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ASSESSMENT OF THE TOXIC EFFECTS OF DIFFERENTLY PROCESSED NIGELLA SATIVA BASED DIETS ON THE HAEMATOLOGICAL INDICES OF ALBINO RATS

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ABSTRACT

The effects of differently processed *Nigella sativa* seed-based diets on haematological indices of albino rats were investigated. The aim was to test if *N. sativa* seeds processed using different methods would have toxic effects on some haematological parameters of albino rats; adopting completely randomized design. The experimental animals were fed with diets supplemented with 10% raw (RAN), Parboiled (PAN), Boiled (BON), Roasted (RON) and control diet at 0% supplementation. The animals were fed for six weeks before being sacrificed and blood collected from them for analysis. The white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), packed cell volume, mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, mean corpuscular volume, monocytes, neutrophils, lymphocytes, eosinophils and Basophils were determined using standard analytical techniques ans Analysis of variance (ANOVA) as the tools for analysis with the mean separation. The results showed that all treatment groups have higher values (concentrations) of PCV, Hb and WBC than the control group. The animal group that received PAN had highest concentrations of PCV (47.30±2.40), RBC (18.7±0.60) and Hb (19.98±0.70) while the group fed with BON showed least concentration of PCV (45.65±2.92), RBC (7.6±1.63) and Hb (18.92±9.02). RAN with 12.43±8.02 x 10^3mm^3 white blood cells had the highest concentration of WBC while the PAN with 6.81±0.70 x10^3mm^3 white blood cells had the lowest concentration of WBC among the treatment groups. The calculated values for neutrophil/lymphocyte ratio (NLR) are as follow in descending order 0.65, 0.63, 0.58, 0.58 and 0.54 for RON, RAN, PAN, NOD and BON respectively. These values were not too high to cause any major damage to the organisms that were fed with the different formulated diets. The results showed that *Nigella sativa* seeds, processed in all the methods assessed, has no toxic effect on the haematological parameters of albino rats.

Keywords: *Nigella sativa,* heamatological parameters, neutrophils, lymphocytesase soil quality.

INTRODUCTION

Medicinal plants play a crucial role in health care needs of people around the world especially in developing countries (Rao *et al*., 2004). Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family: Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background (Ahmad, 2013). Many research works revealed its wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia. It is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia (Khare, 2004). In obesity and dysporea and are administered internally in intermittent fever (Ahmad *et al*., 2004). The seeds of *Nigella sativa* L. are commonly used as a spice known as black cumin seeds. According to Ahmad *et al*., (2013). *N. sativa* has a lot of traditional therapeutic uses (Botnick *et al*., 2012). Haematological parameters are important indices of the physiological and pathological status for both animals and humans. It can also be used to determine the extent of deleterious effect of foreign compounds, including plant extracts, on the blood of the albino rats (Odeyemi *et al*., 2009). Haematological parameters can also be used to explain blood relating functions of plant extract or its products (Odeyemi *et al*., 2009; Adeneye *et al*., 2006; Akinnawo, 2002).

A lot of works had been carried out by many researchers on the nutritional qualities and medicinal purposes using oils or extracts of the seeds. This work, however, aims at carrying out the toxicological testing of the seeds with different processing methods (normal rat diet, raw, parboiled, boiled and roasted) to formulate diets, determine the effects on the haematological parameters of the rats.

MATERIALS AND METHODS

Location of the study

The study was carried out at the Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora.

Rat feed source

Nigella sativa seeds (imported from Saudi Arabia) was purchased from a local herb store in Oyo Town, Oyo-State, Nigeria. The seeds were cleaned under running tap water for 10 minutes. Rinsed twice with distilled water and air dried in an oven at 40°C overnight until a constant weight was attained. Normal rat feed was purchased from commercial animal/rat feed dealer, Al-Had Animal Care, aketan Titun, Oyo. The different experimental feeds were formulated with commercial rat feed plus 10% differently processed *N. sativa* seed powder. The animals were fed with diets formulated with the formulated diets for six weeks.

Experimental design

Experimental design adopted for this was completely randomized design (CRD). A total of thirty male albino rats were used for the experiment. Six (6) rats were randomly selected and assigned to each group as a treatment allocated for a rat feeding feeding experiment group to their respective diets (T1-T5).

Experimental period

The rats were fed their assigned diets for forty-two days (seven weeks) and supplied with clean drinking water.

Treatments

Group 1: Normal rat diet - (Control group)
Group 2: Raw *N. sativa* seed supplemented diet
Group 3: Parboiled *N. sativa* seed supplemented diet
Group 4: Boiled *N. sativa* seed supplemented diet
Group 5: Roasted *N. sativa* seed supplemented diet
All animals were served with clean tap water
At the end of the 35 days feeding experiment, the rats were sacrificed. The rats were anaesthetized with chloroform, cut from the lower abdomen to the thoracic region and the aorta cut to collect blood.

Chemical methods

Five millilitre of blood was collected from the cut aorta of each rat/replicate into separate EDTA sample bottles for the determination of the haematological parameters. Whole blood was collected into heparinized capillary tubes and sealed at one end with plasticine for the determination of packed cell volume (PCV) or haematocrit for each rat.

Determination of Packed Cell Volume (PCV): PVC) was done using the macrohaematocrit method (Dacie and Lewis, 2001).

Determination of Platelets: The platelets were determined by diluting the blood in one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were counted in a definite area using the rulings of an improved Neubaucer counting chamber. Their characteristic Mauve-pink colour was used in their identification

Haemoglobin Estimation

The conventional method (Sahli's haemoglobinometer) was employed for the estimation of haemoglobin (Hb) content of the blood. Using the Sahli haemoglobinometer, the colour of the test solution was filled to 20ml mark with 10N hydrochloric acid. 0.02ml of blood was added and the content of the test tube will be mixed with glass rod. It was left for 5 minutes (for the haemoglobin to be changed into acid haematin). More acid was thereafter added. The mixture was stirred until the colour of the test solution matched that of the coloured glass standard. The level of the fluid in the tube was read and the haemoglobin content will be expressed as a percentage

Determination of MCH, MCHC) and MCV): MCH, MCHC and MCV were calculated from the values obtained from RBC, PCV and Hb contents. They were calculated thus as shown below:

$$\text{Mean corpuscular haemoglobin (MCH)} = \frac{\text{Haemoglobin content}}{\text{Red blood cell counted}} \times \frac{10}{1}.$$

$$\text{Mean corpuscular Haemoglobin concentration (MCHC)} = \frac{\text{Haemoglobin content}}{\text{Packed cell volume}} \times \frac{100}{1}$$

$$\text{Mean corpuscular volume (MCV)} = \frac{\text{Packed cell volume}}{\text{Red blood cell count}} \times \frac{10}{1}.$$

Determination of leucocytes (differential white blood cell count)

The differential white blood cell counts (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were done by making a thin film of blood on a smooth-edged slide. It was allowed to dry on a bench protected from dust, ants, flies, and other insects. The blood film was fixed a covered staining jar of methyl alcohol for 3 minutes. Ten (10) ml of May Grunwald Stain (mixture of 5g of May Grunwald powder and 1 litre of methanol) and 10ml of buffered water (pH 6.8) was mixed thoroughly. The smear was covered with the dilute May Grunwald stain for 3 minutes. The stain was tipped off and replaced with diluted Giemsa's stain (5%) for 9 minutes The stain was washed off with buffered water (pH 6.8) and clean water will be dropped on the slide which was allowed to stay for 30 seconds. The water was tipped off and the slide will be allowed to dry. It was then examined microscopically (McArthur microscope) for the identification of :

Neutrophils (cytoplasm stained pink with small mauve granules),

Eosinophils (cytoplasm stained pink with large red granules),

Basophils (cytoplasm contained dark mauve-blue granules)

Monocytes (cytoplasm stained dull grey-blue)

Lymphocytes (cytoplasm stained blue).

DATAANALYSIS

The results obtained from the data were subjected to Analysis of variance (ANOVA) according to Onuh and Igwemma (1999). Significant means at $p \leq 0.05$ were separated using Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

Table 1 showed the results of the effects of differently processed *Nigella sativa*-based diets on the haematological indices of albino rats. The result showed that the values ranged between 47.30 (PAN) and 45.60 (BON) for packed cell volume, 19.98 and 15.08 $10^6/mm^3$ for haemoglobin concentration, 18.7 and 7.67 % for red blood cell, 59.50 and 57.64 % for mean corposcular volume, 19.74 and 16.20 % for mean corpuscular haemoglobin, and 33.79 and 24.90 % for mean corpuscular haemoglobin concentration for the highest and the lowest respectively.

The parboiled and roasted seeds had a significant increasing effect ($p \leq 0.05$) on the RBC, PVC, Hb and MCV. This may be as result of some residual anti-nutrients present in the feed which must have affected the parameters. On the other hand, the result showed a significant decreasing effect ($P \geq 0.05$) in MCHC and MCH. RBC which is important in the diagnosis of anaemic condition increased significantly in the raw seed when compared with the control, though it was without a definite trend in its increment while MCHC and MCH which are also important in the diagnosis of anaemic conditions all decreased significantly ($p<0.05$) in boiled seeds. MCHC decreased as the concentration of the cooked sample of *N. sativa* seed increased in the feed mix while MCH did not show any definite pattern of decrease as the sample concentration or ratio increased. Since RBC indices are not as important as direct RBC observation, the four substances may still be said to have heamatinic effects.

Table 2 showed the differential counting of leucocytes in the blood of Albino rats fed differently processed *Nigella sativa* seed-based diets. From the results, BON has the highest value for white blood cells (15.44 x $10^3 mm^3$); other values are as follow (x $10^3 mm^3$): 12.43 (RAN); 7.32 (RON); 6.81(PAN) and 6.48 (NOD). The values for monocytes are as follows (in % from highest to lowest): 1.8, 1.16, 1.16, 1.2 and 0.5 for BON, PAN, RON, NOD and RAN respectively; neutrophils: 38.70, 38.33, 36.40, 35.80 and 29.12 for RON, RAN, NOD,PAN and BON respectively; Lymphocytes: 62.80, 61.80, 60.80, 60.50 and 59.50 for BON, NOD, PAN, RAN and RON respectively; eosinophils: 0.66, 0.41, 0.40, 0.16 and 0.15 for RAN, NOD, BON, PAN and RON respectively; basophils:1.2, 0.66, 0.33, 0.2 and 0.00 for BON, RON, PAN, NOD and RAN respectively. The calculated neutrophils/lymphocytes ratios (NLR) gave the following values 0.65, 0.63, 0.58, 0.58 and 0.46 respectively for RON, RAN, NOD, PAN and BON.

Haematological parameters are important indices of the physiological and pathological status for both animals and humans. It can also be used to determine the extent of deleterious effect of foreign compounds, including plant extracts, on the blood of the albino rats (Odeyemi *et al*., 2009). Haematological parameters can also be used to explain blood relating functions of plant extract or its products (Odeyemi *et al*., 2009; Adeneye *et al*., 2006; Akinnawo, 2002). The neutrophils, basophils, eosinophils, monocytes and WBC showed a significant increase ($p \leq 0.05$) in boiled seed-fed animals; there was no significant difference ($P \geq 0.05$) in the control, raw, parboiled and roasted seeds. White blood cells (WBC) are important in defending the body against infection (Aboderin and Oyetayo, 2006), the white blood cell count, however, cannot give a definite or specific information, but the result of a differential WBC counts narrows down to give specific information about infections, toxicity allergy and immuno-suppression and poisoning (Aboderin and Oyetayo, 2006). Calculated neutrophils- to- lymphocytes ratio (NLR) is an important index to determine problems with the animals. The higher the NLR the 'sicker' is the animal. In this study, the values for NLR are as follow, in descending order 0.65, 0.63, 0.58, 0.58 and 0.54 for RON, RAN, PAN, NOD and BON respectively. This means that RON had the highest value and higher damaging effect on the haematological parameter, while BON had the lowest value and the lowest damaging effect on the haematological parameters of the albino rats. An isolated rise in neutrophil count, and, consequently, an elevated NLR, can be observed in several conditions: bacterial or fungal infection (Lowsby *et al.,* 2015; Niu *et al.* 2021) acute stroke (Li *et al.,* 2021), myocardial infarction (Lee *et al.,* 2016), atherosclerosis (Adamstein *et al.,* 2021), severe trauma (Park, 2017), cancer (Lee *et al.,* 2021) , post-surgery complications (Fest *et al.,* 2017) and any condition characterized by tissue damage that activates systemic inflammatory response (SIRS). NLR. Though, is a proven independent prognostic factor of morbidity and mortality in several diseases, its normal cut-off value is still under debate. Forget *et al*. (2017), in a large retrospective case–control study, observed that normal NLR values in an adult, non-geriatric population in good health may be between 0.78 and 3.53. However, the NLR values obtained in this study are not too high to signify any damage to the organisms that were fed with the different formulated diets.

CONCLUSION AND RECOMMENDATIONS

The result of the haematology/toxicity study showed that *Nigella sativa*, processed as raw, parboiled, boiled and roasted, has the potential of defending the body against infection and also has haematinic and blood enhancing quality. Further studies on chronic and histopathological toxicity determination are recommended to be carried out so as to ascertain the effect of *Nigella sativa* seed on the different organs of the albino rats

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Authors' contributions

OLO conceived the study, conducted the analysis and drafted the manuscript.
RMO took part in the study design, data generation and analysis plan.
OTO and AAI provided technical and material support and assisted in the analysis plan.
All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Table 1: effects of differently processed *nigella sativa*-based diets on the haematological indices of albino rats

Experimental groups	Packed cell volume (%)	Haemoglobin ($x10^6/mm^3$)	Red blood cell (%)	Mean corposcular volume (%)	Mean corpuscular haemoglobin (%)	Mean corpuscular haemoglobic concentration (%)
NOD	45.60±2.91	15.08±0.90	7.92±0.55	57.64±0.59	19.04±0.43	33.09±0.43
RAN	46.16±3.92	19.93±5.90	13.43±7.73	58.08±0.77	19.63±0.43	33.79±0.43
PAN	47.30±2.40	19.98±0.70	18.7±0.60	59.26±1.22	19.74±0.55	33.30±0.34
BON	45.65±2.92	18.92±9.02	7.6±1.63	59.5±68.72	16.20±6.23	24.90±3.74
RON	46.06±3.90	19.16±3.90	13.02±5.73	58.00±0.67	19.36±0.13	33.69±1.43

Table 2: Differential counting of leucocytes in the blood of Albino rats fed differently processed *Nigella sativa* seed-based diets

Experimental Group	White Blood cells ($x10^3mm^3$)	Monocytes (%)	Neutophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	NLR
NOD	6.48±0.81	1.2±0.64	36.4±2.84	61.8±3.22	0.4±0.304	0.2±0.13	0.58
RAN	12.43±8.02	0.5±0.32	38.33±2.33	60.5±2.50	0.66±0.61	0.0±0.00	0.63
PAN	6.81±0.70	1.16±0.60	35.8±3.10	60.8±3.40	0.16±0.24	0.33±0.31	0.58
BON	15.44±23.78	1.8±61.22	29.12±31.25	62.8±38.73	0.4±3.23	1.2±53.72	0.46
RON	7.32±0.49	1.16±0.60	38.7±2.23	59.5±2.87	0.16±0.24	0.66±0.31	0.65

Key: NOD= Normal diet (Control); RAN =Raw N. sativa diet; PAN=Parboiled; BON=Boiled; RON=Roasted, NLR = Neutrophil /Lymphocyte Ratio