



(RESEARCH ARTICLE)



The effect of garlic formulation on some hematological parameters and organ weight of male and female Wister albino rats

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Abstract

The study was conducted to investigate the impact of garlic formulation on certain haematological parameters and organ weights in both male and female rats. Haematological data, including the count of red blood corpuscles (RBC), the volume of packed cells (PCV), the count of white blood corpuscles (WBC), and the count of platelets, were evaluated using a fully automated haematology analyser. The liver, right kidney, left kidney, heart, and spleen of the rats were weighed to determine their respective organ weights. A total of thirty (30) albino Wister rats, consisting of both males and females, were randomly assigned to five groups, with six animals in each group. Group 1 acted as the positive control and was given 100% standard feed. Group II rats were given a mixture of 10% garlic and 90% feed. Group III animals were provided with a diet consisting of 20% garlic and 80% feed. Group IV received a diet containing 40% garlic and 60% feed. The final group, Group V, was fed a diet consisting of 80% garlic and 20% feed daily for a period of 21 days. The feed formulation was assessed at concentrations of 10, 20, 40, and 80 g/dl, based on the weight of the animal. The findings indicated a noteworthy ($P < 0.05$) reduction in white blood cell count (WBC) and lymphocyte count (LYM). There were no statistically significant changes ($P > 0.05$) observed in the levels of haemoglobin (HGB), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) when compared to the control group. Furthermore, there was a noteworthy ($P < 0.05$) reduction in HGB, MCV, and MCH when the rats reached a body weight of 80 g/100dl. The results indicated a statistically significant ($P < 0.05$) reduction in the weight of the left kidney, heart, and spleen, as well as in the liver and right kidney weights at 20, 40, and 80 g/dl body weight. There were no statistically significant differences ($P > 0.05$) in the body weight of the liver and right kidney, which were both 10g/dl. Ultimately, the use of garlic formulation in appropriate dosages can impact certain haematological markers and the weights of organs.

Keywords: Garlic; Hematological parameters; Organ weight; Haemoglobin, spleen

1. Introduction

The species *Allium sativum*, often known as garlic, belongs to the genus *Allium* and the family *Amaryllidaceae* (Batiha *et al.*, 2020). The scientific term for garlic is *Allium*. Various species within the genus, particularly different varieties of onions, garlics, cloves, and leeks, are crucial in global cuisine due to the diverse flavours and textures they provide when consumed in raw or cooked form (Sherifat *et al.*, 2020). The plant is native to northeastern Iran, South and Central Asia, and has been utilised as a flavouring agent for millennia by individuals worldwide (Block, 2010). Garlic, scientifically known as *Allium sativum*, is highly esteemed for its gastronomic and therapeutic attributes, and is commonly employed as a seasoning or condiment in various societies. According to Vivekanandini *et al.* (2018), it is widely considered to be one of the most popular supplements. Garlic has been shown in multiple studies to have a wide range of bioactive effects, including its ability to fight against infections, cancer, high blood pressure, liver damage, and insects (Vivekanandini *et al.*, 2018). Garlic has demonstrated anticancer effects in several *in vitro*, *in vivo*, and epidemiological studies. The probable mechanism of action comprises the activation of metabolic enzymes, inhibition of reactive oxygen species,

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scavenging of radicals, reduction of DNA damage, and suppression of cancer growth. Garlic possesses immunomodulatory properties that are facilitated by its ability to regulate cytokine production and enhance the activity of immune cells, leading to the release of antibodies and the initiation of an immune response. Garlic possesses antiallergenic properties via altering the immune response in the airways and inhibiting the production of histamine through antibodies (Sushma *et al.*, 2021). China accounted for 73% of the global garlic production in 2021, according to FAOSTAT (2023). Allium species have been globally employed for ages to combat infectious diseases. The ancient Egyptian papyrus, known as Codex Ebers, documents the utilisation of Allium species for medicinal purposes dating back 35 millennia. According to historical accounts, the slaves who toiled on the construction of the pyramids were commonly provided with onions and garlic, purportedly to enhance their physical strength and endurance. Furthermore, Sherifat *et al.* (2020) mentioned that in ancient Greece, it was customary to provide these plants to athletes as a means of enhancing their energy levels in the days preceding the Olympics. Garlic is cultivated globally, with a yearly per capita consumption of two pounds. China ranked top and India ranked second in terms of average garlic production from 1961 to 2017, according to the Food and Agricultural Organisation of the United Nations (Sushma *et al.*, 2021). Haematology is the scientific discipline that focuses on analysing the quantity and structure of the biological components of blood, including red blood cells (erythrocytes), white blood cells (leucocytes), and platelets (thrombocytes). These findings are then utilised for the purpose of diagnosing and tracking the progression of diseases. Haematological examinations are valuable for diagnosing many disorders and assessing the severity of blood-related damage. Haematological studies have ecological and physiological significance since they aid in comprehending the correlation between blood traits and the environment. Consequently, these studies can be valuable in identifying animals that possess genetic resistance to specific diseases and environmental factors. Haematological measures serve as reliable markers of the physiological condition of animals (Asiwe *et al.*, 2024). Haematological parameters refer to the specific measurements and characteristics associated with the blood and organs involved in blood formation (Waugh *et al.*, 2001; Bamishaiye *et al.*, 2009). Blood serves as a diagnostic indicator of the condition of animals exposed to hazardous substances and other factors. According to the study conducted by Isaac *et al.*, (2013), animals with a favourable blood composition are more likely to exhibit high performance. Performing laboratory tests on blood is crucial for detecting various conditions. Blood is composed of numerous biological structures. When blood is subjected to centrifugation, it undergoes separation into three main components: plasma, buffy coat, and erythrocytes. Blood consists of red blood cells (RBC), white blood cells (WBC), platelets, and plasma.

Therefore, this research aims to investigate the impact of the effects of garlic formulation on some hematological parameters and organ weights of male and female Wister albino rats



Figure 1 Garlic

2. Material and methods

2.1. Collection and preparation of garlic formulation

Fresh garlic was purchased from Relief Market, in Owerri, Imo State, Nigeria. It underwent a thorough cleaning process using running tap water to eliminate any attached trash and pollutants. Additionally, the garlic was peeled and sliced into little pieces. Subsequently, the pieces were left to dry naturally at room temperature until they reached a stable weight. The desiccated garlic was pulverised using a hammer mill to get garlic meal. The garlic meal was incorporated into the formulation of the experimental diets. The experimental diets' compositions were displayed in table 1.

Table 1 Garlic Formulation

Groups	No. of Animals	Feed Formular
I -Positive control	6	100g feed + H2O
II	6	10% Garlic + 90% standard feed
III	6	20% Garlic + 80% standard feed
IV	6	40% Garlic + 60% standard feed
V	6	80% Garlic + 20% standard feed

2.2. Experimental animals

Albino Wister rats of both genders, aged 12 to 16 weeks and weighing 150-200 g, were acquired from the animal house in the Department of Zoology at the University of Nigeria, Nsukka. The rats were kept in well-ventilated laboratory cages located in the animal house of the Department of Biochemistry at FUTO. They underwent a two-week acclimatization period in the laboratory environment before the start of the experiment, during which they were provided with standard animal feeds and *ad libitum* access to drinking water. Afterwards, the rats were categorised into five (5) groups for the experiment.

2.3. Experimental Design

The experiment employed a total of thirty (30) Wistar rats, with each group consisting of five rats (n=6). After the rats had adapted to their environment, they were divided into five groups as follows: Group (1) acted as the positive control, being provided with 100% standard feed (100 g per day) for a duration of 21 days. Group II rats were administered a daily mixture of 10% garlic and 90% feed for a duration of 21 days. Group III animals were given a diet consisting of 20% garlic and 80% feed for the same period of time. Group IV was administered a diet consisting of 40% garlic and 60% feed for a duration of 21 days. In contrast, group V was fed a diet comprising 80% garlic and 20% feed during the entire period. The administration of food in all groups was done orally under sterile conditions, combined with water freely available.

2.4. Hematological parameters and organ samples.

On the 21st night after the 21-day treatment of garlic extract, the animals were subjected to fasting, euthanasia, and blood collection. Haematological parameters were determined by drawing blood samples from the heart of each rat through cardiac puncture into EDTA bottles. Haematological evaluation involved the collection of blood samples from the male rabbits. A 5-ml syringe equipped with a sterile needle was utilised to obtain about 2 ml of blood and promptly transferred to sample vials containing ethylenediaminetetraacetic acid (EDTA). To avoid coagulation, the EDTA sample bottles were gently agitated. The following haematological parameters were measured: hematocrit, haemoglobin, red blood cell count, white blood cell count, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration. The micro-haematocrit method, as reported by Dacie and Lewis (1991), was used to determine the packed cell volume (PCV). The concentration of haemoglobin (Hb) was measured using a spectrophotometer, using the cyanmethemoglobin method proposed by Kelly (1979). The quantification of red blood cells (RBCs) and white blood cells (WBCs), together with their respective 'differentials', was conducted using the Neubauer hemocytometer method, as outlined by Jain (1986) and Feldman (2000). The values for mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined using the methodology described by Jain (1986). After blood collection through cardiac puncture, certain organs including the liver, right kidney, left kidney, heart, and spleen were surgically removed using blades and placed in normal saline to accurately estimate their weight.

2.5. Statistical analysis

The analysis of results employed the Duncan Multiple Range Test. All data were presented as mean \pm SD. Group differences were assessed at a 95% confidence limit, and significance was considered at a probability level of 0.05 ($P < 0.05$).

3. Results and Discussion

Table 2 presented the impact of a garlic formulation on some blood-related characteristics of male and female Wister albino rats. The results indicated a substantial reduction ($P < 0.05$) in white blood cells (WBC) and lymphocytes (LYM) among the various body weights of the animals, as compared to the control group. The red blood cell (RBC), haemoglobin (HGB), haematocrit test (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) all fell within the normal range when compared to the control. The analysis of the red blood cells (RBC) indicates that there was no statistically significant difference ($P > 0.05$) seen among the various body weight concentrations. At a concentration of 80 g/100g RBC, there was a notable decrease ($P \leq 0.05$) in body weight, whereas MCH exhibited a substantial rise ($P \leq 0.05$) compared to the control group.

Table 3 presented the impact of a garlic formulation on the weights of several organs in male and female Wister albino rats. There was a statistically significant rise ($P < 0.05$) in the liver, right kidney, left kidney, and heart weights relative to body weight (20, 40, and 80 g/100g) of rats, but there was a significant decrease in spleen weight compared to the control group.

Table 2 The effects of garlic formulation on some hematological parameters of male and female Wister albino rats

GROUPS	Dose (g/100g)	WBC ($\times 10^3$)	RBC ($10^6/\mu\text{l}$)	HGB (g/dl)	HCT (%)	MCHC (g/dl)	MCV (μm^3)	MCH (pg)	LYM (%)
Control		15.38 \pm 3.53 ^a	7.45 \pm 0.84 ^a	11.68 \pm 1.09 ^a	49.30 \pm 5.58 ^a	23.78 \pm 2.16 ^a	66.14 \pm 6.64 ^a	15.78 \pm 1.17 ^a	14.32 \pm 3.60 ^a
Garlic	10	9.88 \pm 4.34 ^b	6.85 \pm 0.84 ^a	10.50 \pm 1.18 ^a	43.74 \pm 6.76 ^{ab}	24.12 \pm 1.42 ^a	63.70 \pm 3.46 ^a	15.36 \pm 0.52 ^a	9.04 \pm 4.11 ^b
Garlic	20	11.02 \pm 2.56 ^{ab}	7.13 \pm 0.39 ^a	10.88 \pm 0.51 ^a	45.44 \pm 2.57 ^a	23.18 \pm 1.34 ^a	63.82 \pm 4.43 ^a	15.28 \pm 0.63 ^a	10.46 \pm 2.37 ^{ab}
Garlic	40	13.22 \pm 4.45 ^{ab}	6.67 \pm 0.59 ^a	10.90 \pm 10.97 ^a	47.84 \pm 3.64 ^a	22.46 \pm 1.48 ^a	70.26 \pm 3.65 ^a	15.72 \pm 0.49 ^a	11.66 \pm 4.02 ^{ab}
Garlic	80	10.02 \pm 2.40 ^b	5.72 \pm 0.99 ^c	10.76 \pm 1.28 ^a	46.60 \pm 4.92 ^a	23.10 \pm 0.73 ^a	82.60 \pm 9.54 ^b	19.02 \pm 1.72 ^b	9.56 \pm 2.34 ^b

Mean \pm SD values in the column with different superscripts are significantly different ($P < 0.05$), while Mean values with the same superscripts are not significantly different ($P > 0.05$).

Table 3 The effects of garlic formulation on some organ weights of male and female Wister albino rats

Groups	Dose (g/100g)	Liver(g)	R. Kidney(g)	L. Kidney (g)	Heart (g)	Spleen(g)
(control)		3.44 \pm 0.51 ^a	0.47 \pm 0.11 ^a	0.45 \pm 0.05 ^a	0.42 \pm 0.64 ^a	3.38 \pm 0.15 ^a
10% garlic	10	3.45 \pm 0.29 ^a	0.89 \pm 0.14 ^a	0.85 \pm 0.13 ^b	0.69 \pm 0.95 ^{bc}	0.82 \pm 0.24 ^b
20% garlic	20	3.70 \pm 0.73 ^b	0.85 \pm 0.13 ^b	0.84 \pm 0.92 ^b	0.90 \pm 0.24 ^c	0.29 \pm 0.35 ^c
40% garlic	40	3.91 \pm 0.57 ^{ab}	1.06 \pm 0.21 ^{ab}	0.90 \pm 0.15 ^b	0.58 \pm 0.12 ^{ab}	0.37 \pm 0.11 ^c
80% garlic	80	4.45 \pm 0.96 ^b	0.99 \pm 0.19 ^{ab}	0.78 \pm 0.19 ^b	0.77 \pm 0.30 ^{bc}	1.41 \pm 0.28 ^c

Mean \pm SD values in the column with different superscripts are significantly different ($P < 0.05$), while Mean value with the same superscripts are not significantly different ($P > 0.05$).

The findings demonstrated a substantial reduction in white blood cell count in comparison to the control group. This was consistent with the study conducted by (Isaac *et al.*, 2013), which reported a decline in white blood cell count in rats exposed to petroleum compounds. The decrease in white blood cell (WBC) count seen when comparing the positive

control to the treated groups can be related to the ameliorative capacity of antioxidants in safeguarding lymphocytes against oxidative damage, as evidenced in lung cancer (Dacie and Lewis, 2021). The results also indicated that there was no significant variation in red blood cell count, except for the rats with a body weight of 80g/dl, which exhibited a substantial drop (group V). The findings of Leighton, (2019) align with the result, indicating a decrease in red blood cell count and a rise in haemoglobin level in rats exposed to Prudhoe Bay crude oil. Haemoglobin count (HGB) increases as the amount of oxygen-carrying iron in red blood cells rises, resulting in the release of energy for nutrition metabolism. Alternatively, HGB may also rise when the overall blood volume decreases, such as in cases of dehydration (Giovannucci *et al.*, 2019). Therefore, the groups that received treatment (Group II, Group III, Group IV, and Group V) did not show any significant difference ($p>0.05$) in HGB levels compared to the positive control group. These findings corroborate with Cody *et al.*, 2021. The observed rise in the treated groups can be due to the ameliorative capacity of the ingested antioxidant, which counteracts the effect caused by the chemical present in the prepared feed (Giovannucci *et al.*, 2019). Although a decrease in haematocrit is a feature of anaemia, there was no significant rise in HCT. The blood indices (MCHC, MCV, MCH) play a crucial role in diagnosing anaemia in most animals (Shalaby *et al.*, 2006). The elevation in MCHC, MCV, and MCH in the blood of the experimental group compared to the control group can be attributed to a defensive response against *A. sativum*, which stimulates the production of red blood cells. This finding contrasts with the research conducted by Fazlolahzadeh *et al.*, 2011, which demonstrated a notable reduction in MCH and MCV in fish that were fed the maximum dosage of *Allium sativum*. The lymphocyte count (LYM) exhibited a notable reduction, indicating an increased susceptibility to infection, leading to lymphocytopenia (Tavakolpour *et al.*, 2020). There was a notable rise in the size of the liver, right and left kidneys, and heart organs. Conversely, the concentration of the spleen decreased. These findings align with the research conducted by (Jeorg and Lee, 1998), which demonstrated that *Allium sativum* contains certain components that can potentially stimulate the immune system and affect the functioning of organs involved in the production of blood cells, such as the thymus and bone marrow.

4. Conclusion

The study examined the impact of a garlic formulation on haematological parameters and organ weights in male and female Wister albino rats. The results demonstrated that the inclusion of garlic in the rats' diet, along with regular feed, led to enhanced performance, improved haematology, blood profile, and increased organ weights. Therefore, it possesses the capacity to enhance the immune system, promote the synthesis of antibodies, and boost the body's ability to fight diseases. The study has also shown that *Allium sativum* can be incorporated into the diet of rats to enhance nutrient utilisation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The entire study was adapted to the Department of Biochemistry, federal university of Technology Owerri ethics committee rules.

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