

Effect Of Nanoparticles of Allium Sativum in Phenylhydrazine-Induced Anemia in Male Albino Rats

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Abstract

Background and Objective: This study was aimed the possibility of using garlic (extract and nanoparticles) in alleviating of anemia induced by phenylhydrazine, in adult male rats.

Materials and methods: The garlic NPs was prepared according to (Naji *et al.*, 2021) Anemia was induced by phenylhydrazine intraperitoneal (20 mg/kg) for 2 consecutive days. A total of 60 adult male albino rats were used in the present study, at the age of 2 months with body weight 195±15gm were divided randomly to six equal groups (10 rats for each) and treated as follows for 8 weeks :-

Control group:- in this group animals left without any treatment like negative control. **T1:** animals in this group was induced anemia and untreated as positive group. **T2:** animals in this group still normal (no anemic) but treated with daily dose 35.4mg/kg of extract garlic given orally by stomach tube. **T3:** animals in this group were induced anemia and treated with daily dose 35.4mg/kg of extract garlic given orally by stomach tube. **T4:** animals in this group still normal (no anemic) but treated with daily dose 35.4mg/kg of nanoparticles garlic given orally by stomach tube. **T5:** animals in this group were induced anemia and treated with daily dose 35.4mg/kg of nanoparticles garlic given orally by stomach tube. At the end of the experiment, all animals were sacrificed and blood samples (5ml) were collected directly from the heart by the cardiac puncture .

Results: Induction of anemia significantly ($P < 0.05$) decreased PCV, hemoglobin concentration (Hb), and red blood cell count (RBC), while mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and white blood cell count (WBC) significantly increased of anemic not treated rats compared to normal control rats. Also the platelets and erythrocyte sedimentation rate showed significant increase in anemic group when compared with control group. After treatment the platelet and ESR recorded decrease. The total protein and albumin showed significantly decreased in anemic group but fibrinogen recorded significantly increase in anemic group as compared with control group. When rats received garlic extract and nanoparticles reported increase in the blood electrolytes potassium and iron showed significantly increase in anemic group as compared with control group. While, sodium and calcium recorded significantly decreased. After treatment with extract and nanoparticles of garlic the K^+ and Fe^+ decrease in all treatment groups and Na^+ , Ca^{++} increased in all treatment groups. **Conclusion:** This study suggests may be use of garlic NPs as antianemic and ability to prevent hemolysis best from garlic extract. Confirmation of the studied indicator of blood that gave the best results as evidence of treatment.

Key words: Garlic NPs, Anemia, Blood parameters, Biochemical parameters

1. Introduction

Anemia, a common public health problem, is characterized as decrease in erythrocyte mass or hemoglobin concentration in the blood leading to reduction in its oxygen carrying capacity [1]. More than two billion people around the world suffer from anemia [2]. Anemia is more common health problem in the developing countries [3]. Dietary changes and iron supplementation are commonly preferred for the management of anemia. Oral iron therapy has many disadvantages such as insufficient absorption and lack of compliance [4]. Furthermore, consumption of high quantity of these iron supplements can lead to serious health-related complications such as some neurogenic disorders or cancer [5]. All these facts demonstrate the need to have safe and effective alternative for the management of anemia.

Medicinal plants have been a source to control many diseases and anemia is no exception. In traditional systems of medicine including Ayurveda, many plants are claimed to be useful for anemia [6]. Previous studies reported antianemic potentials of several Indian medicinal plants [7]. Few polyherbal formulations are reported to be effective for the treatment of anemia [8]. These herbal based formulations are preferred by the community as they are cost-effective and have less side effects.

Garlic (*Allium Sativum* L.) is a member of the Alliaceae family, is one of the best essential vegetables all over the world. The importance of garlic is due to its use only for culinary but also for therapeutic and medicinal resolves in both traditional and modern medicine [9]. Garlic contains a higher concentration of sulfur compounds(such as allicin, diallyl disulphide, S-allylcysteine, and diallyl trisulfide) which are responsible for its various kinds of medicinal effects .It is eaten up either as raw vegetable(fresh leaves or dried cloves), or after processing in the form of garlic oil, garlic extracts, and garlic powder with changes in chemical composition and bioactive compounds content between the various forms[10].

2. Materials and methods

2.1. Animals

Sixty albino male rats were supplied by the animal house of (University of Babylon - College of Science). Their ages at the start of experiments were 8 weeks, and their weight was 195 ± 15 grams. The rats have been kept under suitable environmental situation . The rats have been housed in cages made up from plastic with dimension 12 x 15 x 29 cm. and had free excess to food (standard pellets) and water (ad libitum). The ground sawdust of cages have been changed every week. For adaptation, the rats were remained in animal house about 2 weeks before beginning the experiment.

2.2 Induction of anemia

An intraperitoneal injection of 20 mg/kg phenylhydrazine was applied for two consecutive days to develop hemolytic anemia on the 4th day after the 1st injection in 30 male albino rats.

2.3 Design of experiment

NC Group:

animals in this group still normal without any treatment as negative control.

Group T1 :

animals in this group was induced anemia and treated with distilled water as positive group.

Group T2 :

animals in this group was treated daily with 34.5 mg/kg of extract garlic given orally by stomach tube.

Group T3 :

animals in this group was induced anemia and treated daily with 34.5 mg/kg of extract garlic given orally by stomach tube.

Group T4 :

animals in this group was treated daily with 34.5 mg/kg of garlic loaded CSNPs given orally by stomach tube.

Group T5 :

animals in this group was induced anemia and treated daily with 34.5 mg/kg of garlic loaded CSNPs given orally by stomach tube.

2.4 Sample collection

At the end of the experiment, all animals were sacrificed and blood samples(5ml) were collected directly from the heart by the cardiac puncture . One ml was put into EDTA tubes for CBC measured, another one ml was put into tube with sodium citrate for obtained plasma which was used for fibrinogen biomarker while the remaining 3 ml pushed slowly into disposable tubes containing separating gel and allowed to clot at room temperature for 30 minutes and then centrifuged at 3000×g for approximately 3 minutes. Then the sera were obtained stored at(-20°C) until physio- biochemical analyses carried out which includes total protein, albumin, and electrolytes.

2.5 Hematological studies

All of hematological profile (CBC) have been done by use of an automated auto-analyzer (Horiba A) Biomerieux. In this test, the blood is placed in the vibrator, after which the power switch is pressed. Blood 20 µl is taken by probe, and taken out of the device, after a minute the result was appeared.

2.6 Biochemical measurement

2.6.1 Determination of total protein, albumin, and electrolytes

All of which determined fully automated chemistry analyzer Genotech (USA) – SMART-150 .

2.6.2 Determine plasma Fibrinogen

- 1- 10 µl serum + 190 µl buffer in test tube(Hitach cup).
- 2- 150 µl of above solution was added to cuvette.
- 3- Added of one magnetic bead into the cuvette.
- 4- Added of 50 µl of liquid fibrinogen reagent into the cuvette.
- 5- Measurement was done using Mindray Semi Automated Coagulation Analyzer (China).

2.7 Statistical analysis

Data were subjected to analysis using SPSS program .The statistical analysis was carried out for data on this experience according to complete randomize design(CRD). Least significant differences (LSD) was used to assess the differences among means at (P≤ 0.05).

3. Results

3.1 Red blood corpuscular parameters (RBC count, Hb, PCV)

The present study of RBCs data in (table 3.1) showed a significant increase in (T2, T4, and T5) and there is no significant ($P \leq 0.05$) difference between them, but still a significant when compared with T1 and negative control group. On the other hand blood sample of RBCs showed prominent reduction in T1 group that received PHZ when compared with control and among treated group. In addition to the our data showed a significant decrease in Hb and PCV to recorded mean value (9.92 ± 0.04 and 25.6 ± 0.38) respectively at $P \leq 0.05$, on the other aspect the data of Hb and PCV appear a significant increase to recorded mean value of Hb (13.1 ± 0.6 , 12.9 ± 0.17 , 13.67 ± 0.21 , and 13.8 ± 0.091) respectively, and mean value of PCV (38.01 ± 1.3 , 36.1 ± 0.58 , 41.08 ± 0.59 , and 41.15 ± 0.39) respectively.

3.1: Effects of garlic Table nanoparticles on Erythrocytes parameters of anemic male rats.

Groups of experiment	RBCs $\times 10^6 / \text{mm}^3$	Hb g/dl	PCV %
NC	6.05 ± 0.27 D	12.06 ± 0.60 B	41 ± 0.39 A
T1	3.43 ± 0.051 E	9.92 ± 0.40 C	25.6 ± 0.38 C
T2	8.21 ± 0.19 A	13.1 ± 0.60 A	38.01 ± 1.3 B
T3	7.20 ± 0.12 B	12.9 ± 0.17 A	36.1 ± 0.58 B
T4	7.72 ± 0.16 A	13.67 ± 0.21 A	41.08 ± 0.59 A
T5	8.2 ± 0.13 A	13.80 ± 0.091 A	41.15 ± 0.39 A
LSD $P \leq 0.05$	0.48	0.97	2.01

The value represent mean \pm S E, N=10 for each group, Different capital letters indicated significant ($P \leq 0.05$) among groups. NC: normal control, T1: anemia positive control, T2: normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles, and T5: anemic group received garlic nanoparticles.

3.2 Red blood corpuscles indices

The mean differences ($P \leq 0.05$) of (MCV, MCH, and MCHC) in anemic group showed a significant increase as compared with NC in (table 3.2), while treatment groups (T4 and T5) recorded a significant decrease when compared with anemic group and negative control group.

3.2: The effect of garlic nanoparticles on RBCs indices of anemic male rats.

Groups of experiment Table	MCV μm^3	MCH Pg	MCHC g/dl
NC	53.9 ± 0.40	18.8 ± 0.27	34.5 ± 0.86

	B	B	B
T1	56.3 ± 1.48 A	19.4 ± 0.32 A	37.4 ± 1.7 A
T2	53.8 ± 0.65 B	18 ± 0.31 B	35.6 ± 0.82 B
T3	55.1 ± 0.42 A	18.16 ± 0.31 B	36.2 ± 0.49 B
T4	53.7 ± 0.73 B	17.9 ± 0.28 BC	32.9 ± 0.38 C
T5	51.4 ± 0.7 C	17.4 ± 0.29 C	32.4 ± 0.39 C
LSD P ≤ 0.05	2.32	1.47	2.55

The value represent mean ± SE, N=10 for each group. Different capital letters indicated significant (P ≤ 0.05) among groups. NC: normal control, T1: anemia positive control, T2 : normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles , and T5 : anemic group received garlic nanoparticles.

3.3 Platelets count and ESR

The platelets in (T2, T3, T4, and T5) revealed a significant decrease (P<0.05) in mean value (673 ± 28, 568± 5, 554 ±3, and 622± 2) respectively as compared with NC and T1(727±7.8, 984± 9.2) respectively. While T2 group recorded no a significant variation (P≤ 0.05) in mean value (673±28) when compared with NC group in (table 3.3).

The ESR in induced anemia rats (positive control group T1) showed a significant increase (P ≤ 0.05) in mean value (3.34 ± 0.28) when compared with the negative control group and all others treatment groups in mean values (2.8 ± 0.16, 2.60 ±0.16, 2.53 ±0.16, 2.32 ±0.16, and 2.3 ±0.16) respectively.

Table 3.3: The effect of garlic nanoparticles on platelet count and ESR of anemic male rats.

Groups of experiment	Platelets mm ³	ESR mm/h
NC	727 ± 7.8 B	2.8 ± 0.16 B
T1	984 ± 9.2 A	3.34 ± 0.28 A
T2	673 ± 28 BC	2.60 ± 0.16 B
T3	568 ± 5 C	2.53 ± 0.16 B
T4	554 ± 3 C	2.32 ± 0.16 C
T5	622 ± 2 C	2.3 ± 0.16 C
LSD P ≤ 0.05	130.4	0.38

The value represent mean ± SE, N=10 for each group . Different capital letters indicated significant (P ≤ 0.05) among groups. NC: normal control, T1: anemia positive control, T2 : normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles , and T5 : anemic group received garlic nanoparticles.

3.4 The White blood cells.

In the present study, the data in the table 3.4 confirmed highly significant (P ≤ 0.05) elevation in WBC, monocyte, granulocyte, and lymphocyte especially in group that received PHZ to induce anemia in mean values (21.3±0.06, 11.7±0.08, 17.85±1.72, and 83.4±0.71) respectively when compared with negative control and treatment groups. While the treatment groups showed a significant (P ≤ 0.05) reduction as compared with control group. Animal group that orally received garlic nanoparticles(T5) at dose 34.5 mg/kg showed a significant decrease (P ≤ 0.05) in WBC and monocyte as compared with anemic group and negative control group.

Table 3.4: The effect of garlic nanoparticles on WBC of anemic male rats.

Groups of experiment	WBC×10 ³ /mm ³	Monocyte%	Granulocyte %	Lymphocyte%
NC	9.67 ± 0.53 B	9.21 ± 0.16 B	10.66 ± 0.91 B	74.7 ± 2.1 C
T1	21.3 ± 0.60 A	11.7 ± 0.80 A	17.85 ± 1.71 A	83.4 ± 0.71 A
T2	9.54 ± 0.59 B	8.12 ± 0.81 C	9.85 ± 1.26 B	80.7 ± 1.52 B
T3	11.98 ± 0.59 A	7.7 ± 0.33 C	9.51 ± 0.82 B	83.5 ± 1.05 A
T4	9.56 ± 0.32 B	7.7 ± 0.21 C	10.34 ± 0.41 B	71.4 ± 2.24 C
T5	9.30 ± 0.52 C	7.60 ± 0.31 C	9.64 ± 1.20 B	79.9 ± 1.45 B
LSD P ≤ 0.05	0.35	1.60	3.18	0.76

The value represent mean ± SE. N=10 for each group. Different capital letters indicated significant (P ≤ 0.05) among groups. NC: normal control, T1: anemia positive control, T2 : normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles , and T5 : anemic group received garlic nanoparticles.

3.5 Biochemical parameters

3.5.1 Electrolytes of blood

Table 3.5 demonstrated the average values of the serum potassium concentration mmol/L in all groups. The present study confirmed that there was a significant (P ≤ 0.05) increase in all mean values of serum K⁺ concentration in T2, T3, T4, and T5 groups as

compared with NC, on other hand found a significant increase ($P \leq 0.05$) in T1 anemic group as compared with negative control group. Serum sodium and calcium concentration in anemic group recorded a significant ($P \leq 0.05$) decrease in mean value (127.4 ± 11.1 and 9.06 ± 0.11) respectively as compared with others groups. While (T2 and T5) groups recorded a significant increase as compared with anemic group. While iron in T1 reveal high significant ($P \leq 0.05$) increase in mean value (207 ± 26.9) as compared with NC group and all treatment groups.

Table 3.5: The effect of garlic nanoparticles on blood electrolytes of anemic male rats.

Groups of experiment	Potassium mmol/L	Sodium mmol /L	Calcium mg/dl	Iron mg/dl
NC	7.36 ± 0.57 B	138.5 ± 9.2 C	10.6 ± 0.16 C	164 ± 8.01 C
T1	9.98 ± 0.45 A	127.4 ± 11.1 D	9.06 ± 0.11 D	207.7 ± 26.9 A
T2	8.21 ± 0.062 AB	145.4 ± 11.6 A	11.69 ± 0.17 A	166.3 ± 10.5 C
T3	8.58 ± 0.20 AB	143.7 ± 12.5 B	11.21 ± 0.11 AB	169.5 ± 3.2 C
T4	6.64 ± 0.10 B	141.9 ± 12.5 B	11.15 ± 0.17 B	170.9 ± 12.7 C
T5	9.45 ± 0.22 A	147.4 ± 14.7 A	11.4 ± 0.11 A	163.3 ± 22.8 C
LSD $P \leq 0.05$	0.93	3.43	0.39	45.4

The value represent mean \pm SE. N=10 for each group . Different capital letters indicated significant ($P \leq 0.05$) among groups. NC: normal control, T1: anemia positive control, T2 : normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles , and T5 : anemic group received garlic nanoparticles.

3.5.2 Total proteins, albumin, and fibrinogen

Data in table 3.6 confirmed that serum total protein concentration in the all treatment groups showed clear significant elevation ($P \leq 0.05$) as compared with anemic group. Although the (T3, T4, and T5) recorded no significant ($P \leq 0.05$) variation between them. Albumin (g/L) concentration in group that injection by PHZ recorded highly significant ($P \leq 0.05$) reduction when compared with control and all others treated groups. On other hands (T3 and T4) showed no significant ($P \leq 0.05$) difference between them.

In addition, the mean difference of fibrinogen showed a significant ($P \leq 0.05$) increase in anemic group as compared with negative control group and decrease in all treatment groups when compared with positive control group.

Table 3.6: The effect of garlic nanoparticles on total protein, albumin, and fibrinogen of anemic male rats.

Groups of experiment	Total protein g/dl	Albumin g/dl	Fibrinogen g/L
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NC	5.95 ± 0.13 C	4.04 ± 0.095 B	269 ± 10.12 B
T1	5.14 ± 0.17 D	3.69 ± 0.13 C	329.4 ± 7.70 A
T2	7.26 ± 0.37 A	4.45 ± 0.15 A	245.7 ± 18.7 B
T3	6.97 ± 0.16 AB	4.25 ± 0.07 AB	239.4 ± 17.6 B
T4	6.37 ± 0.14 B	4.21 ± 0.12 AB	289 ± 9.4 B
T5	6.92 ± 0.32 AB	4.50 ± 0.15 A	244.3 ± 12.1 B
LSD P ≤ 0.05	0.60	0.35	53

The value represent mean ± SE. N=10 for each group. Different capital letters indicated significant (P≤ 0.05) among groups. NC: normal control, T1: anemia positive control, T2 : normal received garlic extract, T3: anemic group received garlic extract, T4: normal group received garlic nanoparticles , and T5 : anemic group received garlic nanoparticles.

4. Dissection

4.1 Blood parameters

4.1.1 Red blood corpuscular parameters (RBC count, Hb, PCV)

Results of the current study show a direct effect of Phenylhydrazine in creating anemia, wherein a decrease in hemoglobin levels, hematocrite, and the number of red blood cells are found in T1. It has been demonstrated in several studies, that there is a significant association of diagnostic values between RCB, Hb, PCV and blood indices in both humans and rats when exposed to PHZ.

In a study conducted by **Igwe et al.**, [11] for the purpose of inducing anemia in rats by using PHZ, it was found that PHZ leads to a decrease in the levels of hemoglobin and red blood cells with a significant increase (P≤ 0.05) in the level of white blood cells compared to the control group, the reason was attributed to the fact that PHZ alters iron metabolism, interfering with the binding of Erythropoietin receptors , forming Heinz bodies in red blood cells [12].

Our results of studies concerning the group of animals that received PHZ and the effects on hematological variables represented by the RBC count, PCV, and the Hb, which is the major variable indicator for anemia agreement with the studies of **Beshel et al.**, [13], the results of these studies indicated that the values of these investigated variables are decreased due to the toxicity of PHZ caused by oxidative stress represented by the generation of free radicals that attack biomolecules that cause damage to the biological system [14,15].

Giving doses(34.5 mg/kg) of garlic extract and garlic nanoparticles to rats, as shown in table (3.1), leads to a significant increase in Hb concentration, PCV, as

well as RBCs compared to the group received PHZ, this is attributed to the role the garlic that play in inhibiting the active radicals produced by PHZ by providing the animals with the natural antioxidants possessed by the plant such as polyphenolic compounds and antioxidant activity. These compounds are antioxidants that get rid of free radicals and improve normal blood cells production [16,17].

The present study agreement with **Suha, [18]** who reported that there was increased in Hb, PCV, and RBCs when treatment groups with garlic. The increase in Hb concentration, PCV, and RBCs count at garlic powder and garlic nanoparticles groups compared with anemic group may be possible related to the end product of garlic metabolism in the body that stimulates the kidney directly to cause formation and secretion of erythropoietin (a potent stimulator of the bone marrow) [18,19].

4.1.2 Red blood corpuscles indices

Red blood indices (MCV, MCH, and MCHC) are particularly important for the diagnosis of anemia in most animals [18]. In the present study the induction with phenylhydrazine (PHZ) caused increased values of MCV, MCH, and MCHC, as observed in table 3.2 and highly decrease in treatment groups (T4 and T5) that received garlic extract and NPs at dose 34.5 mg/kg. Erythrocytes that have a normal size or volume (normal MCV) are called normocytic, whereas high and low mean values indicate macrocytic and microcytic respectively. Erythrocytes with normal of haemoglobin concentration (MCHC) are normochromic, whereas, abnormally high and low mean values indicate hyperchromic and hypochromic conditions respectively, though there is no hyperchromic condition [11] these results are partially agreement with our current study. So the MCV, MCH and MCHC values in this work were normal suggesting macrocytic hyperchromic anemic condition.

These result contrasted with those of **Tchogou et al., [20]** which obtained RBC indices decrease after phenylhydrazine injection but they was in agreement with [21, 22]. The effect of garlic extract and garlic nanoparticles on RBC indices showed a significant decrease. So, it is assumed that the decrease or increase of blood indices may be attributed to a defense reaction against *Allium sativum*, which occurs by stimulation of erythropoiesis [23].

4.1.3 Platelets count and ESR

Results of the current study showed a direct effect of anemia on platelets count of T1 compared with negative control and all treatment groups.

Scharbert et al. [24] that reported a significantly increased in velocity of platelets aggregation in anemic blood sample as result of low hemoglobin concentration. Our study agreement with **Antwi-Baffour et al. [25]** who found the mean platelet count which was significantly higher in the sickle cell anemia participants compared to the control, which was attributed to the auto-splenectomy and/or loss of splenic function that frequently occurs in SCD patients.

The platelet count showed a significant decrease in all treatment groups that received extract and nanoparticles of garlic. Plants of the genus *Allium* such as onion and garlic are often consumed as a source of compounds which inhibit human platelet activity, with the

goal of decreasing vascular diseases. Antiplatelet activity of these plants is in part due to the concentrations of organosulfur compounds [26].

The aqueous extracts of garlic inhibit platelet aggregation induced by collagen and arachidonic acid in a dose-dependent manner in vitro, furthermore, raw garlic was found to inhibit cyclooxygenase activity in a non-competitive, dose-dependent and irreversible manner [27].

Also, the ESR recorded a significant increase in anemic group as compared with other groups in present study that agreement with **Ibrahim et al.** [28] who recorded anemia increase ESR there was negative correlation between ESR and PCV. In anemia, with the haematocrit reduced, the velocity of the upward flow of plasma is altered so that red blood cells sediment faster [29].

Other factors may also affect ESR red cell changes are especially prone to effect ESR. If hematocrit is reduced, red cell aggregates sediment faster. The more severe the anemia the higher the ESR [30].

After period of treatment (60 days) of rats in anemic group observed decrease in level of ESR these result agreement with **Zare et al.** [31] who found that garlic can be effective in reducing ESR in end stage renal disease (ESRD). Garlic can be considered as a useful natural herb in inhibition of inflammation. The advantageous effects of garlic on health are due to organosulfur compounds in it [32]. The result of **Asgharpour et al.** [33] also demonstrate a significant decrease in inflammatory biomarkers such as ESR by using the garlic extract which is compatible with the results of the present study.

4.1.4 The White blood cells

The results of the current study, as shown in table 3.4, show significant differences ($P \leq 0.05$) in the numbers of WBCs was observed a significant increase in the numbers of white blood cells of anemic group compared to the control group. The reasons for this can be explained as an increase in the numbers of white blood cells, that are one of the defense methods for inflammatory conditions especially in the liver and kidneys [34].

The number of white cells increases greatly in cases of infections and is attributed to the presence of a substance in the plasma that stimulates the red bone marrow to increase the production of WBC, and this substance was called leukocytosis-inducing-factor [35].

Regarding to differential WBC changes, PHZ injected rats showed increased in monocytes, granulocyte, and lymphocyte comparing to control rats. These result agree with **Bansode et al.** [36]. White blood cells are defensive mechanisms used by the body to combat cell infiltration by foreign agents or infections; thus the increased proliferation may indicate an immunological response due to acute infections, cell damage or inflammation [37] which might have been caused by the introduction of toxic substance such as PHZ [38].

The increased in the lymphocyte at garlic extract and NPs(T3 and T5) compared with control group came into agreement with the earlier reports in that supplementation of garlic had significantly improved leukocyte count, indicating the immune stimulant properties of garlic [23].

Moreover, the increased in lymphocytes and neutrophils counts at garlic extract groups may be related to an increase in the production of some chemical cytokines (interferon, interleukins and complement proteins). These cytokines stimulate other arms of the immune system and increase the activity of natural killer cells as well as T- and B-lymphocytes [39,40,41]. Furthermore, it has been found that garlic contains a therapeutic factor (Germanium) which enhances natural killer cell and macrophage activity in experimental animals that stimulate the immune function [18,23].

4.2 Biochemical parameters

4.2.1 Electrolytes of blood

In this study, we observed that serum sodium levels were significantly lower ($P \leq 0.05$) and serum potassium levels ($P \leq 0.05$) were significantly higher in anemic group when compared with negative control group. This result agreement with [42]. In our study, the serum sodium levels were significantly lower and serum potassium levels significantly higher in anemic group which is accordance with results of [43, 44]. Previous studies have stated that normal red cells have high level of intracellular potassium and low level of sodium within the extracellular environment. On the other hand, the level of potassium is low in the extracellular environment while that of sodium is high. Na^+ and K^+ ions are restricted to their compartment but can penetrate the cellular membrane through Na^+K^+ ATPase pumps. The red cell Na^+K^+ ATPase is a ubiquitous enzyme and plays a central role in the regulation of intra- and extra-cellular cationic homeostasis[45].

This study reveals that pooled mean sodium levels in anemia patients had lower than control patients, which was due to dehydration which triggered by movement of sodium into the sickle cell and potassium pooled mean level was reported higher in sickle cell patients as compare to control. The possible mechanism was that sickle cell patients usually encountered Cell dehydration and hypoxia, which leads to the loss of potassium from the cell into the extracellular fluid [46]. These were in confirmation with studies done by [45, 47,48, 49] .

The results obtained after treatment with extract and nanoparticles showed increase in both Na^+ and K^+ especially in T5 when compared with anemic group. The serum levels of sodium and potassium also increased significantly with higher doses of garlic. This finding is suggestive of a mild hyperkalaemic and hypernatremic effects. The drug in addition to its other actions may favour an improvement in renal function by increasing sodium and potassium reabsorption. The relieve of hypertension by allicin component of garlic may partly be explained by its secondary effect on possible increase in renal blood flow which enhances renal reabsorption of basic electrolytes like sodium and potassium [50] that agreement with present study.

This result is to some extent against the study conducted by **Safdar et al., [51]** who indicated that garlic had no significant effect on the serum level of sodium in broiler chicks because the chicks were bred in an open shed in the extreme hot and dry climate of DIKhan district, so it is possible that probably due to heat stress endured by the broiler chicks the sera level of sodium were not significantly different for any of the herbal extract.

While calcium high decrease in anemic group in our results, these result may be due to decrease level absorption of calcium in intestinal, present study disagree with **Asowata et al., [52]** who found iron deficiency anemia significantly increase calcium absorption in the duodenum but had no impact in the jejunum and ileum.

After treatment with garlic extract and nanoparticles the concentration of calcium increase in all treatment groups especially in T5 as compared with anemic group and negative control group this may be because the garlic contain calcium this results agree with **Safdar et al., [51]** that showed garlic has increased level of calcium in the sera of broilers, the aqueous extract of garlic probably enhanced the intestinal absorption of calcium by modulating the activity of Ca-ATPase enzyme present in the plasma membrane. Also, the present study is to some extent in agreement with the study conducted by **Mukherjee et al., [53]**, which suggested the significant effect of oil extract of garlic by promoting intestinal transference of calcium in rats.

Iron homeostasis must be maintained so that cells have sufficient iron for cell growth, but not excess due to its toxicity **[54]**, in this study, positive control group that received PHZ showed increased serum iron concentration. This results could be explained by a total iron passage across the enterocytes apical membrane transporters divalent metal transporter 1(DMT1) to the blood **[55]**.

These results agree with **Zangeneh et al. [56]** who mentioned that injection of PHZ into rats induced a hemolytic anemia and sequential changes in iron metabolism tests. The greater quantities of iron released from destroyed red blood cells primarily caused hyperferremia **[57]**.

When treatment with garlic extract and NPs the iron concentration decrease due to garlic is one of the well-known plants with remarkable antioxidant properties **[58]** and inhibitory effects on iron availability.

Ma et al., [59] suggesting that the bioactive garlic polyphenols inhibit iron absorption in a dose-dependent manner in human intestinal Caco-2 cells. **Tuntipopipat et al., [60]** confirmed that garlic polyphenolic compounds are able to inhibit iron absorption by forming iron complexes in the intestine, making dietary iron less available for absorption.

4.2.2 Total proteins, albumin, and fibrinogen

Intraperitoneal injection of PHZ generated a significant ($P \leq 0.05$) decrease in total protein and albumin in T1. Our study disagreement with **Andongma, [61]** who found the total protein and albumin showed no significant change in anemic group that may indicate that the synthetic function of the liver has not been significantly affected.

Igwe et al. [11] recorded that TP and ALB decrease in anemic group as compared with normal control. The liver and kidney biomarkers which were significantly elevated by the PHZ agent as shown in the untreated rats. Studies have shown that intravascular hemolysis in any condition may damage the liver and other vascular organs **[62]** apart from hemolysis

Finduced liver injury. After treatment with extract and nanoparticles of garlic observed increase in total protein and albumin these result agreement with **Thanikachalam et al.[41]** that found the serum total protein and albumin increase in fish after 20 days of feeding with garlic that result may be thought to be associated with stronger innate immune response of fish **[63]**.

Ghiasi et al.[64] who found garlic aqueous extract reduced serum value of albumin and no significant changes in total protein due to that garlic has substances such as allicin and diallyl disulfide which are active components of garlic and all of these result is due to mentioned component. Also **Al-Sayed et al.[65]** reported that no alteration in the levels of total protein and albumin but increase in total globulins was found in rats that received garlic at 5% these result come in accordance with **Jafari et al.[66]** who mentioned that garlic powder increase serum γ -globulin in broiler chicks, immune- stimulant effect of garlic may be due to its component**[67]**.

Also after treatment fibrinogen level decrease. Our result agreement with **Reddy et al.[68]** that reported decrease of fibrinogen after administration of garlic. One Indian study showed that intake of garlic in a regular diet could remove fibrin clots could remove fibrin clots and reduce the incidence of cardio vascular disease **[69]**. Almost all human researches on fibrinolytic activity of garlic have been found to have positive effect in fibrinolysis **[68]**.

Garlic reduced fibrinogen level in hyperlipidemic rats after 4 weeks of treatment, compared with control group. This reduction due to garlic has a potent fibrinolytic activity **(70)**.

5. References

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