Haemoprotective effects of ginger (Zingiber officinale) on wistar rats fed with iron treated water

Abstract

This research examines the impact of simultaneously administering of ginger(zingiber officinale Roscoe) extract in wistar rats fed with iron treated water considering the health benefits of ginger in treating different ailments. A cohorts of 25 wistar rats, 6-8 weeks with an average weight of 100-129g and indeterminate gender. The experimental rats were grouped into four groups each consisting of six animals. Group A (-ve control) received 8.75mg of ferrous sulphate containing 2.8mg of iron in one liter of water.

Group B (+ve control) received 2% of ginger powder mixed with basal diet.

Group C received a combination of 2% ginger in basal diet + 2.8mg iron in one liter of water.

Group D (normal control) received normal feed and water.

The study monitored the packed cell volume (PCV), Haemoglobin, Total white blood cell (WBC) count, differential WBC count and change in body weight following the administration of various treatment which includes ginger powder , Iron, distilled water and a combination therapy of both ginger powder and Iron supplement. All the data collected were subjected to statistical analyses. Results were expressed as mean ±SD. The Post Hoc Tests and one-way ANOVA were used for comparison. The values were considered significant when $p < 0.05$.

The research findings indicates the haemoprotective effects of ginger and iron when administered to wistar rats, showed no significant alternation in the haematological parameters.

key words: *Ginger*, *iron*, *haematological parameters*

1.0 INTRODUCTION

Ginger (Zingiber officinale Roscoe) is an herbaceous perennial plant belonging to the order Scitamineae and the family Zingiberaceae. It is used for both medicinal and culinary purposes. it is a root crop and a typical herb extensively grown across the world for its pungent aromatic underground stem and rhizome which make it an important export commodity in the world trade. Ginger's origin is not well established though it is generally thought to be native of Asia, where it was first cultivated (Ezra et al., 2017). Ginger is the underground rhizome of the ginger plant with a firm striated texture. The flesh of the ginger rhizome can be yellow, white or red color, depending upon the variety. It is covered with a

brownish skin that may either be thick or thin, depending upon whether the plant was harvested when it was mature or young (Shafi, 2020). Other notable members of this plants family are turmeric, cardamom and galangal.

Zingiber officinale was also one of the first oriental spices to be grown to the Europeans, it was introduced to northern Europe by the Romans who got it from Arab traders and was one of the most popular spices in the middle ages (Kala, et al., 2016).
The Arabs also took the plant from India to East Africa in the thirteenth century while the Portuguese took it to West Africa and other part of the tropics in the sixteenth century.
Ginger was introduced to Nigeria in 1927. Nigeria is the third largest exporter of ginger in the world after China and India. Nigeria ginger is on high demand across the globe because of its Gingerols (ginger pungency). The highest non-oil export foreign exchange earner for Nigeria produced 723,971.56 tons of Ginger and 90% was exported 2019. Nigeria ginger is darker in colour and higher in monoterpene content, giving a more pungent aroma with camphoraceous notes; it has a high oil content and level of pungency, therefore it is usually preferred for the production of oils and oleoresins (Otaiku, 2020).

Nigeria ranked first in terms of the percentage of total hectares of ginger under cultivation but her contribution to total world output is too low compared to other countries. This can be attributed to the fact that most of production is undertaken by smallholder and traditional farmers with rudimentary production techniques and low yields. In addition, the major constraints militating against ginger production in the study area are inadequate input, poor transportation facilities, high cost of labour and inadequate credit (Ezra et al., 2017).

Ginger (Zingiber officinale Roscoe) is a common and widely used spice. To understand the beneficial characteristics of ginger, especially its physiological and pharmacological activities at the molecular level, the biological effects of ginger constituents, such as monoterpenes (cineole, citral, limonene and α/β-pinenes), sesquiterpenes (β-elemene, farnesene and zerumbone), phenolics (gingerols, [6]-shogaol, [6]-paradol and zingerone) and diarylheptanoids (curcumin) (Kiyama, 2020).

Accumulated investigations have demonstrated that ginger possesses multiple biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, cardiovascular protective, respiratory protective, antiobesity, antidiabetic, antinausea, and antiemetic activities (Mao et al., 2019). Ginger has staring potential for treating several ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. It also has anti-inflammatory and anti-oxidative properties for controlling the process of aging. Furthermore, it has antimicrobial potential as well, which can help in treating infectious diseases, anti-ulcer and as fertility enhancer and thus we can get a cheap, safe and effective medicinal agent for multipurpose (Rehman and Fatima 2018).

In Nigeria, the use of ginger as medicine is vast, it is also used for spicing almost all kinds of food including tea and it is one of the major ingredients of "zobo" a local drink in Nigeria. The powdered form in combination with garlic is used for the treatment of dysentery, rheumatism, high blood pressure, body pains and eye related problems (Isaac *et al*., 2014). Ginger consumption before or after exercise might reduce delayed onset muscle soreness (DOMS) that presents as pain, soreness, or swelling. Further studies needs to prove long-term effects and the safest dosage that can be consumed for maximum benefits (Cheshier & Jacobson, 2021).

Recently, a great deal of function hasp focused on the protective biochemical function of naturally occurring antioxidants in biochemical systems against toxic heavy metals. Hence it is believed that in the case of iron overload due to comorbidities from chronic inflammatory disorders, ginger can potentially reverse the adverse impacts and restore iron balance (Ooi et al., 2022). Ginger is considered to be a safe herbal medicine with only few and insignificant adverse/side effects (Supu *et al*., 2018).

Figure 1: Ginger (*Zingiberofficinale*)

(Source: Isaac *et al*., 2014)

Hematological and biochemical analyses after treatment with plant extracts in experimental animals are among the important methods of assessing the safety and toxicity of these plant extracts in animals and human being as well. Therefore; we measured some hematological parameters for evaluation of the effect of ginger on rats fed with Iron treated water (Vehedi *et al.,* 2017).

From ancient times, man has recognized the special role of iron in health and disease. Iron had early medicinal uses by Egyptians, Hindus, Greeks, and Romans.During the 17th century, iron was used to treat chlorosis (green disease), a condition often resulting from the iron deficiency (Zhang *et al.,* 2015).However, it was not until 1932 that the importance of iron was finally settled by the convincing proof that inorganic iron was needed for hemoglobin synthesis (Abbaspour *et al*., 2014).

1.1 Iron as a micronutrient

Iron is an essential element of various metabolic processes in humans, including DNA synthesis, electron transport, and oxygen transport. In the human body, iron exists mainly in erythrocytes as the heme compound haemogbobin, in muscle cell as myoglobin, to a lesser extent in storage compounds as ferritin and hemosiderin and are also found bound in proteins as hemoprotein. It also plays an important role in DNA synthesis (Ems, et al., 2017). At the same time, excess iron in the body is associated with toxic effects and poses health problems. However, because human beings have no active mechanism to control iron excretion, excess iron, regardless of the route of entry, accumulates in parenchymal organs and threatens cell viability (Lebda, 2014).

Iron is an abundant element on earth and is a biologically essential component of every living organism. It is an essential micronutrient for growth, development and long-term survival of most organisms(Abbaspour *et al*., 2014). However, excess iron causes an increased production of reactive oxygen species leading to cell dysfunction or death, tissue damage and organ disease (Corradini al., 2020).

As a redox-active transition metal, iron generates reactive oxygen species (ROS) via the Fenton and Haber–Weiss reactions. Excess iron in the human body can lead to toxic effects such as cardiomyopathy, hepatic fibrosis, glucose intolerance, impotence, arthropathy, and even hematological disorders(Abbaspour *et al*., 2014). Increasing clinical evidence has proven that iron chelation therapy can improve hematological parameters and reduce transfusion requirements, indicating that Iron deficiency or iron overload can impact hematopoiesis and is associated with many hematological diseases (Sinha al., 2021). At the same time, excess iron in the body is associated with toxic effects and poses health problems. However, because human beings have no active mechanism to control iron excretion, excess iron, regardless of the route of entry, accumulates in parenchymal organs and threatens cell viability (Lebda, 2014).

Iron is present in ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. Ferrous salts are 3 times more absorbed than ferric salts. $FeSO_4$ tablet is the most commonly used oral form of iron. Other iron preparations are Ferrous Fumarate and Ferrous Gluconate. Oral iron tablets are easy to use, cheap, and easily available hence no hospital staff or methods are required for their use (Nadir *et al*., 2015).

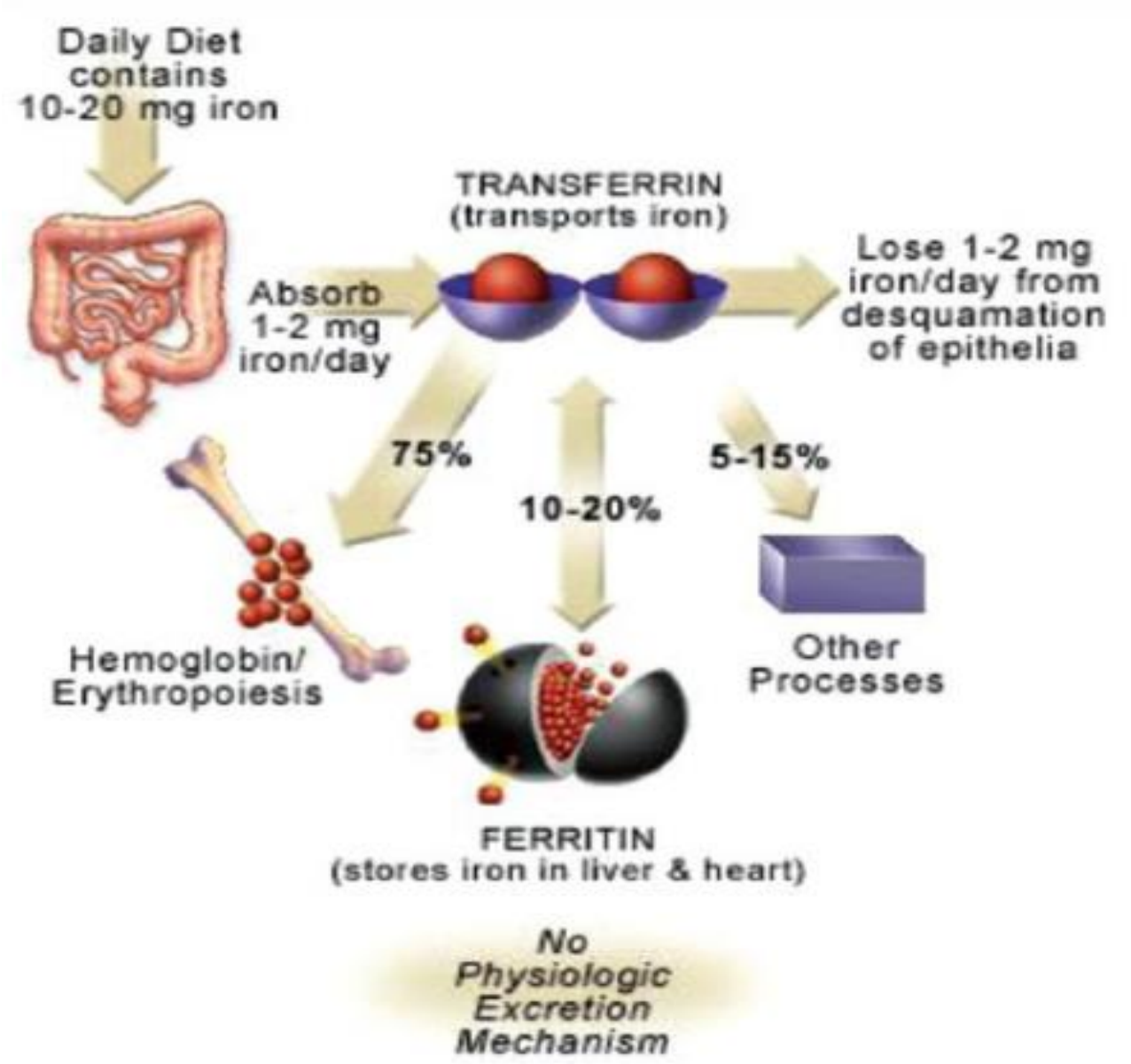

Figure 2: Iron cycle in the body.

(Source: Abbaspour *et al.,* 2014)

Iron is recycled and thus conserved by the body. Figure 1 shows a schematic diagram of iron cycle in the body. Iron is delivered to tissues by circulating transferrin, a transporter that captures iron released into the plasma mainly from intestinal enterocytes or

reticuloendothelial macrophages(Nadir *et al*., 2015). The binding of iron-laden transferrin to the cell-surface transferrin receptor (TfR) 1 results in endocytosis and uptake of the metal cargo. Internalized iron is transported to mitochondria for the synthesis of heme or iron-sulfur clusters, which are integral parts of several metalloproteins, and excess iron is stored and detoxified in cytosolic ferritin (Abbaspour*et al.,* 2014). The body requires iron for the synthesis of its oxygen transport proteins, hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions (Nadir *et al*., 2015).

1.2 Bio-availability of Iron

Dietary iron occurs in two forms: heme and nonheme. The primary sources of heme iron are hemoglobin and myoglobin , cytochromes b and c . Red meat is an excellent source of heme iron while the less bioavailable nonheme form is found in large amounts in milk products and vegetables (Buzala, et al., 2016).

Heme iron is highly bioavailable (15%-35%) and dietary factors have little effect on its absorption, whereas nonheme iron absorption is much lower (2%-20%) and strongly influenced by the presence of other food components. On the contrary, the quantity of nonheme iron in the diet is many folds greater than that of heme-iron in most meals.

The administration of ginger extract and iron may show no significant effect.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample Procurement and Identification

Chemicals and medicinal plants were procured from local markets in Onitsha, Anambra State, Nigeria, and authenticated by a taxonomist. The experimental animals, Wistar Albino rats, were procured from Chris Farms in Awka, Anambra State, Nigeria. Twenty-four (24) Wistar Albino rats, aged approximately 6-8 weeks old and with an average weight ranging

from 100 to 120 grams, were utilized for the project. These rats were selected based on their suitability for the experimental protocol.

2.1.2 Apparatus:
Various laboratory apparatuses, were utilized, including analytical weighing balance, syringes, centrifuge, microscope, spectrophotometer, pH meter, etc..

2.1.3 Chemicals/Reagents:
Standard reagents such as Turk's, Drabkin's, Leishman, stain, chloroform, Ferrous sulphate, and others were used.

2.1.4 Animal Studies:
All experimental animals were housed in gang cages within a controlled environment room, maintaining a 12-hour light-dark cycle. Following a period of 10 days for acclimatization, the animals were subjected to randomization into four experimental groups, each consisting of six rats.

Specialized cages with bar lids were utilized to securely hold water bottles and feed, minimizing the risk of contamination with urine or feces. Throughout the experiment, rats were provided with ad libitum access to food, ensuring their nutritional needs were met without restriction.

This standardized housing and feeding protocol aimed to maintain consistent environmental conditions and ensure the welfare of the animals while facilitating the experimental procedures conducted in the study.

2.2 METHODS

2.2.1Experimental Design:
A total number of 24 Wistar rats were utilized in this experimental study. Upon arrival, the rats were acclimatized for 10 days in gang cages within a controlled environment room, maintaining a 12-hour light-dark cycle.

Following the acclimatization period, the rats were randomly allocated to one of four experimental groups, each consisting of six rats:

1. **Group A (Negative Control):** Rats in this group received 2.8mg of iron in 1L of water, replicating the average elemental iron concentration found in typical Awka water sources. This group served as the negative control to establish baseline physiological parameters.
2. **Group B (Positive Control):** Rats in this group were provided with a diet supplemented with 2% ginger powder mixed with a basal diet. This group served as the positive control to evaluate the effects of ginger supplementation alone.
3. **Group C (Combination Treatment):** Rats in this group received a combined treatment consisting of 2% ginger incorporated into their feed and 2.8mg of iron per liter of water. This group aimed to assess the potential synergistic or antagonistic effects of ginger and iron supplementation.

4. **Normal Control:** Rats in this group received regular feed and water without additional treatments, serving as the standard control for comparison with the experimental groups.

This systematic experimental design allowed for the investigation of the effects of various treatments on the physiological parameters of the Wistar rats, providing insights into the potential therapeutic or adverse effects of ginger supplementation, iron administration, and their combination. The standardized housing and feeding protocols ensured consistent environmental conditions and animal welfare throughout the study.

2.2.2 Measurement of Water pH:

The pH of the water utilized in this study was determined using a standard pH meter to ensure the consistency and suitability of the aqueous environment for experimental procedures. A calibrated pH electrode was submerged into the water sample, and the pH reading was recorded.

The water's pH was measured at 7.4, indicating a neutral pH level. This neutral pH value is crucial for maintaining stable physiological conditions during the experimental procedures, thereby minimizing potential confounding factors that could influence the study's outcomes.

2.2.3 Blood Collection Protocol

Following a three-week treatment period, euthanasia was performed on the experimental animals for blood sample collection. The cardiac puncture method, utilizing sterilized syringes, was employed to ensure the integrity of the samples and minimize any potential sources of contamination.

Samples were swiftly transferred into EDTA bottles to prevent coagulation, maintaining blood integrity for subsequent analysis. This protocol adhered to ethical guidelines, prioritizing animal welfare while facilitating essential sample acquisition for our research investigation.

2.2.4 Administration of Ferrous Sulfate

Ferrous sulfate was meticulously prepared into a powdered form and dissolved in distilled water to ensure uniformity and accuracy of dosage. The solution was administered orally to the experimental subjects at a precise dosage of 8.75 mg/L daily, containing 2.8 mg of elemental iron.

2.2.5 Measurement of Packed Cell Volume (PCV):

Packed cell volume was determined using the Wintrobe method (1962), a standard technique for assessing the proportion of whole blood occupied by red cells.

Principle: PCV, expressed as a ratio (liter/liter), represents the volume occupied by red blood cells about the total blood volume. Anticoagulated blood was loaded into glass capillary tubes and centrifuged in a microhematocrit centrifuge at 12,000 rpm for 5 minutes to pack the red cells consistently. PCV values were then read from the scale of a micro hematocrit reader.

Procedure:

1. EDTA anticoagulated blood was carefully loaded into capillary tubes, filling them to about three-quarters of their capacity.
2. The unfilled end of the capillary tube was sealed with plasticine or briefly exposed to a small flame from a Bunsen burner, ensuring a secure closure.
3. The capillary tubes were positioned in designated slots of the microhematocrit rotor, with the sealed end against the rim gasket.
4. Centrifugation was performed for 5 minutes at 12,000 rpm to sediment the red blood cells.
5. PCV readings were promptly obtained using the microhematocrit reader immediately after centrifugation.

2.2.6 : Estimation of Hemoglobin Method

The estimation of hemoglobin concentration was conducted using the Cyamethaemoglobin (Hemoglobin-cyanide) method, which is internationally recognized for its accuracy in determining hemoglobin levels. The principle of this method involves diluting blood in a solution containing potassium cyanide and potassium ferricyanide. The latter converts hemoglobin (Hb) to methemoglobin, converted to cyanmethaemoglobin (HiCN) by potassium cyanide. The absorbance of the resulting solution is measured using a spectrophotometer at a wavelength of 540nm or in a colorimeter with a yellow-green filter. The procedure involved

- adding 1.5 ml of Drabkin's solution to a test tube,
- gently inverting the blood sample and
- drawing 0.02 ml into the hemoglobin (Hb) pipette.

The pipette was then placed into the tube containing Drabkin's solution, and the blood was slowly expelled into the solution, ensuring thorough mixing. After allowing the solution to stand undisturbed for 5 minutes, the absorbance at 540nm was measured using a spectrophotometer, with Drabkin's solution as the blank. The hemoglobin concentration was calculated using a standard curve, providing precise information on hemoglobin levels in the samples analyzed.

2.2.7: Total White Blood Cell (WBC) Count

The Total White Blood Cell (WBC) Count test determines the number of white blood cells in a blood sample. The method employed for this purpose is the visual haemacytometer, developed by Louis-Charles Malassez in 1934.

The principle underlying this method involves diluting whole blood at a ratio of 1 in 20 with a solution containing glacial acetic acid. This dilution leads to the hemolysis of red blood cells, while gentian violet, a basic dye, stains the nuclei of white blood cells. These stained nuclei are then counted using a Neubauer-ruled counting chamber under a microscope.

The procedural steps included in this method are as follows:

1. Prepare a 1 in-20 dilution of blood by adding 0.02ml of blood to 0.38ml of Turk's solution and mix thoroughly.
2. Prepare the counting chamber by cleaning it and placing a clean cover glass over the grid areas until rainbow colors are observed.
3. Remix the blood dilution and use a clean pipette to fill one of the grids of the chamber with the sample, ensuring that the area is not overfilled.
4. Allow the counting chamber to remain undisturbed for 2 minutes to allow the white cells to settle. Then, place the chamber in a petri dish on dampened tissue to prevent fluid drying and cover it with a lid.
5. Dry the underside of the chamber and place it on a microscope slide.
6. Use a 10× objective lens to count the white cells present in the four outer squares of the chamber.

2.2.8: Differential White Blood Cell (WBC) Count

The Differential White Blood Cell (WBC) Count involves preparing a peripheral blood film and subsequent staining to differentiate and count various types of white blood cells, including eosinophils, neutrophils, basophils, lymphocytes, and monocytes.
Thin Film Preparation:

1. An aliquot of blood was placed on a clean, dry slide.
2. A smooth edge spreader was used to create a thin film covering about 2/3 of the slide.
3. The film was air-dried by gently waving the slide back and forth.
4. Once completely dry, it was fixed using absolute methanol.

Staining the Blood Film with Leishman Stain: Principle: The Leishman stain contains methylene blue and eosin in methanol, which acts as a fixative. Methylene blue stains acidic components such as DNA and cytoplasmic RNA, while eosin stains essential components like hemoglobin and eosinophilic granules pink.
Procedure:

1. The blood film was covered with an undiluted stain for 2 minutes.
2. The volume of pH 6.8 buffered water (twice the amount of stain) was added without overflowing. The mixture was allowed to sit for 8 minutes to ensure proper staining.
3. The stain was washed off with tap water, and the slide was wiped clean on the back. It was then placed on a draining rack to allow the smear to dry.

2.2.9: Statistical Analysis

All the data collected were subjected to statistical analyses. Results were expressed as mean ±SD. Post-hoc tests and one-way ANOVA were used to compare the results. The values were considered significant when $p < 0.05$.

3.0 : **RESULT**

3.1: Packed Cell Volume (PCV).

The PCV of all the treatment groups showed no significant variation from the control. As shown in table 1.

Table 1: Mean Packed cell volume (PCV).

GROUP	PACKED CELL VOLUME (%) ± S. D
A (NEGATIVE CONTROL)	43.80 ± 2.45
B	38.40 ± 1.49
C	36.20 ± 1.73
NORMAL CONTROL	40.60 ± 0.42

3.2: Hemoglobin (HB) Level in (g/dl)

The hemoglobin levels of all the treatment groups showed no significant variation from the control, as shown in Table 2.

Table 2: Mean Hemoglobin (Hb) level in (g/dl)

GROUP	HAEMOGLOBIN (g/dl) ± S. D
A (NEGATIVE CONTROL)	12.88 ± 2.45
B	11.32 ± 1.43
C	10.66 ± 1.73
NORMAL CONTROL	11.96 ± 0.42

3.3: Percentage Neutrophil

The neutrophil level of all the treatment groups showed no significant variation from the control, as shown in Table 3.

Table 3: Percentage Mean Neutrophil

GROUP	NEUTROPHIL (%) ± S. D
A (NEGATIVE CONTROL)	29.20 ± 11.28
B	25.60 ± 12.92

C	33.60 ± 17.57
NORMAL CONTROL	38.80 ± 23.82

3.4: Percentage Change in Weight of Animals

Table 4 shows the overall growth performance of all the treatment groups and control. The dietary treatments marginally affected body weight gain and feed conversion ratio.
% Weight change = Δ in weight / initial weight × 100
Δ in weight = Final weight – Initial weight.
Table 4 shows the percentage increase in weight of various groups.

GROUP	INCREASE IN WEIGHT (%)
A (NEGATIVE CONTROL)	27
B	24
C	25
NORMAL CONTROL	23

4. DISCUSSION

Iron is essential to several oxygen transport and utilization proteins, including hemoglobin (Abbaspour et al., 2014). Blood can also act as a pathological and physiological indicator of animal health (Jorum et al., 2016).
In this research, as seen in Tables 1, 2, and 3, no significant alteration ($p>0.05$) in the PCV, Haemoglobin, and Neutrophil levels was observed at 8.75mg of $FeSO_4$, containing 2.8mg of elemental Iron, respectively. This study agrees with earlier findings by Sahin, Sari, Bidev, Bozkurt, Dizdar, and Oguz (2023), showing that although iron supplementation improves hematological parameters and prevents morbidity and mortality, the administration of iron in small doses only marginally improves hemoglobin levels and iron stores and reduces the risk of developing iron-deficiency anemia in those receiving supplements. However, Joerling and Doll (2019) observed an alteration in PCV and hemoglobin values in rats exposed to oral iron supplementation of 150mg daily, equivalent to 56 mg of elemental Iron. This study suggests that higher doses of iron supplementation raise the PCV values significantly.
Red blood cell indices reflect the size (MCV) and level of hemoglobin content (MCH and MCHC) of the red blood cells and aid in diagnosing the cause of anemia. The red blood cells of the exposed rats were normocytic, as their MCV values did not differ significantly from the controls. Thus, it is observed that at an iron concentration of about 2.8mg/L, the destruction of red blood cells beyond the bone marrow's production capacity is not increased (Abdul-Wahed, 2014).

Ginger, the rhizome of Zingiber officinale, is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment for various foods and beverages (Ajayi, 2013).
The study revealed no significant change in the hematological parameters when 2% ginger (in basal diet) was administered alone and when a combination of 2% ginger (in basal diet) and 2.8mg/L of Iron (in water) was compared to the control group. This agrees with the findings of Vahedi et al. (2017) and Zomrawi (2013) that dietary treatments with ginger (Zingiber officinale) have no effect ($P > 0.05$) on Hb, PCV, RBcs, MCV, MCH, and MCHC percentages.
The change in weight of the experimental animals in all the treatment groups (recorded in percentage) showed only a marginal increase over the control, as seen in Table 4. The dietary treatments did not affect body weight gain and feed conversion ratio.

5.0:CONCLUSION

The conclusion drawn from this study indicates that administering 2.8mg/L of iron, 2% ginger, or a combination of both 2.8mg/L of iron and 2% ginger in the basal diet does not yield significant changes in the hematological profile of the experimental animals.

Consent:
Informed consent was obtained from all participants involved in the study.
Ethical Approval:
The Institutional Ethics Committee approved the study protocol.

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