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A Comparative Study on the Effects of Dietary Supplementation with Garlic (*Allium Sativum*) and Clove (*Syzygium Aromaticum*) on Ghrelin Levels and Some Physiological Parameters

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Abstract

This study focused on evaluating the effects of garlic powder and clove powder supplementation on ghrelin levels, body weight, haematological parameters, and metabolic markers in male rats. Methods: Thirty adult male Swiss albino rats were divided into three groups and studied over two months. Group 1 (control) received a standard rat diet, Group 2 was supplemented with 5% garlic powder in their standard diet, and Group 3 received clove powder at a daily dosage of 30 mg/kg body weight in their diet. At the end of the experiment, blood samples were collected, and the rats' final body weights were measured. Garlic supplementation significantly reduced ghrelin levels ($p \leq 0.05$), whereas clove supplementation resulted in a significant increase ($p \leq 0.05$) relative to the control group. Body weight analysis showed a significant increase in the clove group from the fourth week onward, while the garlic group exhibited a significant decrease from the first week. Haematological analysis demonstrated a notable elevation in RBC count, WBC count, and haemoglobin concentration in both treatment groups, while platelet count decreased significantly. Additionally, cholesterol and blood glucose levels significantly decreased in both groups, with triglyceride levels notably lower in the garlic group. These findings suggest that garlic and clove supplementation have contrasting effects on ghrelin levels and body weight while exerting beneficial influences on haematological and metabolic parameters.

Keywords: Ghrelin; Garlic; Clove; Body weight; Haematological parameters; Metabolic markers.

1. Introduction

Ghrelin is a gastric-derived peptide hormone with appetite-inducing and growth hormone-releasing activities [1]. While it is mainly secreted by the stomach mucosa, ghrelin is additionally found in various other tissues, including the lungs, hypothalamus, liver, and kidneys [2].

Ghrelin has a crucial role in appetite regulation and energy homeostasis [3]. It primarily signals hunger to the brain and directly influences feeding behaviour. Ghrelin, primarily secreted by endocrine cells in the stomach, travels through the bloodstream to the hypothalamus, specifically targeting the arcuate nucleus, which contains neurons responsible for regulating feeding [4]. Ghrelin activates orexigenic neurons, such as those expressing agouti-related peptide (AgRP) and neuropeptide Y (NPY), to stimulate hunger and promote food intake [5]. Simultaneously, it inhibits anorexigenic signals from pro-opiomelanocortin (POMC) neurons, which are responsible for suppressing appetite [6].

The use of medicinal plants has received significant attention because of their health benefits and natural healing properties [7], [8]. One such plant is garlic [9]. Garlic (Allium sativum) is a herbaceous plant that belongs to the Amaryllidaceae family and is well-known for both its culinary and medicinal uses [10]. It contains at least 33 sulphur-containing compounds, such as allicin, which contribute to its characteristic aroma and health-promoting properties [11], [12]. Additionally, garlic contains fructooligosaccharides (FOS), primarily in the form of inulin and oligofructose, which are dietary fibres predominantly fermented in the caecum and colon [13]. These naturally occurring prebiotic fibres can help suppress appetite by reducing ghrelin levels and enhancing the secretion of anorexigenic gut hormones, including peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) [14], [15]. In garlic, these FOS compounds can make up approximately 9% to 16% of its dry weight, contributing to its prebiotic and metabolic effects [16]. This can lead to a decrease in food consumption, enhanced satiety, and a decrease in overall caloric consumption [17], [18].

Garlic has been investigated for its potential pharmacological effects, which include antimicrobial, antifungal, antiparasitic, immune-boosting, antioxidant, and cardioprotective properties [19], [20]. It has been shown to contribute to blood pressure regulation [21], blood glucose level management [22], inhibition of platelet aggregation, reduction of hyperlipidaemia, and prevention of atherosclerosis in the arterial system [23].

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Another example of a medicinal plant is clove (Syzygium aromaticum), a spice obtained from the desiccated flower buds of the clove tree, which belongs to the Myrtaceae family [24]. Clove has a distinctive nail-like shape and is native to the North Maluku Islands in Indonesia, where it has been traditionally used as a natural remedy for treating various ailments, including dental diseases [25]. Research has shown that clove possesses antifungal, anti-allergic, antimicrobial, and anti-mutagenic properties [26], [27]. Additionally, clove is known to contain a substance similar to ghrelin, which may have comparable physiological effects, including appetite stimulation, energy balance regulation, and the promotion of growth hormone release, similar to those of endogenous ghrelin [28].

The present study sought to assess the effects of garlic and clove supplementation on ghrelin levels, as well as certain haematological and metabolic parameters in male rats.

2. Material and methods

2.1. Animals and experimental design

Thirty adult male Swiss rats, with body weights ranging from 160 to 180 grams, were procured from the Medical Entomology Research Institute in Giza, Egypt. The rats were housed in standardized cages with natural ventilation, maintained under a 12-hour light/dark cycle at a controlled temperature of 23 ± 3°C. To allow acclimatization to the laboratory environment, the rats were provided with a two-week adjustment period during which they had ad libitum access to food and water. The rats were provided with a standard rodent diet formulated with precisely calculated nutritional components, including L-cystine (3 g/kg), casein (200 g/kg), cellulose (50 g/kg), sucrose (500 mg/kg), corn starch (150 g/kg), corn oil (50 g/kg), vitamin mix V10001 (10 g/kg), and mineral mix S10001. The study was carried out in the Physiology Department of the Faculty of Veterinary Medicine at Cairo University, following approval from the Institutional Animal Care and Use Committee (IACUC) of the same faculty (Approval Number: Vet Cu131020241054).

The rats were randomly assigned to into three groups, each comprising 10 animals (n = 10). Group 1 (Control Group) received a standard rat diet. Group 2 (Garlic Powder Supplement Group) was fed a standard rat diet supplemented with 5% garlic powder [29]. Group 3 (Clove Powder Supplement Group) was given a standard rat diet enriched with clove flower powder at a daily dosage of 30 mg/kg body weight in their diet. The experiment was carried out for a duration of two months [30].

2.2. Chemical composition of Garlic and Clove

Garlic (*Allium sativum*) is rich in bioactive compounds, with a chemical composition that includes 60–65% carbohydrates, mainly inulin and oligofructose (9–16%) as prebiotic fibres, 10–15% protein, and about 0.5% fat. It contains 1–3% sulphur compounds, such as alliin (0.5–1.8%) and allicin (0.4–0.5%), along with essential minerals (1–2%) and vitamins like C and B6. Garlic also has 0.2–1% phenolic compounds [16], [31], [32].

Clove (*Syzygium aromaticum*) consists of essential oils (15–20%), primarily eugenol (70–85%), along with β-caryophyllene (5–12%), acetyl eugenol, and vanillin. It contains carbohydrates (30–50%), fiber (10–20%), proteins (5–10%), and fats (8–15%), along with essential minerals (1–3%) [33]. Notably, it contains a ghrelin-like substance at 4070.75 pg/mg, with levels exceeding those found in human salivary glands, rat kidneys, human kidneys, and human stomachs [28].

2.3. Preparation of Garlic and Clove powder

Garlic powder and dried clove flowers were purchased from a local market (Harraz, Egypt). For Group 2, garlic powder was incorporated into the standard rat diet at a concentration of 5% (50 g/kg of diet). Meanwhile, for Group 3, dried clove flowers were coarsely ground using a grinding machine and administered daily at a dose of 30 mg/kg body weight.

2.4. Body weight

The rats were weighed weekly to monitor changes in body weight. To ensure accurate measurements, access to feed and water was restricted for 12 hours prior to weighing.

2.5. Sampling

At the end of the experiment, final body weights were recorded, and fasting blood samples were collected from each rat using the orbital sinus puncture method after anaesthesia with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) [34]. Blood samples intended for serum analysis were collected without anticoagulant, allowed to clot, and the resulting serum was stored at -20°C for biochemical and hormonal analysis. Extra blood samples were collected in EDTA (0.1 mg/5 ml of blood) for haematological analysis and in fluoride tubes for blood glucose assessment. After sample collection, all rats were humanely euthanized under anaesthesia.

2.6. Hormones assay

Ghrelin levels in the serum were determined through a double-antibody immunoassay using ELISA kits provided by LEADER TRADE Company in Egypt.

2.7. Haematological examination

The red blood cell count (RBCs), haemoglobin concentration (Hb), white blood cell count (WBCs), and platelet count were measured using a Coulter counter (automated haematology analyser) [35].

2.8. Biochemical parameters

Serum samples were used to measure cholesterol, triglyceride, and glucose levels using diagnostic kits obtained from Biodiagnostic Company, Egypt. The parameters were assessed using a UV spectrophotometer.

2.9. Statistical analysis

Data analysis was conducted using SPSS statistical software, version 11.0 (SPSS, 2001, Chicago, IL, USA). Results were presented as the mean ± standard error of the mean (SEM). To determine significant differences between groups, a one-way ANOVA was utilized and Tukey's test was applied, with statistical significance defined at $p \leq 0.05$.

3. Results

3.1. The Effects on Ghrelin Levels.

The results presented in Figure (I) indicate that garlic supplementation led to a significant reduction ($p\leq0.05$) in ghrelin levels. Conversely, clove powder led to a significant elevation ($P \leq 0.05$) in ghrelin levels relative to the control group.

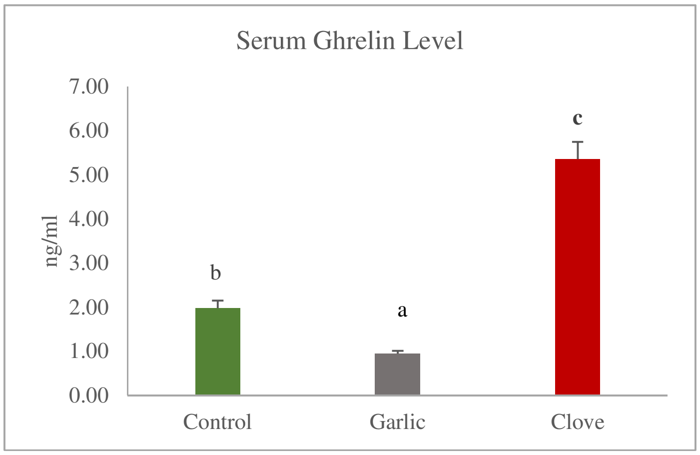

Figure I: The effects of garlic and clove supplementations on serum ghrelin level in adult male rats. The data are presented as mean ± SEM (n = 5 rats per group). Values with different letters (a, b, c) indicate statistically significant differences at $p \leq 0.05$.

3.2. The effects on body weight

The data presented in Table (I) indicate a significant increase ($P \leq 0.05$) in body weight in the clove powder group compared to the control group, beginning in the fourth week of the study. Additionally, the clove powder group demonstrated a higher body weight than the garlic group starting from the second week. Conversely, the garlic group showed a significant reduction ($P \leq 0.05$) in body weight relative to the control group from the first week of the experiment.

Table I: The changes in body weight in grams during the experiment.

Age (wks.) \ Groups		Control (Group 1)	Garlic (Group 2)	Clove (Group 3)
1st month	Initial body weight	162.51±0.88[a]	162.35±1.09[a]	162.23±0.65[a]
	Week 1	166.51±0.79[b]	161.90±1.11[a]	163.85±0.51[ab]
	Week 2	171.41±0.64[b]	158.79±1.12[a]	171.26±0.69[b]
	Week 3	175.29±1.37[b]	155.77±1.06[a]	178.93±1.06[b]
	Week 4	179.65±1.26[b]	153.44±1.26[a]	186.89±1.44[c]
2nd month	Week 5	184.36±1.08[b]	150.61±1.07[a]	194.71±1.90[c]
	Week 6	189.70±0.87[b]	146.85±1.19[a]	202.93±1.73[c]
	Week 7	194.70±0.82[b]	143.06±1.08[a]	211.68±1.71[c]
	Week 8	199.02±0.62[b]	139.44±1.21[a]	221.24±1.67[c]

The data are presented as mean ± SEM (n = 5 rats per group). Values with different letters (a, b, c) within row indicate statistically significant differences at $p \leq 0.05$.

3.3. The effects on Final Body Weight

The data illustrated in Figure (II) demonstrated a significant decline in the final body weight of the garlic-supplemented group relative to the control group. In contrast, the clove-supplemented group showed a significant increase ($P \leq 0.05$) in final body weight

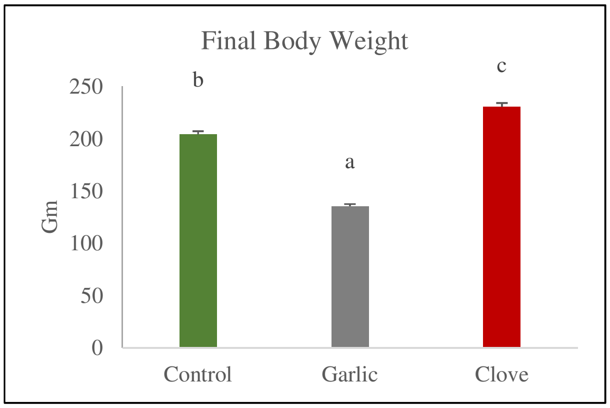

Figure II: The effects of garlic and clove supplementations on some haematological parameters in adult male rats. The data are presented as mean ± SEM (n = 5 rats per group). Values with different letters (a, b, c) indicate statistically significant differences at p ≤ 0.05.

3.4. The effects on Haematological Parameters

The results shown in Figure (III) indicate a significant increase (p≤0.05) in RBC count, WBC count, and haemoglobin concentration in both the garlic-supplemented and clove-supplemented groups. Conversely, both groups demonstrated a significant decline in platelet count relative to the control group.

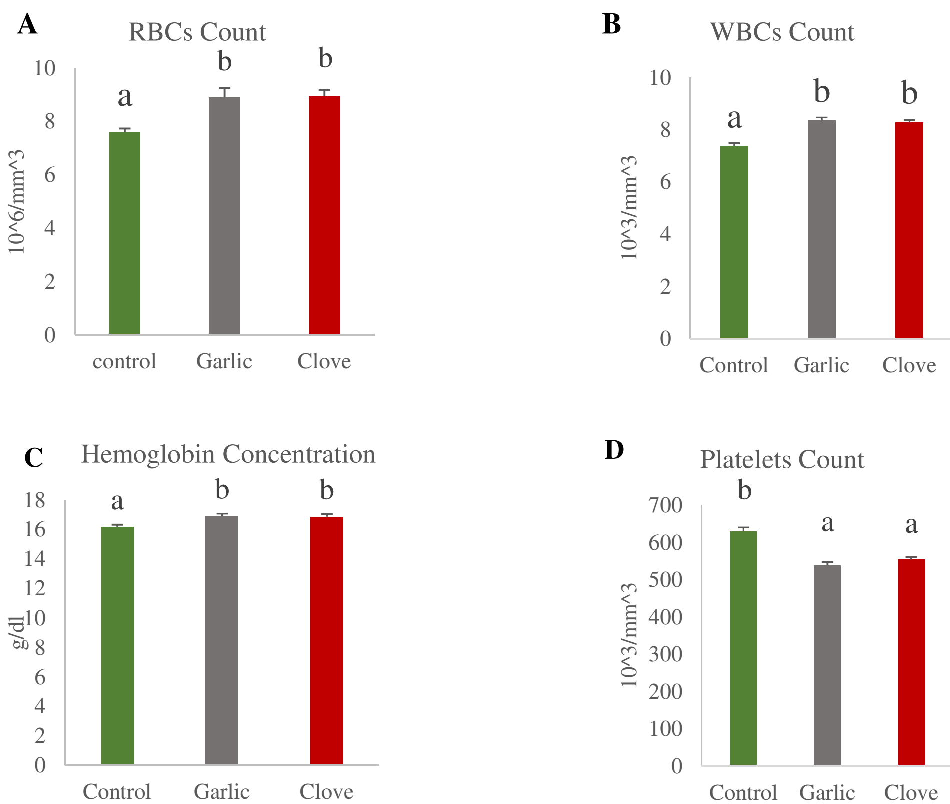

Figure III: The effects of garlic and clove supplementations on some haematological parameters in adult male rats. The data are presented as mean ± SEM (n = 5 rats per group). Values with different letters (a, b, c) indicate statistically significant differences at p ≤ 0.05.

3.5. The effects on cholesterol, triglyceride, and blood glucose levels

The data in Figure (IV-A) demonstrate a significant decrease ($p \leq 0.05$) in cholesterol levels in both the garlic- and clove-supplemented groups relative to the control group. Furthermore, the garlic-supplemented group exhibited a significant decline in triglyceride levels relative to the control. Similarly, the results in Figure (IV-B) reveal a significant decrease in glucose levels in both the garlic- and clove-supplemented groups relative to the control group.

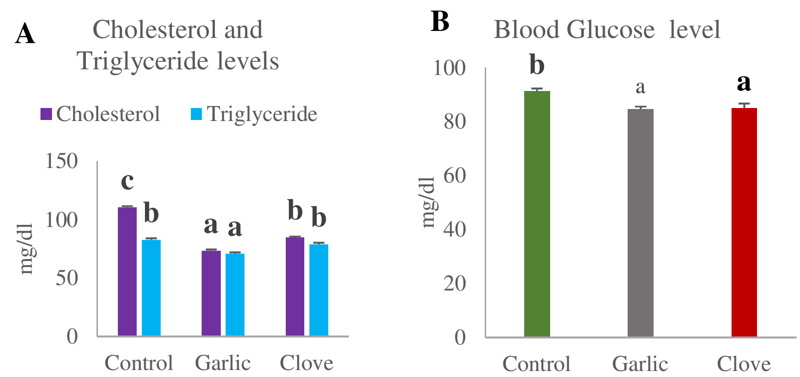

Figure IV: The effects of garlic and clove supplementations on cholesterol, triglyceride and blood glucose level in adult male rats. The data are presented as mean ± SEM (n = 5 rats per group). Values with different letters (a, b, c) indicate statistically significant differences at $p \leq 0.05$.

4. Discussion

This study focused on examining the effects of garlic powder and clove powder supplementation on ghrelin hormone levels. The results indicated a significant reduction in ghrelin levels in the garlic-supplemented group relative to the control group. Additionally, garlic powder supplementation resulted in alterations in feed intake and a reduction in final body weight relative to the control group. Oligofructose and inulin, dietary fibres present in garlic, have been shown to promote weight loss and reduce appetite by lowering ghrelin levels [36] and increasing plasma glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) concentrations [18], [37]. These findings align with a study conducted on adult male rats, which showed that supplementation with 5% or 30% crude garlic mixed with a standard diet resulted in a reduction in final body weight [19]. Additionally, another study found that administering 200 mg/kg of garlic juice for 56 days led to a significant reduction in final body weight [38]. Similarly, a study found that administering oligofructose alone or in combination with inulin to rodents resulted in a reduction in ghrelin levels [15]. Inulin has been associated with weight loss through mechanisms such as reducing caloric intake, modulating hunger hormones, increasing satiety, improving insulin sensitivity and enhancing gut health [39]. It forms a gel-like substance in the digestive tract, which stimulates gut receptors to promote fullness. Clinical trials indicate that inulin and its subgroup, oligofructose, decrease ghrelin (hunger hormone) levels and increase peptide YY (PYY), which suppresses appetite, aiding weight loss [40].

On the other hand, the findings revealed that incorporating clove powder into the rat diet led to a significant elevation in ghrelin hormone levels and final body weight. These results align with a study conducted on female rats, which reported that clove acts as an appetite-stimulating plant containing a ghrelin-like compound that elevates ghrelin levels, thereby increasing appetite and body weight [28]. Studies suggest that clove contains bioactive compounds, such as eugenol and flavonoids, which may mimic ghrelin-like activity or stimulate its secretion [41]. For instance, Hussein et al. (2023) reported that the inclusion of clove oil in broiler diets not only improved growth performance but also increased feed intake and body weight gain, further supporting the appetite-enhancing potential of clove [42]. Similarly, Abdel-Azeem and Abd El-Kader (2022) demonstrated that clove powder supplementation led to increased feed consumption and body weight in rabbits [43]. This consistent pattern across different animal species suggests that clove's appetite-stimulating effects are not species-specific but rather a result of its active compounds promoting ghrelin production, enhancing digestive efficiency, and encouraging food intake. Therefore, the increased body weight observed in clove-supplemented group can be attributed to both elevated appetite and improved feed utilization.

The findings revealed that supplementing the rat diet with garlic and clove resulted in a significant increase in RBC and WBC counts, as well as haemoglobin (Hb) concentration, accompanied by a notable decrease in platelet count. These results align with a study on male rats, which found that administering 200 mg/kg of garlic juice daily for 30 days led to a significant increase in RBC count, haemoglobin (Hb) concentration, and total white blood cell (WBC) count in the garlic-treated groups relative to the control. Additionally, the study found a significant rise in neutrophil, monocyte, and lymphocyte levels, which likely led to the overall increase in total WBC count [44]. Similarly, a study conducted on broiler chickens reported that the inclusion of 0.2% garlic powder significantly increased total WBC count, Hb concentration, and PCV, with no observed changes in RBC count [9]. In contrast, a different rat study found that administering 200 mg of garlic per day did not affect these haematological parameters, while a 100 mg per day dose resulted in a notable elevation in all parameters [45].

Similar to the current findings, Abdel-Azeem and Abd El-Kader (2022) reported that clove powder supplementation resulted in a notable rise in RBC and WBC counts in rabbits [43]. Usman et al. (2021) found that a dose of 1.5 kg of clove powder per 100 kg of feed resulted in a significant elevation in haemoglobin concentration in broilers [46]. In contrast, Naser et al. (2023) reported that adding clove powder to poultry feed did not have a notable effect on total white blood cell (WBC) count [47]. Conversely, a study by Nikoui et al. (2017) found that administering 25 mg/kg of clove oil to dogs significantly reduced the WBC count but did not affect the red blood cell count or haemoglobin concentration [48]. These differences in response could be attributed to the type of clove preparation, dosage, and the species of animal involved.

This study revealed that garlic powder significantly decreased both cholesterol and triglyceride levels, while clove supplementation notably reduced cholesterol but did not markedly impact triglyceride levels relative to the control group. Similarly, a study on rats demonstrated that garlic powder supplementation effectively lowered both cholesterol and triglyceride levels [49]. Garlic lowers cholesterol by suppressing enzymes involved in cholesterol biosynthesis and reducing its absorption in the blood and liver [50]. Inulin supplementation, the prebiotic fibre found in garlic, has been shown to improve metabolic markers, including reducing cholesterol and triglycerides in individuals with obesity [51]. Thomson et al. (2006) found that administering a water-based extract of raw garlic at doses of 50 mg/kg and 500 mg/kg over a four-week period led to a substantial decrease in serum triglyceride and cholesterol levels [52].

Similar to the current findings, a study by Gashlan and Al-Beladi (2016) found that administering high doses of clove oil (600 mg/kg) resulted in a significant decrease in serum triglyceride and cholesterol levels [53]. Balasasirekha and Lakshmi (2012) reported that clove led to a substantial decrease in cholesterol and triglyceride levels in individuals with hyperlipidaemia [54]. Additionally, Abdel-Azeem and Abd El-Kader (2022) reported that in rabbits fed a diet containing 250 mg of cloves per kg, clove powder supplementation resulted in a reduction in total cholesterol levels [43].

This study found that adding garlic powder and clove powder to the diet of rats significantly lowered blood glucose levels. Garlic has been extensively studied for its potential glucose-lowering effects, primarily attributed to active compounds such as allicin and sulphur-containing components [55]. Additionally, inulin contributes to reducing blood glucose levels and improving insulin sensitivity. It slows carbohydrate absorption and prevents blood glucose spikes [39]. A study by Thomson et al. (2006) found that a high dose of garlic extract (500 mg/kg) caused a significant decline in glucose levels [52]. Additionally, research by Nasiri et al. (2017) reported that administering garlic extract orally for 30 days resulted in a reduction in serum glucose concentrations in diabetic rats. Their findings suggest that garlic may enhance insulin secretion from the remaining pancreatic β cells [56] .

Consistent with the present findings, Gashlan and Al-Beladi (2016) observed that a 600 mg/kg dose of clove oil significantly reduced glucose levels in diabetic rats [53]. Similarly, adding 250 mg of cloves per kg of diet led to a significant reduction in serum glucose levels in rabbits [43]. Cloves are rich in antioxidants and bioactive compounds like eugenol, which may help regulate blood glucose levels [57], [58]. The glucose-lowering effect of clove may be attributed to the stimulation of active pancreatic β-cells, which enhances insulin release, or potentially to the regeneration of β-cells. Additionally, eugenol's ability to substantially decrease fasting plasma glucose levels in diabetic rats is thought to result from its potential to promote secretion of insulin from existing islet β-cells and increase glucose uptake by tissues [59].

5. Conclusions

This study demonstrates that dietary supplementation with garlic powder significantly reduces ghrelin levels and body weight in male rats, whereas clove powder supplementation increases ghrelin levels and body weight. Both garlic and clove positively influenced haematological parameters by increasing RBC and WBC counts and haemoglobin concentration, while platelet count decreased. Additionally, both supplements contributed to improved metabolic health by lowering cholesterol and glucose levels. These findings suggest that garlic and clove have potential as natural modulators of appetite and metabolic regulation. Further research is warranted to explore their underlying mechanisms and potential applications in human health.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

This research, including its writing and publication, did not receive financial support from any organization. Additionally, the authors have no relevant financial or non-financial conflicts of interest.

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