic [13,18,22]. Moreover, black garlic exhibits stronger antioxidant activity than fresh garlic, as evidenced by both *in vivo* and *in vitro* studies [41].

Animals administered MSG exhibit obesity, which triggers the accumulation and activation of pro-inflammatory cytokines, such as IFN-γ and COX-2, due to the growth of intra-abdominal adipose tissue [42-45]. A study suggested that glutamate triggered ROS production [45]. ROS triggers inflammation and the secretion of various pro-inflammatory cytokines, which contribute to the inflammatory reaction in hepatic tissue, ultimately resulting in liver damage [46]. In the present study, MSG administration led to elevated levels of COX2 and IFN-γ levels. Our results are consistent with those of previous studies, indicating that MSG exacerbates inflammation by increasing the levels of pro-inflammatory cytokine levels [17,42,47]. COX-2 is a controlling enzyme generated at the site of tissue damage in most tissues and stimulates the production of prostaglandin E2 (PGE2). This molecule, which has hormone-like properties, is frequently associated with cellular processes such as inflammation [48]. High levels of IFN-γ release also indicate liver tissue injury [44].

Treatment with a low dose (200 mg/kg of body weight) of BGE was significantly associated with reduced IFN-γ and COX-2 levels, indicating an anti-inflammatory effect. A previous study also demonstrated BGE's ability to attenuate liver damage induced by various agents such as tetra butyl hydroperoxide through its anti-apoptotic, antioxidative and anti-inflammatory properties [49]. BGE's hepatoprotective action is attributed to its promotion of cell survival and reduction of lipid peroxidation, oxidative stress, and inflammation by regulating the Jun amino-terminal kinases (JNK) signaling cascade [15,50]. Furthermore, BGE induces anti-inflammatory effects by deactivating nuclear factor kappa B (NF-κB), upregulating heme oxygenase-1 expression, and

suppressing COX-2 and 5-lipoxygenase activity [13]. Methanol extract of aged black garlic also reduces COX-2 and prostaglandin E2 (PGE2) synthesis through NF-κB deactivation [51].

Our findings suggested that administration of excessive amounts of BGE may adversely affect rats' livers, with no improvement observed at higher doses of BGE (400 and 600 mg/kg body weight). A comparison with fresh garlic, black garlic exhibits potent antioxidant effects but lower anti-inflammatory activity [12].

This discrepancy may be due to the increased concentrations of total phenolics, flavonoids, thiosulfates, and organosulfur compounds during the black garlic aging process [17,20,22,52]. Additionally, the Maillard reaction during black garlic processing increases the concentrations of sugars, possibly influencing its anti-inflammatory effects [53]. Despite its sweet taste, the potential antioxidant and anti-inflammatory effects of sugars in aged black garlic are poorly understood. A study suggested that sugars may trigger inflammation by promoting pro-inflammatory cytokine levels and activating NF-κB [12]. The diminished anti-inflammatory properties observed in aged BGE compared to fresh raw garlic extract may be attributed to the presence of sugars. The presence of sugar combinations, including galactose, glucose, fructose, and sucrose, was more prevalent in the aged BGE than in the fresh raw garlic extract. This resulted in the lower anti-inflammatory activity of BGE [12,20]. We hypothesized that a high dosage of BGE may contain a high sugar content, resulting in decreased anti-inflammatory properties.

This study is the first to investigate the potential effects of BGE on MSG-induced hepatotoxicity in animal model. Consequently, the absence of comparative studies is a limitation of this study, highlighting the need for further research. Additionally, the study employed a posttest-only design without pretest treatment and absence of a positive control group to assess potential differences in the pre- and post-test conditions.

Conclusion

Our study indicated that MSG led to liver injury in rats by elevating AST and ALT levels as well as COX-2 and IFN-γ levels. Low doses of BGE protected against MSG-induced liver damage, lowered liver enzyme parameters to near-normal levels, and lowered inflammatory marker levels. However, the higher dose of BGE did not alleviate inflammation, as indicated by the higher levels of COX-2 and IFN-γ.

Ethics approval

The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (reference no. 143/KEPH/V/2022, 9 May 2022).

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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