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Hematology of 2, 4 (Dinitrophenyl Hydrazine) Induced Anaemic Rat Administered with *Ficus capensis* Fruits and Leave Extract

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JIO and AIA managed the analyses of the study. Author NNU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Anemia is regarded as public health challenge and is predominant in developing countries due to nutritional deficiencies.

Aim/Objectives: The study was carried out to evaluate the biological properties of *Ficus capensis* fruits and vegetables on some haematological parameters in 2, 4 (*dinitrophenyl hydrazine*) induced anemic rat.

Materials and Methods: *Ficus capensis* leaves and fruits were separately plucked, sorted cleaned. Twenty male adult rats were purchased from the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were divided into 4 groups of 5 rats each on the basis of body weight. The rats in all the groups received 2, 4-DNPH (20 mg/kg body weight) once daily for 7 days to induce anaemia. Group I were fed rat chow alone, group 2 were fed rat chow with ferrous sulphate, group 3 were fed rat chow with *Ficus capensis leaves* extract and group 4 were fed rat chow with *Ficus capensis seed* extract. The aqueous extracts of the leaves and fruits were tested

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for haematinic effects in albino rats. Blood parameters such as Packed Cell Volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Haemoglobin concentration (Hb) were measured.

Results: The result showed that the mean PCV baseline of the rats were (38.72-39.24%), mean PCV of anemic rats (33.01- 34.60%) and the mean PCV of the rats after test of recovery were group 1 (34.10%), group 2 (51.81%), group 3 (40.20%) and group 4 (38.20%).The result showed that the mean HB baseline of the rats were (9.67-10.47 g/dl), mean HB of anemic rats (6.50- 7.10 g/dl) and the mean HB of the rats after test of recovery were group 1 (6.51 g/dl), group 2 (12.32 g/dl), group 3 (9.73 g/dl) and group 4 (9.69 g/dl). The results of the effect of the extracts on the haematological parameters indicated that oral administration of the aqueous extract of *Ficus capensis* leaves and fruits after 22 days exhibited a significant ($P < 0.05$) increase in haematinic activity by increasing the blood parameters Hb, PCV, WBC and RBC.

Keywords: Anemia; Ficus capensis; heamatology; fruits; leafy vegetables.

1. INTRODUCTION

Anaemia is one of the most common haematological defects affecting people of all ages [1]. Anaemia constitute a serious health problem in many tropical countries including Nigeria. Anaemia may be due to a single factor such as a nutritional deficiency or multifactorial [2]. Deficiency caused by faulty eating habit is especially prevalent in the children, adolescent and elderly population [3]. The vulnerable groups are infants, young children and women of childbearing age. It can cause developmental delays and behavioural disturbances in children [4]. In women of reproductive age, it reduces physical performance and increases the risk of preterm delivery and of having babies with a low birth weight [5]. With increasing statistics on the number of deaths resulting from haematopoietic disorders and blood related diseases, it has become very crucial to seek both economic and accessible alternative medicine that could serve as natural haematopoietic- stimulating agent [6].

The process of blood formation and haemostasis is termed haemopoiesis [7]. Blood parameters are the major indices of physiological, pathological, and nutritional status of an organism and changes in the constituents of blood when compared to normal values could be used to interpret the metabolic state of an animal [8]. It is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and lower animals [9]. These parameters include red blood cells count, haemoglobin concentration, packed cell volume, platelets count, total and differential white blood cells count [9].

Green leafy vegetables and fruits are particularly important in promoting health because of their rich nutrients content [10]. The fig tree, *Ficus capensis*, is a native of Western Asia and Africa. The first creature (Adam and Eve) ate the figs and made a covering of their naked bodies using the leaves. It is called “Opoto” in Yoruba part of Nigeria [11]. Fig tree belongs to the order of *Urticales* and family of *Moraceae* with over 1400 species. Leaves, fruits, and roots of *F. capensis* are used in native medicinal system in different ways such as gastrointestinal, respiratory, inflammatory, and cardiovascular disorders [12] Fruits of *F. capensis* can be eaten fresh or dried or used as jam [13]. Fig fruits contained the high levels of polyphenols, flavonoids, and anthocyanins and exhibited high antioxidant capacity [14].

Iron supplementation or fortification is commonly recommended to prevent iron deficiency anaemia, but current solutions have drawbacks such as dose limitation, food oxidation and short shelf life [15,16,17]. *Ficus capensis* leaves and fruits are used in folktale medicine in the management of anemia. There seems to be limited scientific evidence on the use of *Ficus capensis* in modulation of haematopoetic function and the mechanism by which this function may occur. Therefore, it is imperative to evaluate *Ficus capensis* leaves and fruits extracts’ against anemic rats.

2. MATERIALS AND METHODS

Ficus capensis leaves and fruits were collected from the ornamental garden in Amankpume Ozzi-Edem Nsukka Local Government Area, Enugu state, Nigeria. The sample was identified and authenticated at the Department of Agronomy, Faculty of Agriculture and Natural Resources, Enugu State University of Science and Technology.

Fig. 1. *Ficus capensis* leaves and fruits

2.1 Animal Experiment

2.1.1 Reagent

A 2, 4 *dinitrophenyl hydrazine* (2,4 DNPH) was obtained from BDH pool, England. All other chemicals and reagents were of analytical grade and were obtained from Sigma Aldian Company Ltd.

2.1.2 Preparation of extract (leaves and seed)

The extract were prepared by decoction of leaves and fruits of *Ficus capensis* for 1 hr, concentration of evaporation of reduced temperature and spray dried to obtain a fine powder. The solid extracts were dissolved in distilled water for trial studies. Ferrous sulphate used as control was supplied from University of Nigeria Nsukka (Medical Centre, Pharmacy Unit).

2.1.3 Experimental procedure

All procedures for using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria Nsukka.

2.1.4 Animal housing/ feeding

Twenty male adult rats were purchased from the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were divided into 4 groups of 5 rats each on the basis of body weight. The rats were housed individually in cages equipped to separate urine and faeces. The rats were fed on standard rat chow. The rats in all the groups received 2, 4-DNPH (20 mg/kg body weight) once daily for 7 days to induce anaemia. The *Ficus capensis* extract was made to provide 0.11 mg/day iron to the rats. The study lasted for 28 days. A 7- day acclimatization, a 7-day inducing anemia and a 14 - day feeding trial. The extract was administered orally through drinking water bottles ad libitum for 14 days. Commercial hematinic - ferrous sulphate was used as control. Group I were fed rat chow alone, group 2 were fed rat chow with ferrous sulphate, group 3 were fed rat chow with *Ficus Capensis leaves* extract and group 4 were fed rat chow with *Ficus Capensis* fruits extract. The weights of animals were recorded each day. Daily food intake and extract were also recorded to calculate nutrient intake.

2.1.5 Extract reconstitution

The leave and fruits extract obtained from 100 g sample of *Ficus capensis* extracted with 100 ml water was estimated. During acclimatization period, the least quantity of water taken by a rat per day was 5 ml. The recommended iron for rats per day is 0.11 mg/dl. The extract was made such as 5 ml of the extract would provide 0.11 ml/dl iron to the rats. Using dilution of standard solution equation

$$C_1V_1=C_2V_2 \quad (1)$$

2.2 Blood Sample Collection

Anemia was induced to the rats by administering 2, 4 DNPH (20 mg/kg body weight) once a day for the second 7 days of the study. The blood was collected from ophthalmic venous plexus located in the orbital sinus of the rat using a heparinized-capillary tube. Blood was collected on day 0, 7 and 22 for haematological determinations.

2.3 Heamatological Indices

The following biochemical indices were carried out. The haemoglobin level, red blood cell count, white blood cell and PCV.

2.4 Haemoglobin Level

The cyanomethamoglobin method as specified by The International Committee for Standardization in Haematology ICSH, [18] was used.

2.5 Red Blood Cell Count (RBC) and White Blood Cell Count (WBC) Materials

The method recommended by [19] for the estimation of RBC and WBC was used.

2.6 Statistical Analysis

Data collected was subjected to analysis of variance (ANOVA). Means was separated using Duncan's Least Significance Difference (LSD) Test [20]. Analysis were run using SPSS version 22 statistical Package for Social Science at $P<0.05$ significance.

2.7 Haematological Parameters of *Ficus capensis* Leaves and Fruits

Deficiency of iron is as a result of reduced hematological parameters. These blood parameters such as haemoglobin level, red blood cell count, white blood cell and PCV are useful and sensitive for the diagnosis of iron deficiency anaemia. They have been used to test the efficacy of *Ficus capensis* leaves and fruiuts.

3. RESULTS

3.1 Packed Cell Volume

The result of the mean Packed Cell Volume of the rat from day 0- day 22 were shown in Table 1. It was observed that at day 0, which was the first day after acclimatization, the mean PCV of the rats were between 38.72-39.24%. At day 7 when anemia was confirmed the mean PCV level decreased and were between 33.01-34.60%. At day 22, after treatment the PCV of the rats fed rat chow alone (group 1) decreased from 34.24-34.10%, the group 2 that received rat chow and ferrous sulphate increased from 33.01 to 43.81%, the group 3 that received rat chow and Ficus leaves extract increased from 33.70 to 39.20% while group 4 that received rat chow and Ficus fruits extract increased from 34.60 to 38.20%. There is significant different between the mean PCV of rat that received extract and the rats that received rat chow alone ($p< 0.05$). There is also significant different between the mean PCV of rat that received extract and the control that received rat chow and ferrous sulphate ($p< 0.05$). There was no significant different in the mean PCV of the rats that received both leaves and fruits extract ($p< 0.05$).

Table 1. Mean percentage packed cell volume (PVC) of rats fed rat chow alone, rat chow with ferrous sulphate, rat chow with *F.* leaves extract and rat chow with *F.* fruits extract in percentage (%)

Day	Rat chow	Rat chow with ferrous sulphate	Rat chow with *F.* leaves extract	Rat chow with *F.* seeds extract
0	$38.72^{a} \pm 0.16$	$39.24^{a} \pm 0.08$	$39.10^{a} \pm 0.35$	$38.98^{a} \pm 0.11$
7	$34.24^{a} \pm 0.33$	$33.01^{a} \pm 0.23$	$33.70^{a} \pm 0.56$	$34.60^{a} \pm 0.09$
22	$34.10^{c} \pm 0.28$	$43.81^{a} \pm 0.01$	$39.20^{b} \pm 0.10$	$38.20^{b} \pm 0.46$

Values are mean ± standard deviation of 3 replicate. Mean with different superscript letters along the sample row are significantly different at (P<0.05).

Keys: Day 0 = first day after acclimatization; Day 7 = When anaemia was confirmed; Day 22 = Last day of recovery

3.2 White Blood Cell

The result of the mean WBC of the rat from day 0- day 22 were shown in Table 2. It was observed that at day 0, which was the first day after acclimatization, the mean WBC of the rats were between 2.62- 2.92 (Counts/µL) ×10^3. At day 7 when anemia was confirmed the mean WBC level decreased and were between 1.22 – 1.30 (Counts/µL) ×10^3. At day 22, after the treatment the mean WBC of the rats fed rat chow alone (group 1) increased from 1.22-1.24 (Counts/µL), the group 2 that received rat chow and ferrous sulphate increased from 1.24-3.35 (Counts/µL), the group 3 that received rat chow and Ficus leaves extract increased from 1.30-2.48 (Counts/µL) while group 4 that received rat chow and Ficus fruits extract increased from 1.28-2.72 (Counts/µL). There is significant different between the mean WBC of rat that received extract and the rats that received rat chow alone ($p< 0.05$). There is also significant different between the mean WBC of rat that received extract and the control that received rat chow and ferrous sulphate ($p< 0.05$). There was no significant different in the mean WBC of the rats that received leaves and fruits extract ($p< 0.05$).

3.3 Red Blood Cell

The result of the mean RBC count of the rat from day 0- day 22 were shown in Table 3. It was observed that at day 0, which was the first day after acclimatization, the mean RBC count of the rats were between 2.89- 3.02 (Counts/µL) ×10^3. At day 7 when anemia was confirmed the mean RBC count level decreased and were between 1.36 – 1.79 (Counts/µL) ×10^3. At day 22, after the treatment the mean RBC of the rats fed rat chow alone (group 1) decreased from 1.47-1.37 (Counts/µL), the group 2 that received rat chow and ferrous sulphate increased from 1.37-3.32 (Counts/µL), the group 3 that received rat chow and Ficus leaves extract increased from 1.36-2.69 (Counts/µL) while group 4 that received rat chow and Ficus fruits extract increased from 1.79-2.70 (Counts/µL). There is significant different between the mean RBC count of rat that received extract and the rats that received rat chow alone ($p< 0.05$). There is significant different between the mean RBC count of rat that received extract and the control that received rat chow and ferrous sulphate ($p< 0.05$). There was no significant different in the mean RBC count of the rats that received leaves and fruits extract ($p< 0.05$).

3.4 Haemoglobin

The result of the mean HB level of the rat from day 0- day 22 were shown in Table 4. It was observed that at day 0, which was the first day after acclimatization, the mean HB level of the rats were between 9.67-10.47 (g/dl).At day 7 when anemia was confirmed the mean HB level

Table 2. Mean white blood cell of rats fed rat chow alone, rat chow with ferrous sulphate, rat chow with *F.* leaves extract and rat chow with *F.* fruits extract in (Counts/µL) ×10^3

Day	Rat chow	Rat chow with ferrous sulphate	Rat chow with *F.* leaves extract	Rat chow with *F.* seeds extract
0	2.92^a ±0.06	2.62^a±0.82	2.88^a ±0.40	2.90^a±0.01
7	1.22^a ±0.29	1.24^a ±0.04	1.30^a ±0.20	1.28^a±0.72
22	1.24^c ±0.01	3.35^a ±0.10	2.48^b ±0.51	2.72^b±0.24

Values are mean ± standard deviation of 3 replicate. Mean with different superscript letters along the sample row are significantly different at (P<0.05); Keys: Day 0 = first day after acclimatization; Day 7 = When anaemia was confirmed; Day 22 = Last day of recovery

Table 3. Mean red blood cell of rats fed rat chow, rat chow with ferrous sulphate, rat chow with *F.* leaves extract and rat chow with *F.* fruits extract in (Counts/µL) ×10^6

Day	Rat chow	Rat chow with ferrous sulphate	Rat chow with *F.* leaves extract	Rat chow with *F.* seeds extract
0	3.02^a±0.22	2.97^a±0.35	2.89^a±0.28	3.00^a ±0.01
7	1.47^b±0.24	1.37^c±0.70	1.36^c±0.56	1.79^a±0.10
22	1.37^c±0.01	3.32^a±0.98	2.69^b±0.33	2.70^b±0.20

Values are mean ± standard deviation of 3 replicate. Mean with different superscript letters along the sample row are significantly different at (P<0.05); Keys: Day 0 = first day after acclimatization; Day 7 = When anaemia was confirmed; Day 22 = Last day of recovery

Table 4. Mean haemoglobin level of rats fed rat chow, rat chow with ferrous sulphate, rat chow with *F.* leaves extract and rat chow with *F.* fruits extract in (g/dl)

Day	Rat chow	Rat chow with ferrous sulphate	Rat chow with *F.* leaves extract	Rat chow with *F.* seeds extract
0	10.67^{b}±0.47	10.87^{b} ±0.98	10.47^{a} ±0.02	10.40^{a} ±0.23
7	6.50^{a} ±0.30	7.10^{a} ±0.00	6.89^{a}±0.76	6.91^{a} ±0.54
22	6.51^{c} ±0.13	12.32^{a} ±0.36	10.73^{b} ±0.24	10.69^{b} ±0.40

Values are mean ± standard deviation of 3 replicate. Mean with different superscript letters along the sample row are significantly different at (P<0.05)

Keys: Day 0 = first day after acclimatization; Day 7 = When anaemia was confirmed; Day 22 = Last day of recovery

decreased and were between 6.50- 7.10 (g/dl). At day 22, after the treatment the mean HB level of the rats fed rat chow alone (group 1) increased from 6.50- 6.51 (g/dl), the group 2 that received rat chow and ferrous sulphate increased from 7.10-12.32 (g/dl), the group 3 that received rat chow and Ficus leaves extract increased from 6.89-9.73 (g/dl) while group 4 that received rat chow and Ficus fruits extract increased from 6.91- 9.69 (g/dl). There is significant different between the mean HB level of the rat that received extract and the rats that received rat chow alone (p< 0.05). There is also significant different between the mean HB level of the rat that received extract and the control that received rat chow and ferrous sulphate (p< 0.05). There was no significant different in the mean HB level of the rats that received the leaves and fruits extract (p< 0.05).

4. DISCUSSION

4.1 Packed Cell Volume

The result indicated that Packed Cell Volume of the rat in day 0 which was the day after acclimatization ranged from 38.72 to 39.24%. Group 2 which is the control had highest value of PCV and group 1 had the lowest value for PCV but there were no significant different (P<0.05) among all the groups. On day 7 when anemia was confirmed, the mean PCV values ranged from 33.01 to 34.60. Group 4 rats have the highest mean PVC value while group 2 had the least mean PCV value. There was no significant different among the groups of rat on day 7 (p< 0.05). This was because day 0 was the day after acclimatization and all the rats were healthy having a mean PVC that ranges between 38.72 to 39.24. Day 7 was the day anemia was confirmed after administration of 2, 4-*dinitrophenyl hydrazine* to induce anemia. This was as a result of the fact that they were given the same treatment. A significant increase was observed in the PCV of animals in group 2 (rat chow and ferrous sulphate), 3 (rat chow and leave extract) and 4 (rat chow and fruit extract). The increase in the PCV of the control was statistically difference and higher than the rats fed trial diets. Packed cell volume (PCV) is a measure of the portion of the blood volume that is made up by red blood cells. This is in line with the work of Oyedeji [21] who reported an increase in PCV of two severely anaemic paediatric patients when served *T. occidentalis* extract sweetened with milk. Olaniyan and Adeleke [22] served thirty anaemic pregnant women from rural communities with an oral mixture of *T. occidentalis* leaves, milk and raw egg three times a day for seven days and observed a significant rise in mean packed cell volume (PCV). This further show that the aqueous leaf and fruits extract of *Ficus capensis* has potential to stimulate haematopoiesis together with control ferrous sulphate. The reduction observed in the mean PCV of the group fed rat chow alone on day 22 is due to the fact that rat chow is not good sources of iron.

4.2 White Blood Cell

The mean white blood cell count ranged from 2.62 to 2.92 (Counts/µL) $\times 10^3$ on day 0 which was the day after acclimatization, with group 1 having the highest mean value while group 2 (Rat chow with ferrous sulphate) had the least mean WBC value. There were no significant difference (P<0.05) in all the groups on day 0. This was as a result of the fact that all the groups of the rats were healthy after acclimatization. There was no significant difference in all the groups of rats on day 7 after administration of 2, 4 – *dinitrophenyl hydrazine* to the rat and anaemia was confirmed. On day 22, the group fed rat chow and ferrous sulphate (control) had the highest increase in WBC although the groups fed the trial diet had haematopoietic properties. On day 22, the rat fed with rat chow and *Ficus* *capensis* fruits extract had 2.70 Counts/µL $\times 10^3$ which is within the range of the value for normal white blood cell (2.62 - 2.92 counts/µL) $\times 10^3$) Table 4. There is a significant increase in the mean white blood cell (2.48 Counts/µL) $\times 10^3$) of the group fed with *Ficus capensis* leaves extract and rat chow on day 22 but it was not up to the value within the normal range (2.62 - 2.92 counts/µL $\times 10^3$). White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders [23].

4.3 Red Blood Cell

The mean red blood cell count level of the rats fed rat chow alone, rat chow and ferrous sulphate, rat chow and *Ficus capensis* leave extract and rat chow and *Ficus capensis* seed extract showed that on day 0 which was the first day after acclimatization, ranges from 2.89 to 3.02 counts/µL $\times 10^3$. There was no significant different (P<0.05) on the red blood cell count of the groups studied at this level. There was no significant difference in all the groups of rats on day 7 after administration of 2, 4 – *dinitrophenyl hydrazine* to the rat and anaemia was confirmed. On day 22 which was the day tested for recovery. The rats fed rat chow and *Ficus capensis* fruits extract had 2.70 Counts/µL $\times 10^3$ which showed a significant increase Table 4. There is a significant increase in the mean red blood cell count (2.69 Counts/µL) $\times 10^3$) of the group fed with *Ficus capensis* leaves extract and rat chow on day 22 but is not up to the value within the normal range (2.89 – 3.02 counts/µL $\times 10^3$). Red blood cells are able to carry oxygen because of a special protein called haemoglobin [7]. The decrease observed in group 1 shows that rat chow is not a good source of iron. The increase observed in the RBC count of the rats fed trial diet was as a result of the fact that both leaves and fruits extract of *Ficus capensis* possess an appreciable quantity of heamatinic properties which lead to bioavailability of iron thereby increasing the level of RBC count of the laboratory animals.

4.4 Haemoglobin

On day 0 which was the first day after acclimatization, the mean haemoglobin level ranges from 10.67 to 12.47 g/dl. There was no significant difference (P>0.05) among all the groups of rats. On day 7 which was the day when anaemia was confirmed in all the rats. All the animals were confirmed anaemic and there were no significant difference (P>0.05) among all the groups. However, the mean haemoglobin ranged from 5.50 to 6.10 g/dl. Rahayu et al. [24] observed that the average level of hemoglobin at baseline and depletion period was 12.0±0.3 and 5.7±0.2 g dL^{-1}, respectively. Fukui et al. [25]; Handelman and Levin [26] noted that reticulocytes are immature red blood cells that are released into the blood stream to become mature red blood cells. When the iron status is low, reticulocytes are released into the bloodstream with an insufficient amount of haemoglobin. The result of this study was similar with earlier findings [27] who reported that the rats which were fed an iron-deficient diet, 3 ppm iron, *ad libitum* for 16 days resulted in anemic rats, hemoglobin level was 6.1±0.2 g dL^{-1} and a control diet with standard feed, 48 ppm iron for 16 days, the hemoglobin level was 14.4±0.4 g dL^{-1} with the initial hemoglobin level before fed with standard feed was 11 g dL^{-1}. On day 22 which was the test day for recovery, the mean haemoglobin level ranged from 6.51 to12.32 g/dl. There were a significant increase in the rats fed ferrous sulphate, *Ficus capensis* leaves and seed extract (group 2, 3 and 4) which is within the normal range (Table 4). The results of this study showed that *Ficus capensis* leaves and seed extract significantly increases Hb level because they are good sources of iron and vitamin C. Vitamin C enhances the absorption of non heam iron which is the form of iron in plant food materials. Iron is an essential micro mineral in the formation of hemoglobin level. Hemoglobin is composed of four polypeptide chains of globins that each of which contains heme molecules [28,29]. There are various proteins with important roles in cellular physiology that require iron to operate their functions. In mitochondria, the electron transport chains, ferrochelatase enzyme and hemoglobin are needed for bioenergetics and oxygen transport [30].

5. CONCLUSION

The results of this study indicated that the aqueous extracts of the leaves and fruits of *Ficus capensis* may possibly serve as an acceptable blood booster in an anaemic condition. It is suggested that the extracts may have a direct effect on the body system that produces blood cells and contains constituent(s) that can interact and stimulate the formation and secretion of reticulocytes and erythropoietin growth factors/committed stem cells. This suggests that the aqueous extracts of the leaves of these plants possesses haematinic properties. This haematopoietic effect may be due to the high content of different micronutrient especially iron and vitamin C in the leaves and fruits of this plant. This finding supports the ethno and medicinal use of leaves and seeds extract of *Ficus capensis* for the treatment of anaemia.

ETHICAL APPROVAL

All procedures for using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria Nsukka.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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