2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(3): 774-781.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj79
PII: S232245682500079-15

Effects of Black Garlic (*Allium sativum*) Supplementation on Oxidative Stress and Leptin Level in Male Obese Rats

Ibrahim[1,2], Eti Yerizel[3*], Endrinaldi[4], and Gusti Revilla[5]

[1]Program Doctoral of Biomedical Science, Faculty of Medicine, Andalas University, Limau Manis, Padang, Sumatra Barat 25175, Indonesia
[2]Departement of Medical Laboratory Technology, Syedza Saintika University, Jl. Prof. Dr. Hamka No.228, Air Tawar Tim., Padang, Sumatera Barat 25132, Indonesia
[3]Departement of Biochemistry, Faculty of Medicine, Andalas University, Limau Manis, Padang, Sumatra Barat 25175, Indonesia
[4]Departement of Biomedical, Faculty of Medicine, Andalas University, Limau Manis, Padang, Sumatra Barat 25175, Indonesia
[5]Departement of Anatomy, Faculty of Medicine, Andalas University, Limau Manis, Padang, Sumatra Barat 25175, Indonesia

*Corresponding author's Email: etiyerizel@yahoo.com

ORIGINAL ARTICLE
Received: June 24, 2025
Revised: July 28, 2025
Accepted: August 27, 2025
Published: September 30, 2025

ABSTRACT

Obesity is characterized by excessive fat accumulation in tissues, leading to leptin resistance and chronic inflammation. Black garlic is a well-documented natural remedy capable of improving leptin resistance and oxidative stress through its antioxidant compounds, such as S-Allyl cysteine and polyphenol flavonoids. The present study aimed to evaluate the effects of black garlic on oxidative stress and leptin levels in obese male rats. Twenty-five adult male Wistar rats, aged 10 to 12 weeks and weighing approximately 200 g, were divided into five experimental groups, each comprising five rats. These groups included a negative control group fed a standard diet (K1), a positive control group given a high-fat diet (K2), Treatment group 1 receiving a high-fat diet plus 200 mg of black garlic (P1), Treatment group 2 receiving a high-fat diet plus 400 mg of black garlic (P2), and Treatment group 3 receiving a high-fat diet plus 800 mg of black garlic (P3). The rats received black garlic orally for 28 days. The blood samples were analyzed for markers of oxidative stress, specifically malondialdehyde, as well as leptin levels. The present findings indicated that black garlic significantly decreased malondialdehyde and leptin concentrations in groups P1, P2, and P3 compared to the positive control group. Black garlic, administered at doses of 200, 400, and 800 mg, demonstrated the capacity to diminish oxidative stress and leptin levels in obese male rats.

Keywords: Antioxidant, Black garlic, Leptin, Malondialdehyde, Obesity, Oxidative stress, Rat

INTRODUCTION

Obesity leads to lipid accumulation in adipose tissue (Aziz et al., 2020). It is a multifactorial disease caused by genetic, cultural, hormonal, psychological, and social factors (Lin and Li, 2021; Panuganti et al., 2023). Obesity is influenced by hormones that regulate food intake, including leptin, ghrelin, adiponectin, and cholecystokinin, among other mediators (Sumadewi et al., 2016). In obesity, leptin resistance often develops, leading to increased hunger and food consumption (Subarjati and Nuryanto, 2015; Lin and Li, 2021). Leptin is a hormone synthesized by adipose cells that reduces food intake, increases energy expenditure through specific signals in the hypothalamus, and maintains body weight homeostasis (Amos et al., 2017; Xu et al., 2018). Excessive lipid accumulation associated with obesity can lead to an elevated production of reactive oxygen species (ROS) and free radicals, both in circulating blood and adipocyte cells (Fajrani et al., 2021). Oxidative stress is characterized by increased lipid peroxides, as indicated by elevated levels of malondialdehyde (MDA; Jovie et al., 2022).

Management of free radicals and oxidative stress has been studied using different medications, including both chemical medicines from the statin class and natural remedies such as *Camellia sinensis*, *Allium sativum*, *Syzygium polyanthum*, *Uncaria*, and botanicals rich in antioxidant compounds, including polyphenols, flavonoids, phenolic acids, and vitamins. One of the traditional plants used as herbal medicine is black garlic (Sembiring and Iskandar, 2019). The active organosulfur compounds in black garlic have a higher antioxidant concentration than regular garlic (Ahmed and Wang, 2021). Black garlic is produced from regular garlic and is popular due to its low cost and minimal side effects. In both *in vitro* and *in vivo* antioxidant tests, black garlic extract exhibited dose-dependent free radical scavenging activity. Black garlic significantly extended the lifespan of *Drosophila melanogaster*, increased the activities of superoxide dismutase (SOD), including CuZn-SOD and Mn-SOD, and catalase (CAT) as its concentration increased, and lowered MDA levels. These findings suggested that black garlic extract may prolong the lifespan of *Drosophila melanogaster* by activating SOD and CAT and reducing MDA production (Lei et al., 2014).

Black garlic comprises slightly different compounds compared to white garlic. Fermentation conducted at temperatures between 60-90°C induces the conversion of γ-glutamyl-S-allylcysteine to S-allyl cysteine, resulting in a 4-8 times increase compared to fresh garlic (Chae et al., 2023). Black garlic contains 106.1 mg/kg of S-allyl cysteine, which

To cite this paper: Ibrahim, Yerizel E, Endrinaldi, and Revilla G (2025). Effects of Black Garlic (*Allium sativum*) Supplementation on Oxidative Stress and Leptin Level in Male Obese Rats. *World Vet. J.*, 15(3): 774-781. DOI: https://dx.doi.org/10.54203/scil.2025.wvj79

is five times higher than the S-allyl cysteine content of white garlic, measured at 21.0 mg/kg (Lee et al., 2009; Zhao et al., 2021). This fermentation process triggers numerous chemical reactions in garlic, including enzymatic browning and Maillard reactions, which cause the color to change from white and yellow to dark brown (Lei et al., 2014; Ha and Kim, 2017). Studies on black garlic and its components against different diseases have been extensively conducted in recent years. However, investigations to establish the therapeutic effects of black garlic remain substantially limited. Accordingly, the present study aimed to evaluate the impacts of black garlic supplementation on oxidative stress markers and leptin levels in male Wistar rats with obesity.

MATERIALS AND METHODS

Ethical approval

The present study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Andalas University, Indonesia, and in accordance with ethical standards in medical research (ethics permit number: 520/UN.16.2/KEP-FK/2024, issued on October 4, 2024).

Animals

The present study was conducted in the animal house at the Biomedical Laboratory, Faculty of Medicine, Andalas University, Indonesia. Thirty-five Wistar rats, aged 10-12 weeks and weighing 200 g, were obtained from the Biomedical Laboratory of Andalas University, Indonesia. The rats were kept in a controlled environment with a 12-hour light-dark cycle, a room temperature of 22 ± 4°C, and a humidity level of 5 ± 10%. Each rat was allocated 125 cm^2 of floor space (approximately 49.2 in^2) within a cage measuring 14 cm (5.5 inches) in height. During a seven-day adaptation period, the rats were given unlimited access to water and commercial pellet feed. The commercial pellet feed contained a metabolizable energy of 3.4 kcal/g, comprising 60% carbohydrate, 20% protein, 10% fat, and 6% fiber.

Plant collection and identification

Black garlic is a local Indonesian variety indigenous to Bogor Regency, West Java, and its surrounding regions. The black garlic is produced by fermenting *Allium sativum* garlic for 21 days at a temperature of 70°C and a humidity of 80%. The black garlic utilized in the present study was a product of Serambi Botani IPB, MD 655628, and was weighed according to the respective doses for each rat, namely 200 mg, 400 mg, and 800 mg. The black garlic was ground into a smooth paste and mixed with distilled water to facilitate administration to the rats.

Experimental design

Creating an obesity model in rats

Male Wistar rats weighing 200 g were fed a high-fat diet (HFD; 2 mL goat oil and 1 mL egg yolk) for 12 weeks, administered via a tube every morning between 9:00 and 10:00 a.m. After 16 weeks, the body weight and body length of the rats were measured to determine the Lee obesity index in rats, which is similar to BMI in humans. Rats with a Lee index of 0.300 or higher were considered obese (Fajrani et al., 2021).

Experiment procedure

Based on the sample calculation utilizing Federer's (1991) formula, the minimum sample size required was five for each treatment group. Twenty-five rats were divided into five groups, each consisting of five rats, including the negative control group (K1), which received a regular diet with distilled water, the positive control group (K2), which received an HFD with distilled water, Treatment group 1 (P1), which received an HFD and a black garlic at a dose of 200 mg/rat, Treatment group 2 (P2), which received an HFD and a black garlic at a dose of 400 mg/rat, and Treatment group 3 (P3), which received an HFD and a black garlic at a dose of 800 mg/rat. These treatments were administered orally for 28 days. Body weight, body length, and activity levels of rats were monitored weekly. The body weight of male rats was assessed utilizing a precision digital scale (Sartorius, Germany), while the body length was determined with an animal measuring board. Body length was measured from the nose to the anus using a tape measure after administering chloroform anesthesia to the rats (Fajrani et al., 2021). To measure activity, the rats were observed for aggressive behavior and sedentary behavior.

Malondialdehyde and leptin analysis

The MDA and leptin parameters were measured on the final day of the experiment. Blood samples were collected from the orbital sinuses of the rats and then centrifuged to separate plasma and serum. The serum was utilized to determine the MDA and leptin levels. The MDA levels were analyzed using enzymatic samples (UV-Vis Spectrophotometry, Italy) at a wavelength of 532 nm, and the results were calculated based on a curve equation, yielding

MDA levels expressed in nmol/mL (Fajrani et al., 2021). Additionally, leptin hormone levels were assessed using serum samples via the enzyme-linked immunosorbent assay (ELISA). The ELISA leptin assay kit, specifically the Sandwich-ELISA rat leptin (Elabscience®, Catalogue number: E-EL-R0582, England), was employed, and the results indicated leptin levels as expressed in ng/mL.

Statistical analysis

The present data are presented as mean ± standard deviation (SD). Since the data for MDA and leptin were not normally distributed, the Kruskal-Wallis test was used to find significant differences ($p < 0.05$) between the control and treatment groups. Statistical analysis was done with IBM SPSS Statistics version 22.0.

RESULTS

Table 1 displays that there was a significant difference in body weight in Wistar rats fed HFD for 16 weeks ($p < 0.05$). The body weight was the highest in P3 (326.80 ± 3.768) and the lowest in the negative control group (240.20 ± 12.194). In addition, the body weights were 319.20 ± 4.658, 318.20 ± 4.919, and 325.00 ± 5.244 in the positive control group, P1, and P2, respectively. After 28 days of feeding an HFD supplemented with black garlic, the body weight in Group P3 was lower (300.20 ± 14.923) than that of the positive control group (327.80 ± 6.686), Group P1 (307.20 ± 7.225), and Group P2 (310.00 ± 9.935). However, the concentration was still higher than the negative control group (254.80 ± 12.677). A significant difference was observed among the negative control group, the positive control group, and groups P1, P2, and P3 ($p < 0.05$; Table 2).

Table 3 illustrates the levels of leptin hormone following the administration of an HFD supplemented with black garlic over a period of 28 days in Wistar rats. In Group P3, the leptin level was observed to be lower (1.12560 ± 0.160120) in comparison to the negative control group (1.51440 ± 0.241909), the positive control group (3.69020 ± 2.372940), Group P1 (1.41720 ± 0.238614), and Group P2 (1.54500 ± 0.195533). A statistically significant difference was identified among the negative control group, the positive control group, and groups P1, P2, and P3 ($p < 0.05$).

The Effects of black garlic on MDA levels in Wistar rats at doses of 200 mg/rat, 400 mg/rat, and 800 mg/rat given for 28 days are illustrated in Table 4. The MDA levels observed after administering an HFD enriched with black garlic for 28 days indicated that Group P3 exhibited reduced MDA levels (2.2320 ± 0.24793) in comparison to the positive control group (3.9760 ± 0.38927), Group P1 (2.5280 ± 0.16514), and Group P2 (2.4280 ± 0.12337). Nonetheless, these levels remained higher than those observed in the negative control group (1.9300 ± 0.06364). Significant differences were observed among the negative control group, the positive control group, and groups P1, P2, and P3 ($p < 0.05$).

Table 1. Effects of feeding a high-fat diet for 16 weeks on body weight in Wistar rats

Group	Rats (Number)	Minimum body weight (g)	Maximum body weight (g)	Mean ± SD Body weight of rats (g)
Negative control	5	221	253	240.20 ± 12.194
Positive control	5	314	326	319.20 ± 4.658
Treatment 1	5	312	324	318.20 ± 4.919
Treatment 2	5	319	332	325.00 ± 5.244
Treatment 3	5	321	331	326.80 ± 3.768
Total	25	221	332	305.88 ± 34.264
Significant				0.002

Data are expressed as minimum, maximum, mean ± Standard deviation (SD). Negative control: Rats that received regular diet with distilled water, positive control: Rats that received an HFD with distilled water, Treatment 1: An HFD and a black garlic at a dose of 200 mg/rat, Treatment 2: An HFD and a black garlic at a dose of 400 mg/rat, Treatment 3: An HFD and a black garlic at a dose of 800 mg/rat.

Table 2. Effects of black garlic on body weight in Wistar rats at doses of 200 mg/rat, 400 mg/rat, and 800 mg/rat given for 28 days

Group	Rats (Number)	Minimum body weight (g)	Maximum body weight (g)	Mean ± SD Body weight of rats (g)
Negative control	5	235	266	254.80 ± 12.677
Positive control	5	320	336	327.80 ± 6.686
Treatment 1	5	297	315	307.20 ± 7.225
Treatment 2	5	298	321	310.00 ± 9.935
Treatment 3	5	279	316	300.20 ± 14.923
Total	25	235	336	300.04 ± 26.751
Significant				0.001

Data are expressed as minimum, maximum, mean ± Standard deviation (SD). Negative control: Rats that received regular diet with distilled water, positive control: Rats that received an HFD with distilled water, Treatment 1: An HFD and a black garlic at a dose of 200 mg/rat, Treatment 2: An HFD and a black garlic at a dose of 400 mg/rat, Treatment 3: An HFD and a black garlic at a dose of 800 mg/rat.

Table 3. Effects of black garlic on leptin levels in Wistar rats at doses of 200 mg/rat, 400 mg/rat, and 800 mg/rat given for 28 days

Group	Rats (Number)	Minimum level of leptin	Maximum level of leptin	Mean ± SD Leptin hormone (ng/mL)
Negative control	5	1.139	1.763	1.51440 ± 0.241909
Positive control	5	1.964	7.859	3.69020 ± 2.372940
Treatment 1	5	1.134	1.790	1.41720 ± 0.238614
Treatment 2	5	1.294	1.728	1.54500 ± 0.195533
Treatment 3	5	0.955	1.383	1.12560 ± 0.160120
Total	25	0.955	7.859	1.85848 ± 1.365638
Significant				0.008

Data are expressed as minimum, maximum, and Mean ± standard deviation (SD). Negative control: Rats that received regular diet with distilled water, positive control: Rats that received an HFD with distilled water, Treatment 1: An HFD and a black garlic at a dose of 200 mg/rat, Treatment 2: An HFD and a black garlic at a dose of 400 mg/rat, Treatment 3: An HFD and a black garlic at a dose of 800 mg/rat.

Table 4. Effects of black garlic on malondialdehyde levels in Wistar rats at doses of 200 mg/rat, 400 mg/rat, and 800 mg/rat given for 28 days

Group	Rats (Number)	Minimum level of MDA	Maximum level of MDA	Mean ± SD MDA (nmol/mL)
Negative control	5	1.85	2.02	1.9300 ± 0.06364
Positive control	5	3.59	4.58	3.9760 ± 0.38927
Treatment 1	5	2.34	2.72	2.5280 ± 0.16514
Treatment 2	5	2.29	2.61	2.4280 ± 0.12337
Treatment 3	5	1.85	2.45	2.2320 ± 0.24793
Total	25	1.85	4.58	2.6188 ± 0.75254
Significant				0.001

Data are expressed as minimum, maximum, and Mean ± Standard deviation (SD). MDA: Malondialdehyde, Negative control: Rats that received regular diet with distilled water, Positive control: Rats that received an HFD with distilled water, Treatment 1: An HFD and a black garlic at a dose of 200 mg/rat, Treatment 2: An HFD and a black garlic at a dose of 400 mg/rat, Treatment 3: An HFD and a black garlic at a dose of 800 mg/rat.

DISCUSSION

An HFD is a dietary regimen in which the majority of daily caloric intake is derived from fat, typically contributing around 40-60% of total energy over a period of 8-16 weeks. Obesity is defined as the excessive accumulation of adipose tissue (Panuganti et al., 2023). In numerous cases involving obesity, leptin levels are elevated concurrently with the development of leptin resistance (Sharebiani et al., 2023). Additionally, obesity is associated with heightened systemic oxidative stress, which results from an imbalance between free radicals and antioxidants, often marked by increased MDA levels (Ashar, 2023). In the study conducted by Chang et al. (2017), rats administered an HFD comprising 2 mL of goat fat per 200 g of body weight and 1 mL of egg yolk per 200 g of body weight over a period of 16 weeks via oral gavage exhibited an approximate 62.9% increase in body weight. Similar findings have been reported in a study conducted by Kim et al. (2017), which demonstrated that a diet comprising 60% fat, administered over six weeks, induced obesity in rats. Additionally, an HFD consisting of 45% kcal over ten weeks resulted in notable weight gain. Likewise, Chae et al. (2023) induced obesity in animals using an HFD at 60% over 16 weeks. Jahan et al. (2024) reported weight gain in rats fed a combination of 90% standard pellets and 10% goat fat oil. Sukmawati and Asgap (2017) developed a hyperlipidemia and obesity model using an HFD comprising 50% goat fat mixed with butter, coconut oil, and cholesterol. Furthermore, Maigoda et al. (2016) confirmed the efficacy of goat fat mixtures in inducing obesity. Additionally, Yu et al. (2019) demonstrated that prolonged consumption of egg yolk resulted in increased adiposity in rats.

A study by Nasution (2023) revealed that Sprague-Dawley rats aged 20-24 weeks and weighing in the range of 250 to 300 g, when subjected to a diet comprising a mixture of beef fat, egg yolk, and palm oil for a duration of 30 days, demonstrated a considerable increase in weight gain in comparison to the control groups. Additionally, these subjects exhibited elevated levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL), alongside a reduction in high-density lipoprotein. Aguila et al. (2003) observed that rats fed egg yolks experienced an average weight gain of 32.6 ± 1.3 g over four weeks, surpassing the gains observed in rats fed oats (28.6 g) or wheat bran (25.7 g). Similarly, Omole and Ighodaro (2013) reported that mice subjected to an HFD gained approximately 40% more body weight, along with an increase in total cholesterol by 30%, triglycerides by 11.9%, and LDL from 56.9 to 81 mg/dL. Yu et al. (2019) investigated the effects of a diet incorporating egg yolk powder combined with vanadium, where the HFD (containing 301.2 g per kg of ether extract derived from egg yolk) over five weeks remarkably elevated body weight and markers of oxidative stress. Feeding rats utilizing an HFD was linked to the development of obesity, caused by excessive energy intake and disrupted regulation of satiety and hunger signals, which subsequently led to leptin resistance and chronic inflammation.

Black garlic is a fermented form of garlic characterized by a soft, savory texture, mild aroma, slightly sweet or tangy flavor, and a chewy or jelly-like consistency (Choi et al., 2014; Sukrianto et al., 2022). According to Sailah (2021), raw garlic can be processed through fermentation or prolonged heating. The fermentation process is most effective at a temperature of 70-80°C with a controlled humidity of 70-90% for 21 days, without adding any extra ingredients (Lei et al., 2014). This process leads to an increase in total acid content, amino nitrogen, polyphenols, flavonoids, and certain bioactive compounds such as lactic acid, gly-pro-glu, sorbase, and 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-propanoate (CEHC), while reducing 5-hydroxymethyl furfural levels and enhancing grassy, floral, and fruity aromas (Si et al., 2019). During the present study, rats fed an HFD and supplemented with black garlic for 28 days exhibited different results across the different treatment groups. The rats that received 800 mg of black garlic per rat demonstrated a lower body weight compared to the positive control group and the group receiving 400 mg per rat. The present results support the findings of other studies that highlighted the anti-obesity properties of black garlic. Chang et al. (2017) reported that black garlic supplementation improved HFD-induced obesity, while Chae et al. (2023) demonstrated that aged black garlic mitigates obesity and prevents muscle atrophy. Xu et al. (2018) found that aged garlic extract improved inflammatory and immune responses in obese adults, and Lei et al. (2014) confirmed its role in reducing oxidative stress. Similarly, Kimura et al. (2017) analyzed saccharides and antioxidant activity in black garlic, linking them to weight reduction. In contrast, Sembiring and Iskandar (2019) emphasized the antioxidant and anti-inflammatory effects of black garlic in decreasing both weight and oxidative stress. Other studies, by Nasr and Saleh (2014) and Si et al. (2019), confirmed its pharmacological activity as an antioxidant and anti-obesity agent. Additionally, Ahmed and Wang (2021) demonstrated that black garlic supplementation reduced body weight by 18% and fat mass by 44-63% in obese mice. Also, Chang et al. (2017) exhibited that Wistar rats fed an HFD supplemented with 0.2%, 0.6%, or 1.2% black garlic for six weeks demonstrated significant weight reductions of 4.8-8.9% compared to unsupplemented HFD rats. The beneficial effects of black garlic are attributed to its high antioxidant content, including S-allylcysteine, flavonoids, and polyphenols, which lower oxidative stress and elevate adiponectin levels, a protective hormone secreted by adipose tissue (Setiawan et al., 2021). Mechanistically, black garlic modulates fat metabolism by downregulating the expression of fatty acid synthase (FAS) and inflammatory cytokines, such as interleukin-6 (IL-6; Xu et al., 2018), while increasing the activity of lipolytic enzymes, including ATGL, CPT-1, and HSL, in adipose tissue and the liver. Furthermore, black garlic inhibits lipogenesis by downregulating genes such as SREBP-1c, ACC, FAS, G6PDH, HMG-CoA reductase, and ACAT, while concurrently upregulating CPT-1 expression, thereby promoting fatty acid oxidation (Ha et al., 2015; Lestari and Santika, 2023).

Leptin is a protein hormone composed of 167 amino acids, primarily secreted by adipose tissue (Amos et al., 2017; Nasution, 2023), acting on the central nervous system (CNS) to suppress appetite and increase energy expenditure (Amor et al., 2019; Kim and Kim, 2023). In the present study, supplementation with 800 mg/rat of black garlic resulted in the most significant reduction in leptin levels in rats fed an HFD. These findings are consistent with those of Chae et al. (2023), who reported that black garlic supplementation reduced obesity in HFD-fed mice and lowered leptin levels. Similarly, Chang et al. (2017) indicated that black garlic improved leptin regulation in obese mice, while Xu et al. (2018) demonstrated that aged garlic extract reduced inflammation and modulated leptin and other adipokines in obese individuals. Additionally, Amor et al. (2019) confirmed that HFD-fed mice supplemented with aged black garlic exhibited a marked decrease in plasma leptin concentrations from 19.4 to 9.4 ng/dL.

Moreover, black garlic enhances leptin sensitivity, primarily due to its high Sulfur-containing compounds such as S-allylcysteine and other antioxidant compounds, which reduce oxidative stress and inflammation in the hypothalamus (Amor et al., 2019; Halim and Suzan, 2020). Given that hypothalamic inflammation contributes to leptin resistance, its reduction improved leptin responsiveness and signaling efficiency. Additionally, black garlic has demonstrated effects in modulating the gut microbiota by reducing lipopolysaccharide (LPS), a principal inducer of systemic inflammation. The reduction of LPS levels combined with an increase in short-chain fatty acids contributes to enhanced leptin sensitivity and a decrease in leptin resistance (Ha et al., 2015; Amor et al., 2019; Kim and Kim, 2023; Morawati et al., 2024). Numerous individuals with obesity exhibited high leptin levels without the anticipated decrease in fat mass, which signifies leptin resistance. This condition occurs when increased leptin, caused by greater adiposity, fails to stimulate adequate satiety or energy expenditure. Leptin resistance is frequently linked to chronic inflammation in adipose tissue and the brain. Common signs of leptin resistance include lower levels of soluble leptin receptors, decreased protein-bound leptin, and higher levels of circulating free leptin (Subarjati and Nuryanto, 2015; Halim and Suzan, 2020; Amudi et al., 2021). Black garlic possesses antioxidant and anti-inflammatory properties that aid in leptin resistance by inhibiting the release of pro-inflammatory cytokines such as TNF-α and IL-6, which are recognized for their role in impairing leptin signaling (Salsabila and Busman, 2021). Overall, leptin is crucial in regulating body weight and lipid metabolism. Black garlic directly enhances leptin sensitivity by reducing oxidative stress, inflammation, and regulating fat metabolism, helping to alleviate leptin resistance and support metabolic homeostasis (Amor et al., 2019; Nasution, 2023).

Malondialdehyde, with the chemical formula $CH_2(CHO)_2$, is a reactive dialdehyde widely recognized as a reliable marker of oxidative stress. It is formed as a terminal product of lipid peroxidation, a process that damages cell membranes. Given that MDA is chemically more stable than numerous other peroxidation byproducts, it is frequently employed in experiments as a marker of oxidative damage (Mulianto, 2020; Huang et al., 2023; Widhiani et al., 2023). An HFD facilitates the production of ROS, thereby accelerating lipid peroxidation and elevating MDA levels, which serve as indicators of oxidative stress. Black garlic, rich in antioxidant and anti-inflammatory compounds, can neutralize ROS, decrease oxidative stress, reduce MDA concentrations, and promote cellular health repair. In the present study, rats given the highest dose (800 mg/rat) exhibited significantly lower MDA values compared to the positive control group and other treatment groups. These findings are consistent with the study conducted by Saravanan and Ponmurugan (2010), which demonstrated that S-allyl cysteine, a key component of garlic and black garlic, decreased MDA and oxidative stress in diabetic rats. Si et al. (2019) highlighted the strong antioxidant potential of black garlic in lowering MDA, while Chae et al. (2023) confirmed its ability to mitigate oxidative stress biomarkers. Chang et al. (2017) demonstrated that black garlic supplementation reduced hepatic oxidative stress in obese rats, and Ha and Kim (2017) reported that supplementation up to 1.5% significantly inhibited lipid peroxidation. Furthermore, Ahmed and Wang (2021) found that garlic fermentation reduced MDA levels, supporting its role as a potent antioxidant intervention. Obesity in the United States has been linked to elevated oxidative stress. According to a study by Lee et al. (2009), MDA levels in the control group were reported as 2.33 ± 0.36. In contrast, rats receiving a diet supplemented with 5% black garlic exhibited notably lower levels at 1.32 ± 0.26, representing an approximate reduction of 43.3%. The reduction in MDA with black garlic supplementation is attributed to its S-allylcysteine, flavonoids, polyphenols, and other organosulfur constituents, which act as scavengers of reactive oxygen and nitrogen species (ROS and RNS). By neutralizing these radicals, black garlic suppresses lipid peroxidation, thereby lowering MDA concentrations (Lee et al., 2009; Dampati and Veronica, 2020; Sukrianto et al., 2022). Black garlic enhances the function of superoxide dismutase, which converts superoxide into hydrogen peroxide (H_2O_2), and CAT and glutathione peroxidase, which break down H_2O_2 into water. This enzymatic defense prevents the formation of hydroxyl radicals, one of the most damaging reactive species responsible for lipid peroxidation. Furthermore, black garlic reduces inflammation and inhibits NF-κB signaling (Xu et al., 2018; Salsabila and Busman, 2021). Chronic inflammation contributes to increased ROS through macrophage activation and NF-κB pathway stimulation. By suppressing pro-inflammatory cytokines such as IL-6 and TNF-α, black garlic lowers ROS generation from inflammatory processes, protecting lipids from oxidative damage, and reducing MDA formation (Lei et al., 2014; Kusriani et al., 2022; Morawati et al., 2024).

CONCLUSION

In the present study, leptin levels were assessed after 28 days of administering an HFD supplemented with black garlic at doses of 200 mg, 400 mg, and 800 mg per rat. The present results suggested that black garlic effectively contributes to weight reduction, lowers leptin concentrations, and decreases MDA levels as an indicator of oxidative stress. The 800 mg/rat dose resulted in a significantly greater reduction compared to the positive control group that received only an HFD. A larger sample size is required to enhance the statistical power and generalizability of the findings. Utilizing animals of different sexes or species could yield a more comprehensive understanding of the effects of black garlic. Furthermore, testing different dosages and determining the optimal dose of administering black garlic are essential to establish the boundaries of efficacy and safety.

DECLARATIONS

Acknowledgments

The authors express their gratitude to the Department of Biomedical Laboratory and the Biochemistry Laboratory, Faculty of Medicine, Andalas University, Indonesia, for their assistance with sample analysis.

Authors' contributions

Ibrahim, Eti Yerizel, Endrinaldi, and Gusti Revilla were responsible for conceptualizing the article. Ibrahim collected the data and the manuscript, while Eti Yerizel reviewed it. All authors considered and agreed on the final edition of the manuscript for publication.

Funding

The present study was privately funded by the authors.

Availability of data and materials
The data are available upon reasonable request from the corresponding author.

Competing interests
The authors declared no conflicts of interest.

Ethical considerations
Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

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