Journal of Biosciences and Medicines, 2026, 14(7), 327-349
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Functional Cookies Development Using Snail Powder as an Iron Fortificant and Its *in Vivo* Impact on Behavioral, Hematological, Histopathological and Oxidative Biomarkers in Anemic Rats

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How to cite this paper: Agbor, C.E., Mamekeu, P.N., Djieyep, D.F., Achidi, A.U., Arrey, O.E.A. and Tiencheu, B. (2026) Functional Cookies Development Using Snail Powder as an Iron Fortificant and Its *in Vivo* Impact on Behavioral, Hematological, Histopathological and Oxidative Biomarkers in Anemic Rats. *Journal of Biosciences and Medicines*, **14**, 327-349.
https://doi.org/10.4236/jbm.2026.147026

Received: December 6, 2025
Accepted: July 21, 2026
Published: July 24, 2026

Abstract

Iron deficiency anaemia (IDA) remains a pervasive health challenge especially among vulnerable populations such as adolescent girls, pregnant women and children. Snail meat contains high amounts of minerals especially iron and has demonstrated promising potential in the management of iron deficiency anaemia. Therefore, this study was undertaken to develop cookies rich in iron and other minerals needed to combat IDA. Cookies were prepared with a composite of wheat flour and snail meat (*Archachatina marginata*) flour. The mineral composition and organoleptic properties were conducted. For *in vivo* study, the antianaemic effect of the snail-rich cookies was investigated using 36 male albino rats, grouped into 6 rats per group. Groups 2 to 6 were administered 40 mg/kg body weight of phenylhydrazine (PHZ) to induce anaemia. Groups 1 and 2 received normal basal diet, group 3 received normal basal diet with $FeSO_4$ (0.35 mg/kg), while groups 4, 5 and 6 received snail-rich cookies in different concentrations for 16 days and were sacrificed on day18, receiving food and water ad libitum. Using the Sensory and Iron desirability score, with 60% given to sensory properties and 40% given to iron, for commercial consumer purposes, F4 (30% snail flour), F5 (40% snail flour), and F6 (50% snail flour) were the samples selected from the 10 samples. The rats were sacrificed, and whole blood was collected for serum preparation, which was used for haematology and oxidative stress. Organs were collected, weighed and put in formalin for histopathological studies. Organ homogenates were also prepared and used together with serum for oxidative stress biomarkers (MDA, GSH, CAT,

SOD and NO). The results showed that the formulated cookies had improved micronutrient composition with high iron content. The results for the animal bioassay showed that the cookies restored blood parameters to normal levels in rats with IDA. A histological analysis suggested that the liver, kidney, heart and lungs morphology in the test groups became similar to that of the normal group. Furthermore, the cookies improved antioxidant activities in the serum, kidney, spleen, brain and liver. The level of free radicals significantly reduced with incorporation of snail cookies in the diets. In general, high substitution of the cookies exhibited the best effects in terms of iron supplementation and antioxidant activities. The present findings showed that the formulated cookies might be a potential new approach to solve IDA.

Keywords

Iron, Cookies, Snail, Anaemia, Histology, *Archachatina marginata*, Oxidative Stress, Haematology

1. Introduction

Anaemia is a medical condition in which there is a decrease in the number of red blood cells or haemoglobin in the blood, resulting in impaired oxygen transportation [1]. The World Health Organization defines anaemia as having haemoglobin (Hb) levels lower than 11 g/dL in children, 12.0 g/dL in females and 13.0 g/dL in males [2].

Anaemia affects approximately 1.3 billion individuals worldwide, with an average of 9.6 million children experiencing severe anaemia. It is a major public health issue affecting both developing and developed countries [3].

Causes of anaemia include nutritional deficiencies, particularly iron, vitamin A, B vitamins, folic acid, chronic inflammation, parasitic infections and congenital conditions. However, iron deficiency is considered the leading cause of anaemia worldwide [4].

The World Health Organization has identified iron deficiency anaemia (IDA) as the most widespread nutritional deficiency globally, affecting about 30% of the population [5]. Although more common in children, adolescent girls and pregnant women, adult men can also be affected depending on socioeconomic and health conditions [6].

Iron deficiency can be caused by inadequate consumption of iron, poor bioavailability of dietary iron or excessive loss of iron from the body. Although iron is commonly found in many diets, only a small amount is absorbed, and low bioavailability is a major contributing factor to iron deficiency [7].

Providing foods rich in protein and iron is a key strategy for overcoming iron deficiency anaemia [8]. This food-based approach (FBA) is effective in increasing intake of essential nutrients and improving iron status [9]. However, for FBA to be sustainable, factors such as eating habits, food preferences and food availability

must be considered [10]. A combination of FBA strategies can be used to maximize iron absorption, such as increasing consumption of iron-rich foods, adding absorption-enhancing components and applying food processing techniques such as heating [2].

Functional foods and nutraceuticals are a major focus of research in the food industry. These foods contain added bioactive ingredients that provide health benefits beyond traditional foods [11]. Staple foods and snacks can be fortified with micronutrients to improve nutritional value, providing a cost-effective intervention for anaemia control in vulnerable groups [9].

Many interventions targeting iron deficiency anaemia focus on plant-based non-heme iron sources such as cereals, legumes and vegetables [12]; however, non-heme iron has low bioavailability due to inhibitors such as phytates and polyphenols [13]. Heme iron, found in animal-derived foods such as meat, is absorbed more efficiently (15% - 35%) than non-heme iron (2% - 20%) and is less affected by dietary inhibitors, making it more effective in improving iron status [14] [15]. Systematic evidence also supports the greater effect of heme iron intake on haemoglobin improvement in iron-deficient populations [16].

Underutilized animal foods such as edible snail meat contain measurable levels of iron and other essential minerals and can contribute significantly to dietary iron intake [17] [18]. Incorporating bioavailable iron sources such as snail into functional foods such as fortified cookies, which are widely consumed across age groups, presents a promising strategy for improving iron intake and addressing anaemia.

Snail meat (Archachatina marginata) is a good example of a food that can help prevent iron deficiency. It contains ferrous iron (Fe^{2+}), which is readily absorbed by the body and less affected by dietary inhibitors [19]. This makes snail meat a suitable option for individuals at risk of iron deficiency.

As part of efforts to curb iron deficiency anaemia, the present study aims to develop iron-enriched cookies to combat anaemia using blends of wheat and snail meat powder. Thus, the aim of this work was the formulation of snail-rich cookies and evaluation of their haematological, oxidative stress and histopathological effects on anaemia-induced rats. Specifically, the study involves formulation of cookies using wheat and snail meat flour and evaluation of their nutritional and sensory attributes. Secondly, it evaluates haematological status, oxidative stress and histopathological effects of the formulated cookies in anaemic induced rats.

2. Materials and Methods

2.1. Sample Collection

15-litre bucket of fresh snail meat, particularly *Archachatina marginata*, was purchased from local vendors at Muea Market, Buea. Basic ingredients including wheat flour, margarine, sugar, nutmeg, and baking powder were also obtained from Muea Market.

2.2. Sample Processing

The method described by Tonfack *et al.* [20] was used to process the snail meat. The purchased snails were taken to the laboratory, where the meat was removed from the shell and part of the slime eliminated by washing in potable water. Alum was added to reduce slime formation and ease handling during removal of non-edible parts. After this, salt was used to wash the snails thoroughly, and the samples were rinsed four times with potable water before being soaked for 90 minutes to reduce salt concentration. After soaking, the fresh snail meat was removed and conditioned for further processing.

2.3. Transformation of Sample into Flour

The method of [21] was followed for preparation of snail meat flour (SMF). The freshly washed snail meat was dried at 50˚C for 24 hours. The dried material was then ground using an electric blender and sieved through a 250 µm mesh sieve. The resulting flour was sealed and stored in an airtight plastic container until further use.

2.4. Biscuit Formulation

Biscuit production was carried out following the method of Khullar *et al.* [22]. Nine biscuit formulations (F2 - F9) were prepared using composite blends of wheat flour and snail meat flour, as presented in **Table 1**, where varying proportions of wheat flour were replaced with snail meat flour. The control biscuit (F1) was prepared using 100% wheat flour and standard ingredients.

Margarine and sugar were creamed together for 5 minutes. All dry ingredients except flour were then mixed thoroughly. The mixture was transferred into a bowl, and flour, together with sodium bicarbonate, salt, and baking powder, was added gradually with continuous mixing for 15 minutes until a smooth dough was obtained.

Table 1. Formulation of cookies.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Wheat flour	100	90	80	70	60	50	40	30	20	10
Snail flour	0	10	20	30	40	50	60	70	80	90
Sugar (g)	35	35	35	35	35	35	35	35	35	35
Margarine (g)	48	48	48	48	48	48	48	48	48	48
Milk (g)	10	10	10	10	10	10	10	10	10	10
Sodium bicarbonate (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt (g)	o.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vanilla (g)	2	2	2	2	2	2	2	2	2	2

A portion of the dough was placed on a clean surface and rolled using a rolling

pin to a thickness of 5 mm. The dough was cut into desired shapes using a biscuit cutter and placed on a baking tray. Baking was carried out at 100˚C for 20 minutes. After baking, the cookies were removed and cooled at room temperature on a clean tray. The cooled cookies were packaged in polyethylene sachets and sealed for storage.

2.5. Physical Attributes of the Cookies

The method described by Feumba *et al.* [23] was used to evaluate the physical properties of the cookies. The thickness of the cookies was determined by stacking five biscuit samples, measuring the height with a digital Vernier calliper, and calculating the average value. The diameter of cookies was obtained by measuring the diameter of five biscuit samples placed edge to edge; then the average value was considered. The spread ratio was calculated by dividing the diameter by the thickness. The weight of cookies was measured as average values of five individual cookies with the help of an analytical weighing balance.

2.6. Sensory Evaluation

Sensory evaluation of each biscuit sample was conducted using a 9-point hedonic scale with a panel of 30 untrained assessors. The 9-point hedonic scale, ranging from "dislike extremely" (1) to "like extremely" (9), is widely used for measuring consumer acceptability of food products [24] [25]. The experimental biscuit formulations were prepared alongside a control sample made from 100% wheat flour. Each sample was evaluated for colour, aroma, texture, taste, appearance, mouthfeel, aftertaste, and overall acceptability. Panelists scored the samples according to their degree of liking for each sensory attribute, and mean scores were computed to determine product preference and acceptability.

2.7. Determination of the Micronutrient Composition of Cookies

Minerals (Ca, K, Na, Mg, Fe and Zn) were determined using atomic absorption spectrophotometry (AAS), iCE vo1.3 as described by AOAC [26].

2.8. Best Sample Selection

The best samples were selected based on the results obtained from sensory evaluation (those with the highest overall acceptability for each formulation) and high iron content, using the sensory desirability score and iron desirability score with 60% given to sensory properties and 40% given to iron.

$$Di = \frac{I - I\min}{I\max - I\min!}$$

$$Ds = \frac{S - S\min}{S\max - S\min}$$

$$\text{Compositescore} = 0.5DI + 0.5DS$$

where we assigned different weights for sensory property being 60% and iron being 40%.

2.9. Animal Bioassay

2.9.1. Ethical Clearance

For the use of animals (rats) in this study, ethical clearance was obtained from the University of Buea Institutional Animal Care and Use Committee (UB-IACUC), with reference number UB-IACUC No. 12/2024. All animal procedures were conducted in accordance with established Animal Experimentation Guidelines and relevant institutional and international standards for the care and use of laboratory animals.

2.9.2. Animal Housing and Acclimatization

In this study, 36 seven-week-old male Wistar rats weighing 150 g - 200 g were used and housed in polycarbonate cages. They were kept at room temperature (25˚C) with a cycle of 12 h of light and 12 h of darkness. They had access to their basal diets (65.7 g of Corn starch, 155 g Dextrinase, 100 g Sucrose, 50 g Fiber, 40 g Soil Oil, 35 g Mineral Mix, 10 g Vitamin Mix, 5.5 g Cystine, 0.008 g BHT and 10 g Fish Meal) and water ad-libitum and were fed on standard rat chow during the acclimatization period which lasted for a week so the animals could get used to their new environment.

2.9.3. Animal Grouping, Induction, and Experimental Design

The best samples obtained (F4, F5, F6) from the results of the proximate analysis and sensory analysis were used in the animal study. The animals were divided randomly into six groups of six rats each. The rats were fed a total of 100 g of diet per day. The groups were divided as follows:

Group 1 (normal group = ND): Rats fed with a 100% standard diet;

Group 2 (negative control = NC): Rats induced with anaemia and fed with the standard diet;

Group 3 (positive control = PC): Rats induced with anaemia and fed with iron sulphate (0.35 mg/kg) supplemented feed;

Group 4 (test group 1 = TG1): Rats induced with anaemia and fed with formulated cookies 40% SMF (F4);

Group 5 (test group 2 = TG2): Rats induced with anaemia and fed with formulated cookies 50%SMF (F5);

Group 6 (test group 3 = TG3): Rats induced with anaemia and fed with formulated cookies 60%SMF (F6);

Before the study, the haemoglobin levels of the rats were measured. Phenylhydrazine (40 mg/kg) was then used to induce the rats with anaemia consecutively for 2 days. This was done by intraperitoneal injection. The rats were observed to see changes in their behaviour and physical appearance. The haemoglobin level was then measured to confirm that the rats were anaemic. Rats with haemoglobin levels less than 30% were considered anaemic [27].

2.9.4. Organ Collection, Weighing and Preparation of Homogenates

After euthanasia, the animals were placed in the dorsal decubitus position for the pectoral incision procedure to remove the organs from the carcass. The liver,

heart, heart, brain, spleen and kidney were removed, weighed on a scale and macroscopically analysed. Liver, lungs, heart and kidney tissue samples were stored in formol [28]. A homogenate was prepared, using 2.5 mg of liver, heart, and kidney tissue respectively, and 1 mL 50 mM HCL (pH 7.4). It was crushed in a mortar and then centrifuged at 1000 rpm for 20 minutes and the supernatant was collected.

2.9.5. Serum and Plasma Collection and Preparation

At the end of the 16 days of treatment, the animals fasted for 12 h on day 17 and sacrificed on day 18. Blood was collected by cardiac puncture and introduced in dry tubes and EDTA tubes. After 3 h, the fresh blood in dry tubes was centrifuged at 3500 rpm for 15 min. Serum was obtained and conserved at −18˚C for further analyses.

2.10. Analysis

2.10.1. Evaluation of Behavioural Test

On day 17 of the experiment behavioral test was conducted. An open-field test was used to evaluate the locomotory activity, level of exploration, and emotional reaction of animals [29]. The open field consisted of a surrounding square (40 cm × 40 cm) divided into 16 small squares and 1 centre field (10 cm × 10 cm) wall of 19 cm high (Brown *et al.*, 2007) [30]. On day 17, each rat was placed individually in the centre of the arena. The time spent at the centre, the number of crossings (the number of lines crossed by the rat), grooming (rapid cleaning movements of the forelegs towards the face and or body), rearing (the frequency of rats standing on their hind limbs), and faecal boli (the number of faecal pellets excreted by each individual rat) weight were recorded for a 5-minute duration.

2.10.2. Haematology

Whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and were immediately sent to the laboratory for full blood count, in particular, haemoglobin, erythrocytes, leukocytes, and platelets using an automated blood analyser.

2.10.3. Oxidative Stress

Oxidative stress was analysed by evaluating the level of oxidative stress markers in serum and organ homogenates.

Measurement of Nitric Oxide (NO)/Nitrite:

The NO quantification was done using the organ homogenates of the rats and serum through the griess reagent [31]. The NO quantification was done using the organ homogenates of the rats and serum through the griess reagent. The principle of nitric oxide (NO) determination using the Griess method involves the indirect measurement of its decomposition products, nitrite (NO^{2-}) and nitrate (NO^{3-}). This method necessitates the reduction of NO^{3-} to NO^{2-}, which is then quantified through the Griess reaction. The Griess reaction is a two-step diazotization process where NO-derived nitrosating agent, dinitrogen trioxide (N_2O_3), generated from the acid-catalyzed formation of nitrous acid from nitrite (or au-

toxidation of NO) reacts with sulfanilamide to produce a diazonium ion. This diazonium ion is then coupled to N-(1-naphthyl) ethylenediamine to form a chromophoric azo product that absorbs strongly at 540 nm [31].

The Griess reagent is reared by mixing 1% sulfanilamide and 0.1% naphthyl ethylene diamine in 2.5% phosphoric acid as describe by Fotio *et al.* [32]. The following were mixed in the spectrophotometer cuvette 100 µL of Griess Reagent, 300 µL of sample, 2.6 mL of deionized water. The mixture was incubated for 30 minutes and the absorbance read at 570 nm and the nitrite level was determined by using the sodium nitrite standard curve.

Malondialdehyde (MDA):

The assessment was conducted following Nemmiche *et al.* [33]. A homogenate of tissue (1 mL) was combined with 0.5 mL of a 20% trichloroacetic acid solution and 1 mL of a 0.67% thiobarbituric acid solution. This mixture was then incubated at a high temperature of 90˚C for 10 min in a water bath. Subsequently, the mixture was centrifuged and the absorbance of the supernatant was measured at 530 nm. The MDA concentration was quantified using the extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$, and the results were expressed as micromolar (µM) of MDA per gram of protein.

Reduced glutathione level:

The measurement was conducted following Alisik *et al.* [34]. Organ tissue homogenates (10 µL) were combined with Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) (1500 µL). The mixture was then incubated for one hour at room temperature and the absorbance was measured at 412 nm. The concentration of reduced glutathione was calculated using the molar extinction coefficient of 13,600/M × cm, and the results were expressed as micromolar (µM) of reduced glutathione per gram of protein.

Catalase activity:

The evaluation was conducted following Sinha [35]. Initially, 25 µL of homogenate and 375 µL of phosphate buffer (0.1 M, pH 7.5) were combined. Hydrogen peroxide solution (100 µL, 50 mM) was then added to the mixture, and the reaction was halted after one minute by incorporating 1 mL of a dichromate/pure glacial acetic acid mixture. The tubes were heated to 100˚C for 10 minutes. Following the heating period, the absorbance was measured at 620 nm. The catalase activity was determined using a calibration curve and expressed as micromolar (µM) of H_2O_2 per minute per milligram of protein.

Superoxide Dismutase (SOD) Activity:

The assessment of superoxide dismutase (SOD) activity was conducted following the method described by Datkhile *et al.* [36]. A mixture was prepared by combining 67 µL of liver homogenate with 833 µL of carbonate buffer (50 mM, pH 10.2) and 100 µL of adrenaline solution (0.3 mM). The absorbance of this mixture was measured at 480 nm, 20 seconds and 80 seconds post-introduction of adrenaline. The specific activity of SOD was then calculated and expressed as SOD units per milligram of protein.

2.11. Histology

The organ samples previously kept in buffered formol (10%) were subjected to the following histological techniques. After dehydration, liver samples were fixed, trimmed, pre-embedded, embedded in paraffin (melting point: 56˚C ± 2˚C) [37] to form blocks, 5 µm thick sections from each sample were stained with haematoxylin-eosin and observed under light microscope (Olympus, GHBS, Japan) [38].

2.12. Statistical Analysis

Data was entered into spread sheet using Microsoft Excel and analysed using graphpad prism version 8.4.3 (686) (San Diego, CA, USA). Data was expressed as means ± standard deviation. Difference between group means was compared using one-way analysis of variance (ANOVA). Ordinary and individual analysis of group mean values was done using Turkey's Multiple Comparison Test as the post hoc test significant levels were measured at 95% confidence interval with significant differences set at $P < 0.05$.

3. Results

3.1. Physical Parameters Units of the Formulated Cookies

Table 2 shows the results of the physical parameters of the biscuits: the weight, diameter, thickness, and spread ratio of the samples.

Table 2. Physical parameters units of the cookies.

Formulae	Sample Codes	Weight (g)	Diameter (m)	Thickness (m)	Spread Ratio
F1	F1 (100%W, 0%S)	3.92 ± 0.54^{a}	3.77 ± 0.13	0.33 ± 0.04^{a}	11.42 ± 0.08^{a}
F2	F2 (90%W, 10%S)	2.72 ± 0.05^{b}	3.69 ± 0.09	0.21 ± 0.01^{b}	17.57 ± 0.05
F3	F3 (80%W, 20%S)	2.76 ± 0.07^{bc}	3.69 ± 0.07	0.31 ± 0.01^{ai}	11.90 ± 0.04^{ab}
F4	F4 (70%W,30%S)	2.80 ± 0.04^{bcd}	3.56 ± 0.32	0.22 ± 0.01^{bc}	16.18 ± 0.16
F5	F5 (60%W, 40%S)	3.15 ± 0.22^{bcde}	3.42 ± 0.21	0.27 ± 0.05^{abci}	12.66 ± 0.13
F6	F6 (50%W, 50%S)	3.49 ± 0.28^{aeg}	4.05 ± 0.08	0.56 ± 0.03^{d}	7.23 ± 0.05c
F7	F7 (40%W, 60%S)	3.28 ± 0.18^{cdfg}	3.79 ± 0.11	0.50 ± 0.04^{de}	7.58 ± 0.07^{cd}
F8	F8 (30%W, 70%S)	4.10 ± 0.40^{ah}	3.99 ± 0.14	0.48 ± 0.05^{ef}	8.31 ± 0.095
F9	F9 (20%W, 80%S)	4.10 ± 0.05^{ahi}	4.03 ± 0.13	0.54 ± 0.05^{defg}	7.46 ± 0.09bc
F10	F10 (10%W, 90%S)	3.83 ± 0.12^{afghi}	3.94 ± 0.15^{j}	0.41 ± 0.01^{fh}	9.60 ± 0.08

Note: Values are mean ± SD, values with different superscripts (a)-(i) within column are significantly differently ($p < 0.05$).

3.2. Mineral Composition of the Cookies

The results of mineral content of the fortified cookies were displayed in Table 3. The findings demonstrated that the addition of SMF increased the iron, magnesium, calcium and zinc content of the fortified cookies. On the contrary, the content of potassium, and carbohydrate decreased as the substitution progresses. Results regarding the mineral content, of the samples have shown significant differ-

ences ($p < 0.05$) among all the parameters evaluated in this study.

Table 3. Mineral content of the cookies in mg/100g.

Formulae	Iron	Magnesium	Calcium	Potassium	Zinc	Sodium
F1 (100%W, 0%S)	0.71 ± 0.02^{defg}	24.79 ± 0.79^{a}	199.25 ± 1.06^{a}	130.90 ± 0.71^{a}	0.01 ± 0.01^{h}	41.77 ± 0.69^{a}
F2 (90%W, 10%S)	1.29 ± 0.69^{cdef}	48.57 ± 0.16^{b}	319.25 ± 1.06^{b}	116.75 ± 0.08^{b}	19.05 ± 0.69^{b}	41.32 ± 0.67^{ad}
F3 (80%W, 20%S)	2.46 ± 0.68^{bcdj}	194.45 ± 0.86^{c}	239.25 ± 1.06^{c}	130.86 ± 0.78^{a}	121.98 ± 0.01^{c}	33.06 ± 0.68^{b}
F4 (70%W,30%S)	1.88 ± 0.00^{bcde}	49.04 ± 0.82^{db}	399.25 ± 1.06^{d}	273.49 ± 0.75^{c}	27.32 ± 0.03^{d}	41.79 ± 2.03^{ade}
F5 (60%W, 40%S)	3.04 ± 0.03^{bcij}	243.35 ± 1.48^{e}	239.25 ± 1.06^{ce}	145.73 ± 1.41^{d}	8.86 ± 0.63^{e}	42.25 ± 2.00^{adef}
F6 (50%W, 50%S)	3.62 ± 0.63^{bhij}	63.62 ± 0.88^{f}	375.25 ± 1.06^{f}	102.37 ± 0.80^{e}	6.20 ± 0.03^{ai}	41.83 ± 0.68^{adefg}
F7 (40%W,60%S)	4.21 ± 0.03^{ahij}	193.96 ± 0.17^{cg}	239.25 ± 1.06^{ceg}	77.26 ± 0.10^{f}	6.82 ± 0.68^{ai}	41.33 ± 1.37^{adefgh}
F8 (30%W, 70%S)	4.79 ± 0.00^{ahi}	145.98 ± 0.85^{h}	239.25 ± 1.06^{cegh}	89.23 ± 0.78^{g}	16.14 ± 0.00^{f}	41.81 ± 0.70^{adefghi}
F9 (20%W, 80%S)	5.37 ± 0.69^{ah}	1357.00 ± 0.16^{i}	8559.25 ± 1.06^{i}	116.75 ± 0.09^{bh}	135.40 ± 0.69^{g}	41.32 ± 2.74^{adefghi}
F10 (10%W, 90%S)	5.95 ± 0.06^{a}	149.05 ± 0.84^{bdj}	8619.25 ± 1.06^{bj}	17.22 ± 0.76^{i}	6.70 ± 0.06^{a}	41.68 ± 0.44^{c}

Note: Values are mean ± SD, values with different superscripts (a)-(j) within column are significantly different ($p < 0.05$).

3.3. Sensory Evaluation of Formulated Cookies

This was a crucial aspect of the cookies making process as it helps to assess the quality and acceptability of the final product from consumer's perspective. The results in Table 4 showed that the overall acceptability decreased with increased snail meat flow substitution. The information gotten could be used to make adjustments to recipe ingredients or processing techniques to improve the overall quality and desirability of the cookies. Significant difference in organoleptic properties of different biscuit formulas. Acceptance reduced with increase concentration of SMF since SMF has poor organoleptic properties. 100% wheat flour was the most accepted.

Table 4. Sensory properties of cookies.

Formulae	Colour	Consistency/texture	Flavour	Taste	Overall acceptability
F1	8.1	7.7	7.9	7.7	7.85
F2	7.2	6.6	6.8	6.8	6.85
F3	6.7	6.9	6.6	7.2	6.85
F4	5.7	5.9	6.4	6.9	6.225
F5	7.3	6.9	6.9	7.2	6.95
F6	7.1	7.3	7	6.8	7.1
F7	5	5.8	6.6	7	6.15
F8	5.9	6.7	6.1	6.6	6.32
F9	5.5	6	6.3	6.5	6.07
F10	5.2	7	6.7	6.8	6.42

3.4. Behavioral Test of Animals

The behavioral test (presented in Table 5) was done to assess the effect of anaemia and also the cookies on physical reactions of the rats. Centre duration—the amount of time animal spends in the centre if testing apparatus which indicates effects of treatment on its exploration and risk-taking tendency. Line 9 crossing provides information about animal activity and locomotion. Rearing—the ability of animal standing upright on its hind legs. It's an indicator of it exploratory behavior Grooming—its self-cleaning and maintenance activities performed by animals such as licking, scratching of their skin, increase grooming indicates increase stress.

Table 5. Behavioural parameters of different experimental animals.

Groups	Centre duration	Line crossing	Rearing	Grooming
ND	2.75 ± 0.96^{a}	58.75 ± 11.76^{a}	16.50 ± 3.11	2.50 ± 1.00
NC	7.50 ± 1.73^{b}	32.25 ± 2.22^{b}	11.75 ± 2.99	2.50 ± 0.5
PC	5.50 ± 1.29	60.00 ± 7.35^{ad}	15.25 ± 0.96	3.50 ± 1.73
TG1	5.25 ± 0.50	39.25 ± 7.54^{bc}	15.75 ± 7.27	2.25 ± 1.26
TG2	6.00 ± 1.41	48.00 ± 4.69^{abcde}	13.50 ± 2.65	3.25 ± 1.50
TG3	6.00 ± 1.83	57.50 ± 6.45^{ade}	13.25 ± 1.71	2.75 ± 0.96s

Note: ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)).

3.5. Effect of Treatment on Hematological Measurements of Rats

The hematology test as seen in Table 6 was done to assess the effect of the cookies on hematological parameters such as hemoglobin and others. There was an observed increase in hemoglobin and red blood cells upon consumption of the cookies by rats.

Table 6. Haematological measurements of the experimental rats.

Sample	WBC ($\times10^{3}$µl)	Neutro (%)	Lymph (%)	Mono (%)	RBC ($\times10^{6}$µl)	HB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	Platelet ($\times10^{3}$µl)	MPV (fl)
ND	2.80 ± 1.41^{bc}	21.70 ± 0.02^{af}	73.00 ± 0.01^{a}	5.25 ± 0.02^{bc}	56.05 ± 0.02^{ad}	13.70 ± 0.28^{c}	41.15 ± 0.00^{c}	74.40 ± 0.00^{ac}	24.70 ± 0.13^{c}	3.28 ± 0.25^{a}	10.75 ± 0.07^{c}
NC	2.90 ± 1.31^{b}	16.60 ± 0.01^{b}	62.80 ± 0.03^{b}	5.15 ± 0.04^{b}	33.25 ± 0.04^{b}	9.90 ± 0.14^{b}	14.45 ± 0.01^{b}	112.30 ± 0.68^{b}	29.35 ± 0.07^{b}	8.42 ± 0.67^{b}	9.35 ± 0.04^{b}
PC	3.60 ± 1.41^{a}	25.05 ± 0.03^{a}	73.00 ± 0.02^{a}	6.30 ± 0.01^{a}	55.60 ± 0.28^{a}	16.10 ± 0.00^{a}	48.25 ± 0.00^{a}	83.90 ± 0.00^{a}	27.90 ± 0.00^{a}	3.21 ± 0.54^{a}	10.55 ± 0.07^{a}
TG1	2.30 ± 1.41^{d}	20.25 ± 0.01^{cf}	75.15 ± 0.01^{a}	4.85 ± 0.03^{bcd}	57.85 ± 0.03^{acd}	13.05 ± 0.49^{cd}	41.25 ± 0.02^{cd}	88.55 ± 0.21^{a}	28.20 ± 0.70^{ae}	3.30 ± 0.94^{a}	9.45 ± 0.05^{bd}

Continued

TG2	2.80 ± 2.82^{bce}	11.60 ± 0.03^{d}	81.70 ± 0.04^{c}	6.35 ± 0.02^{a}	60.10 ± 0.02^{cde}	11.05 ± 1.34^{be}	33.20 ± 0.03^{e}	88.90 ± 0.70^{a}	29.55 ± 0.21^{bd}	2.83 ± 0.85^{a}	9.85 ± 0.06^{e}
TG3	3.50 ± 2.12^{a}	15.70 ± 0.02^{be}	87.35 ± 0.02^{d}	5.05 ± 0.03^{bcde}	56.05 ± 2.75^{acde}	16.00 ± 0.56^{a}	44.60 ± 0.21^{acd}	86.40 ± 0.56^{ac}	28.75 ± 0.21^{e}	4.99 ± 0.89^{c}	10.25 ± 0.07^{f}

Note: ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)); RBC = red blood cell count, WBC = leukocyte count, MCV = mean corpuscular volume, MCH= mean corpuscular haemoglobin. Values are mean ± SD (n = 6). Data were analyzed by one-way ANOVA followed by Bonferroni post-test for multiple comparisons shown on the table with letters (a, b, c, d, e). Same letters show the p-values are not significant ($p > 0.05$).

3.6. Effect of Treatment on Oxidative Stress in Organs and Serum

Table 7 shows the oxidative stress marker levels in the different experimental groups of rats. The results indicate an increase in glutathione (GSH), catalase, and superoxide dismutase (SOD) activities, while malondialdehyde (MDA) and nitric oxide (NO) levels were observed to decrease.

Table 7. Oxidative stress in organs and serum.

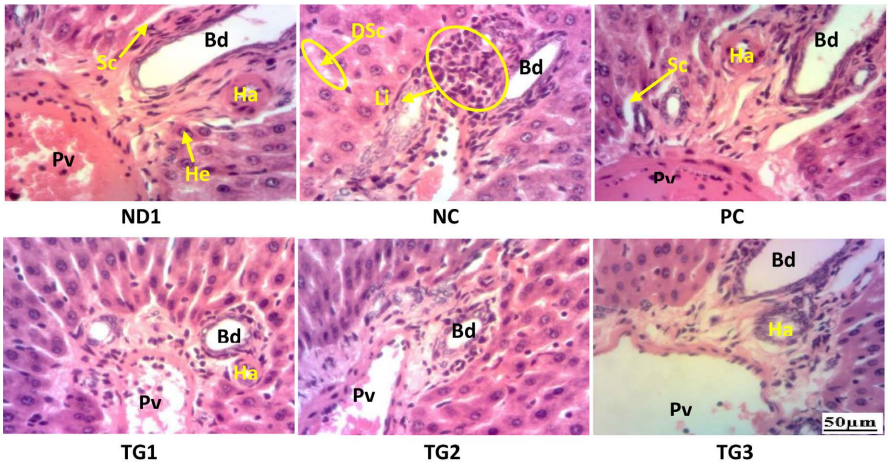
Malondialdehyde (MDA (uM/g))							
Test Groups		**ND**	**NC**	**PC**	**TG1**	**TG2**	**TG3**
Organs	**Brain**	1.55 ± 0.33^{a}	4.58 ± 0.20^{c}	0.78 ± 0.07^{b}	3.41 ± 0.20^{f}1	2.51 ± 0.05^{e}	0.55 ± 0.23^{d}
	Liver	1.62 ± 0.02^{a}	3.31 ± 0.04^{c}	0.41 ± 0.03^{b}	2.64 ± 0.24^{f}	2.10 ± 0.01^{e}	1.86 ± 0.14^{d}
	Spleen	2.60 ± 0.30^{a}	5.48 ± 0.14^{c}1	0.64 ± 0.18^{b}	4.31 ± 0.31^{f}2	3.43 ± 0.20^{e}	2.43 ± 0.07^{d}
	Kidney	1.55 ± 0.04^{a}	1.49 ± 0.03^{c}	1.12 ± 0.02^{b}	3.16 ± 0.03^{f}	2.29 ± 0.06^{e}	1.53 ± 0.02^{d}
Serum		0.04 ± 0.03^{a}	0.25 ± 0.02^{c}	0.15 ± 0.02^{f}	0.04 ± 0.01^{bd}	0.07 ± 0.02^{de}	0.02 ± 0.01^{b}
Glutathione GSH (mol/mg)							
Test Groups		**ND**	**NC**	**PC**	**TG1**	**TG2**	**TG3**
Organs	**Brain**	35.44 ± 0.31^{a}	8.88 ± 0.86^{b}	21.35 ± 0.82^{c}	11.43 ± 1.12^{d}	13.94 ± 1.117^{e}	17.83 ± 0.36^{f}
	Liver	28.01 ± 2.18^{a}	3.78 ± 0.15^{b}	20.85 ± 0.40^{c}	5.55 ± 0.05^{bd}	11.10 ± 0.64^{e}	16.96 ± 0.40^{f}
	Spleen	83.79 ± 2.06^{a}	12.17 ± 1.65^{b}	70.93 ± 6.50^{c}	36.20 ± 3.67^{d}	44.89 ± 1.49^{e}	54.51 ± 2.89^{f}
	Kidney	52.90 ± 0.05^{a}	9.36 ± 2.63^{b}	30.02 ± 3.01^{c}	12.83 ± 0.26^{d}	15.81 ± 1.97^{de}	21.76 ± 0.52^{f}
Serum		31.84 ± 0.38^{a}	12.18 ± 0.41^{b}	19.11 ± 0.00^{c}	12.87 ± 0.07^{d}	18.01 ± 0.12^{e}	18.84 ± 0.23^{cf}
Superoxide dismutase (SOD (U/mg))							
Test Groups		**ND**	**NC**	**PC**	**TG1**	**TG2**	**TG3**
Organs	**Brain**	0.02 ± 0.01^{b}	0.01 ± 0.00^{a}	0.04 ± 0.02^{a}	0.02 ± 0.02^{a}	0.01 ± 0.03^{a}	0.03 ± 0.04^{a}
	Liver	0.08 ± 0.04^{b}	0.03 ± 0.01^{a}	0.06 ± 0.02^{a}	0.04 ± 0.07^{a}	0.05 ± 0.01^{a}	0.06 ± 0.06^{a}
	Spleen	0.01 ± 0.02^{b}	0.01 ± 0.01^{a}	0.01 ± 0.03^{a}	0.05 ± 0.01^{a}	0.09 ± 0.02^{a}	0.10 ± 0.00^{a}
	Kidney	0.05 ± 0.00^{b}	0.01 ± 0.00^{b}	0.04 ± 0.03^{ac}	0.03 ± 0.05^{abcd}	0.03 ± 0.03^{abcd}	0.04 ± 0.01^{abcd}
Serum		0.02 ± 0.01^{b}	0.01 ± 0.03^{b}	0.07 ± 0.01^{c}	0.06 ± 0.02^{bd}	0.05 ± 0.00^{bde}	0.06 ± 0.00^{bde}
Catalase (mmol of H_2O_2/min/mg)							
Test Groups		**ND**	**NC**	**PC**	**TG1**	**TG2**	**TG3**

Continued

Organs	Brain	48.63 ± 9.65[a]	2.46 ± 0.86[b]	31.01 ± 4.05[a]	9.94 ± 2.27[c]	15.82 ± 2.36[d]	22.89 ± 1.62[e]
	Liver	142.44 ± 1.55[a]	21.23 ± 6.25[b]	94.72 ± 1.79[af]	35.59 ± 3.83[c]	50.06 ± 0.98[dg]	59.99 ± 6.58[ade]
	Spleen	113.71 ± 9.13[a]	29.64 ± 6.38[b]	90.21 ± 9.83[a]	42.89 ± 5.57[c]	51.84 ± 6.51[cd]	71.98 ± 1.69[de]
	Kidney	102.47 ± 3.08[a]	14.06 ± 1.49[b]	49.02 ± 2.54[c]	17.68 ± 1.93[d]	23.91 ± 1.39[e]	30.26 ± 5.15[f]
Serum		81.70 ± 3.39[a]	12.05 ± 1.81[b]	44.26 ± 5.56[c]	23.00 ± 0.06[d]	24.30 ± 0.18[e]	33.87 ± 3.48[f]
		Nitrite oxide (NO (mM/mL))					
Test Groups		**ND**	**NC**	**PC**	**TG1**	**TG2**	**TG3**
Organs	Brain	0.01 ± 0.04[a]	0.18 ± 0.02[b]	0.01 ± 0.03[a]	0.11 ± 0.02[c]	0.07 ± 0.07[d]	0.03 ± 0.03[e]
	Liver	0.01 ± 0.01[a]	0.56 ± 0.05[b]	0.02 ± 0.01[ad]	0.26 ± 0.05[c]	0.07 ± 0.01[d]	0.03 ± 0.00[ad]
	Spleen	0.06 ± 0.02[a]	0.43 ± 0.01[b]	0.09 ± 0.01[a]	0.26 ± 0.02[c]	0.21 ± 0.01[cd]	0.18 ± 0.02[de]
	Kidney	0.01 ± 0.01[a]	0.16 ± 0.02[b]	0.03 ± 0.01[c]	0.14 ± 0.01[d]	0.08 ± 0.02[e]	0.05 ± 0.01[f]
Serum		0.01 ± 0.00[a]	0.17 ± 0.01[b]	0.02 ± 0.01[c]	0.13 ± 0.01[d]	0.11 ± 0.00[e]	0.09 ± 0.00[f]

Note: ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3 test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)).

3.7. Effect of the Treatment on Histology of the Liver



Note: He = Hepatocyte, Ha = Hepatic arteria, Pv = Portal vein, OPv = Obstruction of Portal vein, Bd = Bile duct, Sc = sinusoid capillaries, DSc = Dilatation of sinusoid capillaries, If = Inflammatory area, Li = Leukocyte infiltration. ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG 3 test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)).

Figure 1. Microphotographies of the Liver (Hématoxylin-éosine X 200).

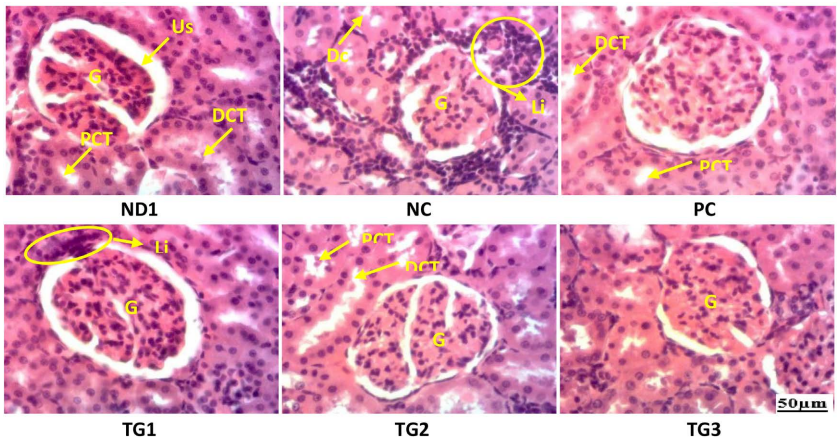
The microphotograph of the liver of normal animals (ND1) showed in **Figure**

1 a normal hepatic architecture with a well-defined portal space. The portal triad consisting of the portal vein (Pv), hepatic artery (Ha), and bile duct (Bd) was clearly observed. Hepatocytes (He) were arranged in normal hepatic cords separated by sinusoidal capillaries (Sc).

In the negative control group (NC), the hepatic parenchyma appeared thinned with an inflammatory focus around the bile duct characterized by diffuse infiltration of leukocytes. There was also evidence of obstruction of the portal vein (OPv) toward the inflammatory area as well as dilation of the sinusoidal capillaries (DSc), indicating hepatic injury and inflammation.

However, the groups of animals treated with the cookies at different doses, as well as the positive control group, showed a marked restoration of the overall liver architecture. At the end of the experiment, the liver tissue of animals treated with the extract appeared similar to that of the normal control group, suggesting a protective or restorative effect of the treatment on hepatic tissue. Similar histological recovery patterns following antioxidant treatment have been reported in experimental liver injury models.

3.8. Effect of the Treatment on Histology of the Kidney



Note: Us = Urinary space, G = Glomerulus, PCT = Proximal Convoluted Tube, DCT = Distal Convoluted Tube, Dc = Degeneration of cell nucleus, Li = leukocytes Infiltration. ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3 test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)).

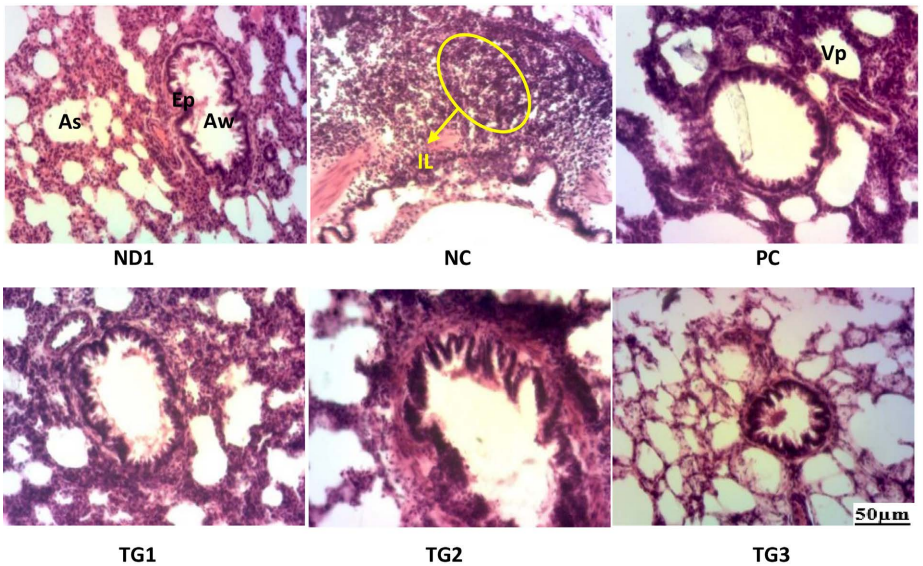
Figure 2. Microphotographies of the Kidneys (Hématoxyline-éosine × 200).

Figure 2 shows photomicrographs of the kidneys of animals from different groups. In the normal control, the kidney presents a normal arrangement with a glomerulus, a urinary space or Bowman's capsule and distal convoluted tubes with an enlarged lumen clearly distinct from the proximal tubules presenting a narrowed lumen. Induction of the pathology resulted in leukocyte infiltration,

tubular clarification, degeneration of hepatocytes, and difficulty in differentiating tubules in the negative control (NC). The animals treated with the cookies, in particular in TG2 and TG3 group, as well as the positive control group, favoured an improvement in renal architecture compared to the negative control. Though TG1 treatment group still presented a slight area of leukocyte infiltration.

3.9. Effect of the Treatment on Histology of the Lungs

Figure 3 below shows the cross sections of the lungs of the experimental rats. The microarchitecture of the lungs of normal animals (ND1) presents disseminated alveolar sacs, bronchi with a large lumen; these are ducts which subdivide into bronchioles to supply the alveolar sacs, a pseudostratified columnar bronchial epithelium with stereocilia, resting on the basal lamina. In negative control animals, a dissemination of leukocytes is observed on the tissue marking inflammatory foci. We also observe emphysema marked by a progressive destruction of the wall of the alveolar sacs making the differentiation of these alveolar sacs impossible. At the end of treatment, the TG1 and TG3 groups of animals as well as the positive control group (PC) present tissue repair with tissue integrity close to the normal control. Animals in the TG2 group present a thickening of the epithelium of the bronchial wall.

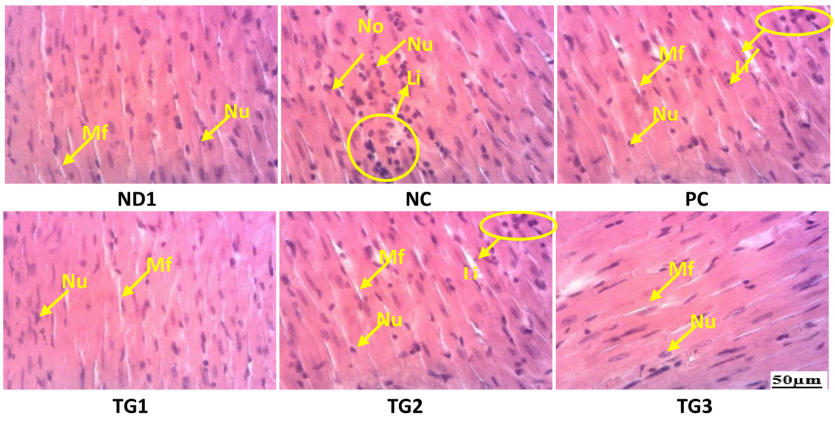


Note: **As** = Alveolar sac, **Aw** = Airway, **Li** = Leukocyte infiltration, **Em** = Emphysema, **Ep** = Epithelium, **Br** = Bronchus, **TEp** = Thickening of the epithelium of the bronchial wall. ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3 test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SMF (F6)).

Figure 3. Microphotographies of lungs (source: Hématoxyline-éosine × 200).

4. Effect of the Treatment on Histology of the Heart

Figure 4 shows the cross sections of the myocardium of the experimental rats. The microarchitecture of the heart presents a well-organized tissue composed of polynucleated cardiac striated muscle fibres. The animals in the control group showed disorganization of the muscle fibres, dispersion of the nuclei of these fibres and leukocyte infiltration. This disorganization is also observable in the positive control animals and the animals of the TG2 group. The groups of animals TG1 and TG3 present a normal structure close to the normal control.



Note: **Mf** = Muscular fibre, **Nu** = Nucleus, **Li** = Leukocyte infiltration. ND: normal group (group 1, Rats fed with a 100% standard diet); NC: negative control (Group 2, Anaemic rats fed with standard diet); PC: positive control (Group 3, Anaemic rats fed with iron sulphate (0.35 mg/kg) supplemented feed); TG1: test group 1 (Group 4 Anaemic rats fed with forrmulated cookies 40% SMF (F4)); TG2: test group 2 (Group 5 Anaemic rats with fed with formulated cookies 50% SMF (F5)); TG3test group 3 (Group 6 Anaemic rats fed with formulated cookies 60% SM (F6)).

Figure 4. Microphotographies of the heart (Hématoxyline-éosine × 200).

5. Discussion

5.1. Physical Properties of the Cookies

These are the properties which determine consumer's acceptance and measuring this can guide products development to meet consumer's preferences. As the percentage of SMF in the biscuit mix increased, both the biscuit's thickness and weight increased. This trend is likely because the addition of SMF boosts the protein content. The spread ratio or diameter is crucial for evaluating the flour's suitability for biscuit-making and the biscuit's ability to expand Akubor *et al.* [39]. As highlighted by Mahajan *et al.* [40], biscuit with a larger spread ratio is generally preferred.

5.2. Mineral Content of the Cookies

Iron serves as a crucial element in heamoglobin and myoglobin, facilitating the transport of oxygen throughout the body's tissues and cells. Additionally, it plays a vital role in enzyme activities that contribute to the synthesis of amino acids,

hormones, and neurotransmitters [39]. The suggested dietary allowance for iron is 10 mg for males and 15 mg for females over the age of 11. The iron concentrations in biscuit samples analyzed varied widely, from 0.71 to 5.95 mg per 100 grams. The cookies made from wheat flour had the lowest iron content (0.71 mg/100g), whereas those made with a blend of 90% snail meat flour (SMF) and 10% wheat flour contained the highest level (5.95 mg/100g). Statistically significant differences ($p < 0.05$) were noted among the iron contents of the different biscuit types. Furthermore, these findings underscore the assertion that snail meat is an excellent source of heme iron.

Magnesium is indispensable for the health of both hard and soft tissues in the body. It is crucial for metabolic processes and helps manage nerve and muscle activity, including cardiac functions Bakare *et al.* [41]. The magnesium levels in the biscuit samples varied considerably, from 24.79 to 1357.00 mg per 100 grams. There was a notable ($p < 0.05$) variation in the magnesium content across the biscuit samples. The suggested daily magnesium intake varies, being between 80 to 130 mg for children aged 1 to 8 years and 240 to 420 mg for adults aged 9 to 70 years Bakare *et al.* [41].

Calcium, alongside phosphorus, forms the structural basis of bones and teeth, contributing to their durability and resilience. Additionally, calcium is essential for proper nerve and muscle functioning, blood coagulation, heart rhythm regulation and cellular metabolism National Academies [41]. The calcium levels in biscuit samples spanned from 199.25 to 8559.25 mg per 100 grams. The cookies made entirely from wheat flour had the lowest calcium content (199.25 mg/100g), whereas those blended with 80% snail meat flour (SMF) and 20% wheat flour exhibited the highest level (8559.25 mg/100g). There were substantial ($p < 0.05$) variations in the calcium content among the biscuit samples.

Zinc is a vital trace element known for its antioxidant properties, membrane stabilization, and involvement in numerous zinc-dependent enzymes' activities Kaur *et al.* [43]. Research indicates that supplementing with zinc and iron enhances haemoglobin levels Nguyen *et al.* [42]. The zinc concentrations in biscuit samples varied widely, from 0.01 to 135.40 mg per 100 grams.

The sodium levels in the biscuit samples varied from 33.06 to 42.25 mg per 100 grams. Sodium is essential for maintaining fluid balance and nerve signaling Bakare *et al.* [41]. However, excessive sodium intake is linked to health problems such as hypertension and congestive heart failure Bakare *et al.* [41].

5.3. Sensory Evaluation of Formulated Cookies

There were notable differences among the biscuit samples in aspects such as colour, flavour, texture, taste, and overall acceptability. The cookies colour was predominantly affected by the SMF level, with higher SMF levels correlating with lower colour ratings. The texture and flavour were more affected by the wheat flour composition than the SMF level. Given that taste is a critical factor in food product approval, the samples' taste diminished as the SMF percentage increased.

Among the combinations tested, the biscuit made with 80% SMF and 20% wheat flour was the least preferred in terms of texture, taste, flavour, colour, and overall acceptability. On the contrary, the biscuit composed entirely of wheat flour was the most preferred [37]. Mahajan *et al.* [40] also reported similar trends in consumer acceptability of composite flour cookies.

5.4. Effect of Treatment on Haematological Parameters

Wistar rats were used for this study because their genetics and biological behavioural characteristics are closely related to those of humans Kaliste *et al.* [44]. Haemoglobin (Hb), the primary component of red blood cells, transports oxygen to different parts of the body Humphry *et al.* [45]. By the end of the study, the Hb levels in the negative control group were lower than those in the normal group due to iron deficiency Sinha *et al.* [46].

Hematocrit (HCT), defined as the ratio of red blood cell volume to total blood volume Khang *et al.* [47], showed reduced values in the negative control group.

MCV, MCH, and MCHC represent red blood cell indices Frerking *et al.* [48]. These were reduced in anaemic rats due to decreased haemoglobin synthesis Suzana *et al.* [49].

5.5. Effect of Treatment on Oxidative Stress of Serum and Organs

The body's primary defense against reactive oxygen species involves enzymes like SOD, CAT and GSH Ighodaro *et al.* [50]. MDA levels indicate lipid peroxidation while nitric oxide reflects inflammation Luo *et al.* [51]. Compared to controls, the negative control group exhibited lower antioxidant enzyme activities and higher oxidative markers Altun *et al.* [52].

At the end of treatment, antioxidant enzyme activities increased while MDA and NO decreased in treated groups. MDA reduction indicates decreased lipid peroxidation Ayala *et al.* [53]. Antioxidant enzymes protect cells from oxidative damage Halliwell *et al.* [54].

5.6. Effects of Treatment on Histology of Rat Organs

Livers of anaemic rats showed inflammation and necrosis but improved after treatment Sasse *et al.* [55]. Kidney damage including fibrosis and degeneration was also reversed Sasse *et al.* [55].

Lung tissue inflammation and cardiac degeneration observed in anaemic rats were reversed after treatment Salama *et al.* [56]. Nutrients and iron in snail-based cookies improved tissue regeneration Salama *et al.* [56].

6. Conclusions

A total of 10 cookie formulations were made. Based on the iron-to-acceptability ratio, three samples, F4 (70% w 30% S), F5 (60% w 40% S) and F6 (50% w 50% S) were selected and used in treating anaemia in test groups 1, 2 and 3, respectively. Results of the sensory evaluation showed that the acceptability of the cookies based

on their sensory attributes decreased with increased concentration of snail meat flour in the cookies.

The proximate and mineral composition indicated an increase in the nutritional profile with increased concentration of Snail meat flour in the cookies. Sample F10 with 10% wheat and 90% snail meat flour had the highest nutrient profile with 38.29% of proteins and 5.95 mg/100g of iron. Haematology results showed that rats which were fed on sample F6 with 40% wheat and 60% snail meat flour had the best haematological parameters with 3.50×10^3 WBC, 15.70% neutrophils, 87.3% lymphocyte, 5.05% monocytes, 56.05×10^6 µl RBC, 16.00 g/dl haemoglobin, 44.60% HC, 86.40 fl MCV, 28.75fl MCH, 4.99×10^3 µl platelets, 10.2 fl MDV. Effects of treatment on Oxidative stress markers indicated that levels of antioxidant enzyme activities (super oxide dismutase, catalase and glutathione) was highest in the serum and organ homogenates of rats which were fed with F_6 (40% wheat, 60% snail) while the indicators of lipid peroxidation (MDA) were found to be significantly lower in test group 3 which received F6 (40% wheat, 60% snail). NO was significantly lower in TG3. Therefore, increasing snail meat substitution increased antioxidant activities and reduced Oxidative stress in the animals. Photo Micrographs of the liver, kidney, lungs and heart of rats indicated that those of rats in the negative control groups had deformities but their overall architecture was found to be reversed after treatment with cookies containing the highest snail concentration, bringing their architecture close to that of the positive control group.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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