

Safety Assessment of Garlic (*Allium Sativum*) Extracts on Body Weight and Haemato-Biochemical Parameters in Male Wistar Rats

Abayomi O. Adeoye. and Ayodele S. Ake

¹Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria.²Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria.**ABSTRACT**

This study evaluated the safety of aqueous garlic (*Allium sativum*) extract by examining its effects on body weight and blood parameters in male Wistar rats. Thirty (30) male six-week-old albino rats were randomly divided into 3 groups of 10 animals each: the control group given distilled water (Group A), Group B rats were administered 30mg/kg of garlic extract daily, and Group C rats were administered 60mg/kg of garlic extract daily. Body weight was monitored weekly, and blood samples were collected for analyses after 14 and 30 days of treatment. Phytochemical analysis of the extract indicated the presence of flavonoids, tannins, and terpenoids. The treated rats showed a significant, dose-dependent increase in body weight gain when compared with the control. No significant changes were observed in the haematological parameters [packed cell volume (PCV) haemoglobin concentration (Hb) and red blood cell (RBC) count], liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP)], blood urea nitrogen (BUN) or creatinine values of the treated groups compared to the control group after 14 and 30 days of the experiment. However, a significant reduction in white blood cell (WBC) count was observed in the treated groups after 30 days, along with an insignificant increase in platelets and lymphocytes, with a reduction in neutrophils. In conclusion, the results suggest that phytochemicals in the aqueous extract of *Allium sativum* may promote weight gain without causing negative effects on blood parameters or liver function. Therefore, the administration of *Allium sativum* is safe and may provide some beneficial effects.

Keywords: Garlic, Weight gain, Haematology, Liver enzymes, Phytochemistry.

Received 02 March 2026

Revised 14 June 2026

Accepted 14 June 2026

Published online: 15 June 2026

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Introduction

For many years, natural products have been a cornerstone of traditional medicine,^{1,2} with global health organizations like the World Health Organization (WHO) estimating that a significant percentage of the global population still relies on herbal remedies for primary health care.^{3,4} Local medicinal herbs, used for centuries to promote wellness and treat illnesses, are a vital part of indigenous cultures. Technological advances are helping to speed up the discovery of new drugs.^{5,6} Garlic (*Allium sativum*) is one of those plants that have been seriously investigated over several years and used for centuries to fight infectious diseases.^{7,8,9} The plant is a member of the *Liliaceae* family and one of the most widely used medicinal herbs due to its rich content of sulfur compounds, calcium, magnesium, enzymes, vitamins, and minerals.¹⁰ It has a higher concentration of sulfur compounds than any other *Allium* species, which are responsible both for its garlic's pungent odor and many of its medicinal properties.^{11,12} Many medicinal properties like cholesterol-lowering, anti-obesity, antioxidant, anti-dementia, antibacterial, and antirheumatic effects have been linked with the garlic-derived sulfur-containing compounds.^{13,10,11} Several herbs are thought to likely cause adverse effects.

Adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life-threatening or lethal. Although many consumers believe that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing adverse reactions in the patient. Herbal remedies can also be dangerously contaminated, and herbal medicines without established efficacy may unknowingly be used to replace medicines that do have corroborated efficacy. Although some of the therapeutic effects of the *Allium sativum* extract have been reported, this study investigated the safety of the extract on body weight and blood parameters. Therefore, the study aims to determine the safety of aqueous garlic (*Allium sativum*) extract on body weight and blood parameters in male Wistar rats.

Materials and Methods*Ethical approval*

The Animal Care Use and Research Ethics Committee (ACUREC), University of Ibadan, gave full approval with assigned number UI-ACUREC/17/0034.

Plant collection and authentication

The fresh bulbs of *Allium sativum* were purchased in April, 2018 at Bodija Central Market, Ibadan, Nigeria, and authenticated by Esimekhuai D.P.O. a botanist at the Herbarium of the Department of Botany, University of Ibadan, Nigeria, with reference no UIH-23823.

Preparation of extract

The plant's bulbs were air-dried at room temperature, pulverized, and 1000g of it was soaked in 10 liters of distilled water for 24 hours and then filtered through Whatman filter paper no. 1 and concentrated at 50°C with a rotary evaporator. Thereafter, the garlic extract was preserved in the refrigerator until use. 1 g of the dried extract

*Corresponding author. mail: akeayodele@gmail.com
Tel: +2347037701891

Citation: Adeoye AO. and Ake AS. Safety Assessment of Garlic (*Allium Sativum*) Extracts on Body Weight and Haemato-Biochemical Parameters in Male Wistar Rats. Trop J Phytochem Pharm Sci, 2026, 5(3); 518 - 522 <http://www.doi.org/10.26538/tjpps/v5i3.5>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

concentrate was dissolved in 50 mL of distilled water to give a concentration of 20mg/mL. To ensure that the active principles are preserved, fresh aqueous extracts were prepared every other day.

Experimental animals

30 adult male albino rats weighing 100-150g were kept in the experimental animal housing unit of the Department of Veterinary Physiology and Biochemistry, University of Ibadan. They were acclimatized for 2 weeks before the commencement of the experiment and fed with commercial Vital® feed and water *ad libitum*.

Plant extracts phytochemical screening

Phytochemical screening of aqueous extract of *Allium sativum* was conducted using the method as described by Trease and Evans.¹⁴

Experimental design

The thirty (30) male albino rats of about 6 weeks in age were divided into 3 groups of 10 animals each. Five rats from each group were sacrificed on day 14 of extract treatment, while the experiment was finally terminated after 30 days of treatment.

Group A: Negative control (distilled water).

Group B: 30mg/kg of extract daily for 30 days.

Group C: 60mg/kg of extract daily for 30 days.

Weight Monitoring

The weights of the rats were monitored at the commencement of the experiment and then every week using an automated electronic scale (Sensor Disc Technology, London). To weigh a rat, a round plastic container was placed on the scale and tared to zero, following which the rat was dropped inside the container and subsequently weighed.

Blood Collection and Analysis

The rats were anaesthetized with diethyl ether, and blood samples were collected from the orbital vein of each rat using sodium heparinized micro haematocrit tubes into separate lithium heparinized sample bottles for evaluation of haematological and biochemical parameters.

Statistical Analysis

All data obtained from the study were presented as means \pm standard error of mean (SEM). The mean of each group was compared by Analysis of variance (ANOVA) using SPSS, and a value of $P < 0.05$ was considered significant.

Result and Discussion

Phytochemical analysis of *Allium sativum* extract

Phytochemical analysis of the aqueous extract of *Allium Sativum* indicated the presence of flavonoids, tannins, and terpenoids, as shown in Table 1. Huzaiifa in 2014, reported the presence of alkaloids, flavonoids, saponins, tannins, and cardiac glycosides in aqueous extract of garlic bulbs,¹⁵ while Boukeria in 2016 observed the presence of saponins, alkaloids, and traces of glycosides in *A. sativum* varieties.¹⁶ Katkar and Dubal recently confirmed the presence of glycosides, flavonoids, steroids, phenols, terpenoids, and flavonoids in aqueous extract.¹⁷ In addition, Neeraj in 2014 screened the aqueous extracts of *Allium sativum* leaf, root, and pod at various stages till 25 days of growth and reported the presence of glycosides and terpenoids in developing pods; tannins, terpenoids, and glycosides in developing shoots, and flavonoids and glycosides in developing roots.¹⁸ These results are in agreement to a large extent. Any discrepancy may be attributed to the differences in the varieties, age, and source of the garlic.¹⁹

Effects of *Allium sativum* extract on weight gain

After 7 days of extract treatment, animals treated with 30mg/kg and 60mg/kg of extract show a significantly ($p < 0.05$) higher percentage weight gain of 15.6% and 18.1% respectively, when compared with control animals that gained 13.6% of their body weight as shown in Table 2. Also, on day 14 of extract treatment, animals treated with 30mg/kg and 60mg/kg of extract showed a significantly ($p < 0.05$) higher percentage of weight gain of 24.3% and 28.1% respectively, when compared with control animals that gained 20.8% of their body weight.

This study observed a dose-dependent increase in percentage body weight gain in extract-treated animals. The growth-promoting potential of garlic has been attributed mainly to its alliin constituent.⁷ Many studies have reported that garlic supplementation led to improved body weight and growth in piglets, lambs, Nile tilapia, and buffalo calves.²⁰⁻²² In contrast with the result of the current study, previous studies on pigs, and chicken reported no positive effects of garlic supplementation on growth performance.^{21, 22} Likewise, diet supplementation with 30 and 60 kg of garlic bulbs per ton of feed showed no effect on growth performance in growing lambs. The inconsistent results may be due to differences in the type, quality, or quantity of the supplemented garlic and also the species and age of the animals.¹⁹

Table 1: Phytochemical constituents of aqueous extract of *Allium sativum* bulb

Component	Test
Alkaloid	-
Tannins	+
Flavonoid	+

+ indicates presence of component
- indicates absence of component

Effects of *Allium Sativum* extract on blood parameters

No significant changes ($p > 0.05$) were observed in the red cell indices (PCV, Hb, and RBC) of extract-treated groups when compared to the control untreated group following 14 days and 30 days of treatment at 30mg/kg and 60mg/kg as shown in Table 3 and 4. However, a significant ($p < 0.05$) reduction in WBC count was observed in groups treated with the extract for 30 days, while an insignificant increase in platelet and lymphocyte count, with a reduction in neutrophil count, was recorded in all extract-treated groups. Also, no significant ($p > 0.05$) changes were observed in the liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP)], blood urea nitrogen (BUN) and Creatinine values of extract-treated animals with doses of 30mg/kg and 60mg/kg for 14 and 30 days when compared with control untreated animals.

The results of the current study indicate no significant changes in the erythron parameters (PCV, Hb, and RBC) of extract-treated groups when compared to the control group at day 30. This suggests that garlic has no deleterious effect on erythron parameters. This finding is in agreement with the report of Jawad in 2007 that there were no significant alterations in PCV, Hb, and RBCs in the group fed 10% raw garlic when compared with the control group.²³ However, there have been conflicting reports on the effect of garlic on blood parameters. An increase in red blood cell parameters in animals fed a garlic-supplemented diet has been reported by some authors,^{24, 19} however Yan observed that prolonged feeding of rats with garlic may result in anaemia.²⁵

Anti-inflammatory activities of the extract were also observed from the suppression of inflammatory cells in extract-treated animals. Leucocyte count was significantly reduced in groups treated with the extract for 30 days, while an insignificant increase in platelet and lymphocyte count, with a reduction in neutrophil count in all extract-treated groups was observed. Shokrollahi in 2016 reported that garlic supplementation led to enhanced WBC and lymphocyte concentrations but reduced that of neutrophils, while Yan in 2011 reported that dietary fermented garlic by *Weissella koreensis* powder doesn't affect lymphocyte, WBC, and RBC in growing pigs.^{19, 25}

In addition, this study observed that aqueous garlic extract has no deleterious effect on liver function, as shown in Table 5, since the plasma liver enzyme, whose elevation is an indication of hepatic damage,^{26, 27} is reduced, though insignificantly, in extract-treated animals compared to untreated groups. This is in agreement with previously observed reports indicating the hepatoprotective effect of aged garlic extract by Amagase,²⁸ who demonstrated in vivo

protective effect of garlic against liver toxins-induced substances like carbon tetrachloride, paracetamol (acetaminophen), and bromobenzene. Borek also reported that garlic inhibits both the formation and bioactivation of liver carcinogenic nitrosamines and has prevented the mutagenic effects of aflatoxin B1.²⁹ The hepatoprotective activities of garlic have been attributed to its organosulphur compounds.³⁰

Table 2: Effects of *Allium sativum* extract on Mean body weight gain of Wistar rat after 14 days of treatment (n = 10, Mean±SEM)

BODY WEIGHT (g)	CONTROL	30mg/kg	60mg/kg
Initial Weight	97.5±2.9	92.0±2.0	95.0±0.5
Weight at day 7	110.8±3.7 (13.6%)	106.3±2.9 (15.6%)	117.5±0.5 (18.1%)
Weight at day 14	117.8±3.7 (20.8%)	114±1.9 (24.3%)	127±2.5 (28.1%)

All values are expressed as mean ± standard error of mean.
Percentage (%) body weight changes in parentheses.

Table 3: Effects of *Allium sativum* extract on haematological parameters of Wistar rat after 14 days of treatment (n = 10, Mean±SEM)

PARAMETERS	CONTROL	14 Days	
		30 mg/kg	60 mg/kg
PCV (%)	41.5±0.3	41.0±1.5	39.0±1.0
HB g/dL	13.7±0.2	13.2±0.4	13.0±0.6
RBC (10 ⁶ /μL)	6.8±0.2	6.9±0.3	6.5±0.1 ^a
WBC (10 ³ /μL)	5437.5±1494.1	6050.0±312.3	5675.0±275.0
PLAT (10 ⁶ /μL)	114750.0±14783.9	151666.7±23254.6	158000.0±26000.0
LYM (10 ³ /μL)	56.8±1.6	59.7±4.4	62.5±0.5
NEUT (10 ³ /μL)	39.0±1.8	37.0±4.0	33.0±1.0
MONO (10 ³ /μL)	2.0±0.4	1.7±0.7	2.0±0.0
EOS (10 ³ /μL)	2.3±0.3	1.7±0.7	2.5±0.5

Statistical significance is reported at α0.05 and indicated with a in the table.

Note: PCV = packed cell volume, Hb = haemoglobin concentration, RBC = red blood cells, WBC = white blood cells, Neut = Neutrophils, Lym = Lymphocytes, Mono = Monocytes, Eos = Eosinophils, Plat = Platelets

Table 4: Effects of *Allium sativum* extract on haematological parameters of Wistar rat after 30 days of treatment (Mean±SEM, n = 10)

PARAMETERS	CONTROL	30 Days	
		30 mg/kg	60 mg/kg
PCV (%)	41.5±0.3	45.3±1.5	36.7±5.6
HB g/dL	13.7±0.2	15.0±0.4	11.9±1.9
RBC (10 ⁶ /μL)	6.8±0.2	7.7±0.2	6.0±1.0
WBC (10 ³ /μL)	5437.5±1494.1	3850.0±180.3 ^a	3200.0±321.5 ^a
PLAT (10 ⁶ /μL)	114750.0±14783.9	139000.0±29816.1	166666.7±6359.6 ^a
LYM (10 ³ /μL)	56.8±1.6	68.0±1.5	69.3±2.4
NEUT (10 ³ /μL)	39.0±1.8	28.3±1.5	27.3±2.7
MONO (10 ³ /μL)	2.0±0.4	1.3±0.3	2.0±0.6
EOS (10 ³ /μL)	2.3±0.3	2.3±0.3	1.3±0.7

Statistical significance is reported at α0.05 and indicated with a in the table.

Note: PCV = packed cell volume, Hb = haemoglobin concentration, RBC = red blood cells, WBC = white blood cells, Neut = Neutrophils, Lym = Lymphocytes, Mono = Monocytes, Eos = Eosinophils, Plat = Platelets

Table 5: Effects of *Allium sativum* extract on biochemical parameters of Wistar rats after 14 and 30 Days of treatment (Mean±SEM, n = 10)

PARAMETERS	CONTROL	14 DAYS		30 DAYS	
		30mg/kg	60mg/kg	30mg/kg	60mg/kg
TP (g/dl)	7.3±0.1	6.3±0.2	7.2±0.2	7.5±0.1 ^a	6.9±0.4
ALB (g/dl)	3.0±0.2	2.5±0.1 ^a	3.0±0.1	3.4±0.2	3.0±0.4
GLB (g/dl)	4.3±0.1	3.8±0.2	4.3±0.2	3.8±0.2	4.0±0.1 ^a

AG RATIO	0.7±0.1	0.6±0.0	0.7±0.1	0.8±0.0 ^a	0.7±0.1
AST (ul)	41.3±0.5	39.7±0.9	37.5±0.5	41.7±0.3	39.0±1.5
ALT (ul)	30.3±0.8	28.7±0.9	25.5±0.5	30.3±0.3	26.7±1.2
ALP (ul)	108.8±5.5	98.0±8.7	104.5±9.5	102.0±2.1	91.0±11.0
BUN (mg/dl)	16.4±0.3	16.3±0.7	16.9±0.9	16.5±0.1	16.0±0.5
CREAT (mg/dl)	0.7±0.1	0.5±0.0	0.6±0.0	0.6±0.0	0.6±0.1

Statistical significance is reported at $\alpha^{0.05}$ and indicated with ^a in the table.

Note: TP = (Total protein), ALB = (Albumin), GLB = (Globulin), AG ratio = albumin globulin ratio, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline Phosphatase (ALP) and blood urea nitrogen (BUN), CREAT= (Creatinine)

Conclusion

This study concluded that aqueous extract of *Allium sativum* contains flavonoids, tannins and terpenoids, which provide an explanation for the observed enhancement of body weight gain, safe effect on blood parameters, and protective effect on the liver.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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Galley Proof