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

Abstract

This study aimed to investigate the effect of increased iron concentration in water and the potential protective efficacy of ginger (Zingiber officinale Roscoe) against hematological disturbances. A cohort of 24 Wistar rats, aged 6-8 weeks before dosage with an average weight of 100-120g and of both genders, was divided into four groups and treated for four weeks. The experimental rats were grouped into four groups, each comprising six animals.

Group A (-ve control) received 8.75mg of ferrous sulphate containing 2.8mg of Iron in one liter of distilled water.

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

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

Group D (normal control) received an un-supplemented diet with distilled water.

The study monitored the Heamatology profile (complete blood count), such as the packed cell volume (PCV), Haemoglobin(Hb), white blood cell (WBC) count, mean cell volume (MCV), Red blood cells (RBC), Mean cell haemoglobin concentration (MCHC) and platelets (PLT) in the serum and change in body weight following the administration of various treatment which includes ginger powder, Iron, distilled water and combination therapy of both ginger powder and Iron supplement. 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.

Hence, the administration of dry ginger powder suggests that its polyphenolic compound, specifically 6-gingerol, may enhance iron absorption in humans, mitigate iron deficiency anemia, and provide protection against iron-induced oxidative damage in various tissues. Iron supplementation improves hematological parameters and prevents morbidity and mortality.

The research findings indicate that the chemoprotective effects of ginger and Iron, when administered to Wistar rats, showed no significant alternation in the hematological parameters; thus, ginger administration is not significant if the p-value is> 0.05.

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). According to (Crichton et al., 2023) "a mechanistic studies support the findings from human clinical trials that ginger may assist in improving symptoms and biomarkers of pain, metabolic chronic disease, and gastrointestinal conditions. Bioactive ginger compounds reduce inflammation, which contributes to pain; promote vasodilation, which lowers blood pressure; obstruct cholesterol production, which regulates blood lipid profile; translocate glucose transporter type 4 molecules to plasma membranes to assist in glycemic control; stimulate fatty acid breakdown to aid weight management; and inhibit serotonin, muscarinic, and histaminergic receptor activation to reduce nausea and vomiting. Additional human trials are required to confirm the antimicrobial, neuroprotective, antineoplastic, and liver- and kidney-protecting effects of ginger".

"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 and Jacobson, 2021).

"Recently, a great deal of function has 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 (*Zingiber officinale*)

(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). "Although iron has an important tasks in the human body such as oxygen transfer, DNA synthesis, and electron exchange, it may also become toxic and harmful in excess. The toxicity of iron poisoning starts to appear with an intake of 20 mg/kg of elementary iron ions, along with GIS symptoms. In iron poisoning, patients usually present with various clinical findings and symptoms such as nausea, vomiting, palpitation, metabolic acidosis, deteriorated respiration, or mental disorders varying up to coma. Intervention in all patients begins with ABC evaluation, obtaining vascular access, monitoring, and (if necessary) providing oxygen support" (Durmuş and Güneysu 2020). "Iron, through its participation in oxidation/reduction processes, is essential for the physiological function of biological systems. In the brain, iron is involved in the development of normal cognitive functions, and its lack during development causes irreversible cognitive damage" (Gleitze et al., 2021)

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 haemoglobin, 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). Iron is a potentially toxic molecule, as it can both donate and accept electrons. Iron can catalyze the formation of free radicals from reactive oxygen species (ROS) via the Fenton reaction, which is the reduction of H2O2 by a single electron to produce a hydroxyl radical [Bresgen and Eckl 2015); this ultimately leads to damage to a wide variety of cellular structures.

"Therefore, the majority of iron is bound to other molecules for storage and/or transport, and only minute amounts of iron are available in the labile pool . Even in this labile pool, however, iron is not completely unbound, as the majority is believed to form a complex with peptides, carboxylates, and/or phosphates" [Laurent et al., 2014). "Despite the abundance of complexes to which iron can bind in the body, iron levels in the body can occasionally exceed the pool of available transferrin molecules. For example, iron toxicity is common during iron overload states associated with genetic factors or acquired factors such as repeated blood transfusions. However, excess iron causes an increased production of reactive oxygen species leading to cell dysfunction or death, tissue damage and organ disease, glucose intolerance, impotence, hepatic fibrosis, arthropathy, and even hematological disorders" (Corradini et al., 2020) and (Abbaspour*et al*., 2014).

"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 chelation has been introduced as a new therapeutic concept for the treatment of neurodegenerative diseases with features of iron overload. At difference with iron chelators used in systemic diseases, effective chelators for the treatment of neurodegenerative diseases must cross the blood–brain barrier" (Nuñez and Chana-Cuevas2018).

"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).

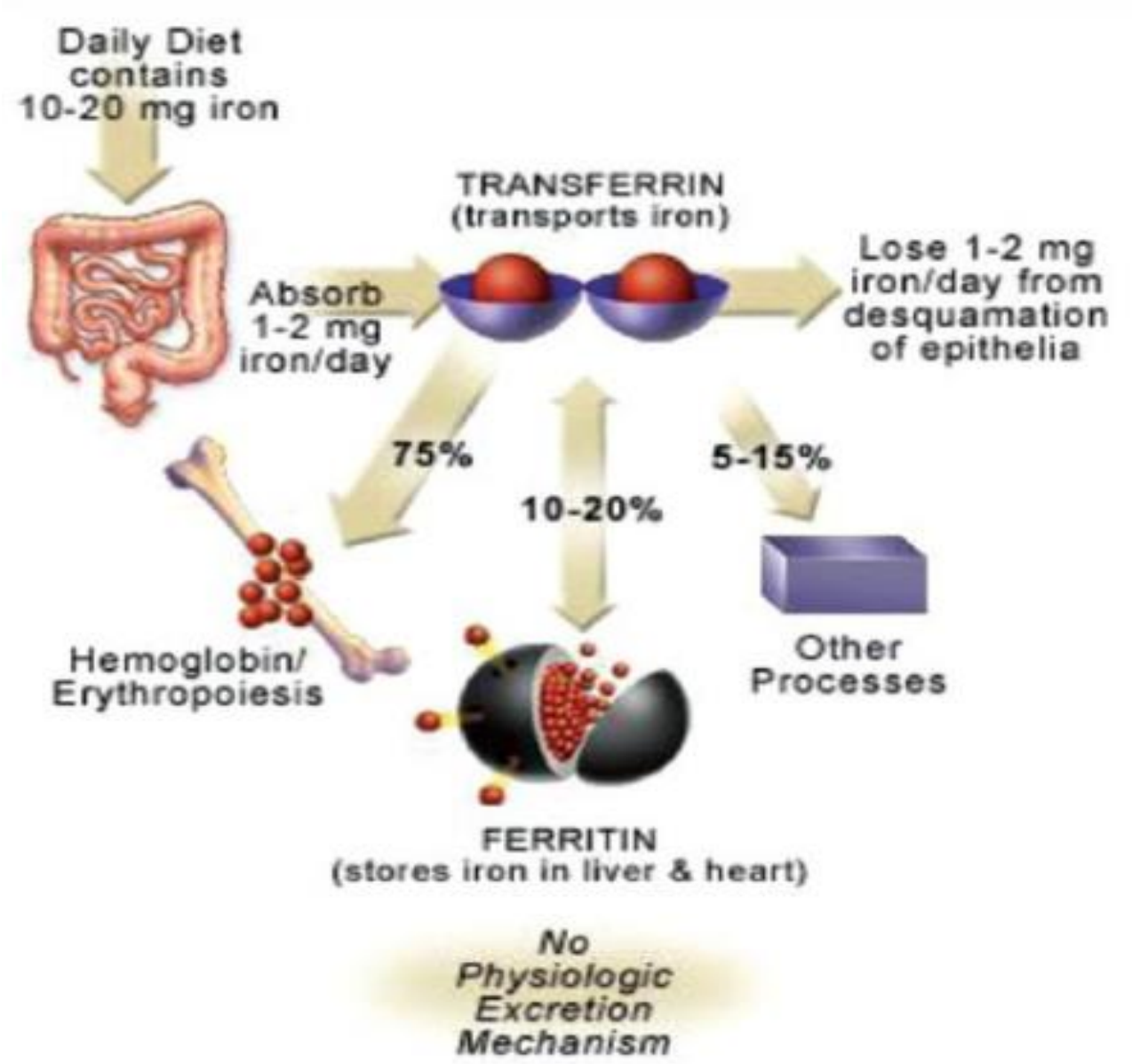

Figure 2: Iron cycle in the body.

(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 reticulo endothelial 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.

2.0MATERIALS 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.

2.2METHODS

2.2.1 Experimental Design:

In this Randomized Controlled Trial (RCT) study, a total number of 24 Wistar rats were utilized. 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 distilled 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% dry 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% dry ginger powder incorporated into their feed and 2.8mg of iron per liter of distilled 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 distilled water without additional treatments, serving as the standard control for comparison with the experimental groups.

Additionally, the study involved giving ginger supplementation to the rats for 3 weeks to assess its effects on 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 distilled 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 Haematological Parameters

Haematology is the study of the morphology and physiology of blood. A haematology laboratory in a healthcare setting is concerned with diagnosing and monitoring diseases of the blood and blood-forming organs. The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutritional and pathological status of an animal (Etim et al., 2014)

Haematological tests are performed on blood samples to diagnose diseases such as leukemia, anemia, and abnormalities of blood coagulation on any given day. A complete blood count or haematology profile is one of the most commonly performed blood tests, as it tells us so much about our health status. It consists of a haemoglobin (Hb), red blood cells (RBC) count, packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), white blood cells (WBC) count and platelets (PLT) in the serum.

2.2.6 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.

2.2.7 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.

2.2.8: 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.

2.2.9: 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.

2.2.10: 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

4.0DISCUSSION

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 (Sahin et al., 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).
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 distilled water) was compared to the control group. The lack of significant effects observed with ginger treatment in the study may stem from various factors. The dosage and duration of supplementation might have needed to be increased to elicit noticeable hematological changes. Bioavailability issues could have hindered the effective delivery of ginger's active compounds. Interactions with iron supplementation

negated potential synergistic effects. Individual rat variability, environmental conditions, and statistical considerations such as sample size could have further influenced the observed outcomes, collectively shaping the study's results. This agrees with the findings of (Vahedi et al., 2017) and (Abd Almajeed and Ibrahim 2022) 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:LIMITATION

One potential limitation of this study is the relatively short duration of the experiment, which lasted only three weeks. This timeframe may have needed longer to fully understand the potential long-term effects of ginger supplementation and iron administration on hematological parameters. Conducting studies over several months could offer a more comprehensive understanding of the sustained effects and potential gradual changes in physiological responses to these treatments.

In addition, the study only used a fixed dosage of dry ginger powder (2% in the basal diet) and iron (2.8 mg/L in distilled water) without investigating dose-response relationships. Exploring different dosage levels could reveal dose-dependent effects that should have been captured in this research. Future studies incorporating various dosage regimens could help identify the optimal doses for achieving desired therapeutic outcomes without adverse effects.

Furthermore, the study primarily focused on hematological parameters and did not assess other potential health indicators or organ-specific effects of ginger and iron supplementation. Including a broader range of physiological assessments, such as biochemical markers and histological examinations of organs, could provide a more comprehensive evaluation of the overall health impacts of these interventions.

Finally, the study used Wistar Albino rats as the experimental model. While rats are commonly used in biomedical research due to their physiological similarities to humans, it is important to interpret these findings cautiously when applying them directly to human populations. Human clinical trials are necessary to validate and translate the observed effects of ginger and iron supplementation in animal models to human health outcomes.

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% dry ginger powder in the basal diet does not yield significant changes in the hematological profile of the experimental animals.

Consent:

Written consent was obtained from all participants involved in the study.

Ethical Approval:
The Institutional Ethics Committee approved the study protocol.

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