

Serum lipid profiles of albino rats fed with differently processed *Nigella sativa*-based diets

¹Oyekunle, L. O.; ²Raji, M. O.; ¹Amuzat, A. I.; & ¹Olaniyi, T. O.

¹Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria. ²Department of Animal Health and Protection, Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria.

Correspondence Author: looyesuccess@gmail.com

Keywords: *Nigella Sativa*, Albino Rats, Lipid Profile, Artherosclerosis, Cardiovascular Disease

Abstract

This study was conducted to evaluate the serum lipid profile of albino rats fed with differently processed *N. sativa*-based diets. Thirty male albino rats (*Ratus norvegicus*) randomly divided into five groups of six each and kept in the separate cages were used for the study. The treatment groups were given rat chow pellets containing raw (RAW), parboiled (PAN), boiled (BON) and roasted (RON) *N. sativa* dose at 10% supplementation while the control group (NOD) was fed with rat chow pellet without supplementation with *N. sativa*. The rats were sacrificed, at the end of 35 days, to collect blood samples for serum lipids evaluation. The plasma levels of Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein (HDL) and Low-density lipoprotein (LDL) were determined. The results showed that RAN gave the highest value (in mg/dl) for TC (56.08 ± 1.72) while BON gave the lowest value (45 ± 1.90). The highest value for TG (72.66 ± 5.32) by RAN and the lowest value by PAN. RAN gave the highest value (28.15 ± 0.83) for HDL and the lowest value by BON (22.83 ± 2.34); for LDL, PAN gave the highest value (13.6 ± 0.94) and the lowest by BON (10.03 ± 0.90). Decrease in HDL and a concomitant increase in LDL and serum cholesterol is indicative of hyperlipaemia, a risk factor of cardiovascular diseases. The increase in total cholesterol observed in the rats fed RAN, PAN, RON-formulated diets above NOD, decrease in the HDL observed in PAN, BON and RON, and increase in the levels of LDL in PAN, RAN and RON, though not too significant, may predispose animals to artherosclerosis and other related diseases. The results,

however, showed that the *N. sativa* seeds processed in any of the methods used in this study is safe for consumption as far as lipid profile is concerned.

Introduction

Plants are the richest bioresources of drugs for traditional systems of medicine, food supplement, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs (Arunkumar and Muthuselvan, 2009). Plant are extensively used in agriculture, human therapy, veterinary and related scientific research (Manssor *et al.*, 2002). Examples of such plant are; garlic, oat, ginkgo biloba, ginger, pigeon pea, *Nigella sativa*, etc (Sultan *et al.*, 2009). *Nigella sativa* Linn is an annual herb that belongs to the family Ranunculaceae and is most extensively investigated for its therapeutic purposes (Aggarwal *et al.*, 2008; Kamal *et al.*, 2010). *Nigella*'s original name originates from the Latin word "niger," which means "black," referring to the plant's seed colour (Mahr, 2009). Black cumin is a genus of annual plants in the Ranunculaceae family with approximately 14 species. English common names of *N. sativa* L. are black cumin, fennel flower, nutmeg flower, black seed, black caraway, Roman coriander, damascena, devil in-the-bush, and wild onion seed (Kulloli, 2016; Sultana *et al.*, 2015). The hierarchal classifications of black cumin are plants (kingdom), vascular plants (subkingdom), seed plants (super division), flowering plants (division), Dicotyledons (class), Magnoliidae (subclass), Ranunculales (order), Ranunculaceae (family), *Nigella* (genus), and *Nigella sativa* L. (species) (Kartesz, 2019). A number of works had been carried out and reported by many researchers on the nutritional qualities and medicinal purposes of the seeds using oils or extracts of the *Nigella sativa* seeds. This work, however, aims looking at the effects of the seeds on the lipid profile of albino rats and so evaluating the toxicological testing of the seeds in another direction by using whole seeds of *N. sativa* used as raw, parboiled, boiled and roasted by using them to formulate diets that were be used to feed albino rats and compare the effects of the different diets on the lipid profiles and those of the normal rat diets.

Materials and methods

Area of the study: The study was carried out in the Department of Science Laboratory Technology (Chemistry Option) of the Oyo State College of Agriculture and Technology, Igboora.

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. *N. sativa* seeds were divided into four portions and the different portions were treated as: Raw seeds, seeds that were parboiled, seeds that boiled and seeds that were roasted. The processed dried seeds were milled and kept in an air tight container to avoid moisture absorption. Each portion was mixed with rat pellet powder and water into different at 10% and the dough was baked in an oven at 40°C until constant weight was obtained

Experimental animals: Thirty (30) male albino rats, aged 7-9 weeks were used for the study. The animals were kept in metabolic cages in groups in hygienic environment. A total of thirty rats, divided into five groups, six rats in each group.

Normal rat feed purchased from commercial rat feed supplier in Ibadan. 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:

Group 1: Normal rat diet (DON)- (Control group)

Group 2: Raw *N. sativa* seeds (RAN)

Group 3: Parboiled *N. sativa* seeds (PAN)

Group 4: Boiled *N. sativa* seeds (BON)

Group 5: Roasted *N. sativa* seeds (RON)

All animals were served with water

Experimental procedure: Animal were weighed on the first day of the experimental feeding as initial weight. Then weekly weighing were systematic done until the end of the seventh week where the final weight was recorded. Feed intake were determined weekly for each replicate by weighing feed that was supplied minus the residue feed. At the end of the experiment, the animals were sacrificed by anaestizing and dissecting from the lower abdomen to the upper thoracic region.

Five ml of blood were collected from the cut aorta of rat/replicate into separate EDTA sample bottles. The serum was obtained by centrifugation of whole blood. All samples were stored at -4°C for later serum analysis

The determination serum lipids was carried out as follows: The total cholesterol (TC) in serum was determined using the enzymatic end-point method of Siedel *et al.* (1983); serum triglyceride (TG) was determined by the enzymatic colorimetric according to the method of Tietz (1990) using commercial kit from Randox Laboratories; serum HDL cholesterol was determined following the of Jacobs *et al.* (1990). and serum LDL concentration was calculated applying the Friedwald equation (Friedwall *et al.* 1972).

Statistical analysis: Data will be presented as Mean \pm standard error of mean. Group comparison will be analysed using ANOVA and significant difference between control and experimental groups will be done by least significant difference(LSD) and student's t-test

Results and discussion

Table 1 showed the lipid profile of albino rats fed with differently processed *Nigella sativa*-based diets while Table 2 showed the ratios of lipid fraction. From Table 1, it could be seen that the total cholesterol (TC) values ranged between 45.33 mg/dl and 56.08 mg/dl; with the rats fed with the diet supplemented with raw *N. sativa* seeds (56.08 mg/dl) having the highest value while rats fed with diet supplemented with boiled *N. sativa* seeds had the lowest value (45.33 mg/dl); the normal rat diet gave a value of 49.50 mg/dl. The highest value for triglycerides (TG) was given by raw *N. sativa*-based diet while the parboiled *N. sativa*-based diet gave the lowest value. These values are however lower than the normal values for albino rats which was given by Ihedioha *et al.* (2013), which is 113.02 mg/dl. For high density lipoproteins (HDL), the highest value (28.15 mg/dl) was recorded for the raw *N. sativa*-based diet and the lowest (22.83 mg/dl) for boiled *N. sativa*-based diet. The highest value of 13.60 mg/dl was given for low density lipoprotein by the parboiled seed while the lowest value (12.44 mg/dl) was recorded for normal rat diet. Ihedioha *et al.* (2013) reported 50.28 mg/dl as the normal value for albino rats. In terms of toxic effects of the differently processed diets, the values given by the TG, TC/HDL and

LDL/HDL are very useful. According to Hodis *et al.* (1999), elevated plasma triglycerides (TG) had been implicated in the development of cardiovascular diseases (CVD) and such elevation is associated with obesity, pro-inflammatory and pro-thrombic biomarkers and type II diabetes which predispose to CVD. So the elevated level of TG in the raw seeds is a pointer that if taken in large quantity in the form it can lead to incidence of CVD. The TC/HDL and LDL/HDL ratios correlate with cardiovascular diseases and are thus useful indices for atherogenicity (Kamisah *et al.*, 2005; Oladipo *et al.*, 2005). Elevated ratios of TC/HDL were recorded in the test diets, but the levels are not as high as to cause any damage to the organs of the body. The same things were seen in the LDL/HDL ratios for test diets. It had also been reported that HDL- cholesterol does not contribute to arteriosclerosis (hardening of the artery due to deposition of thrombotic plaque in the artery) because it favours the delivery of cholesterol from peripheral sites to the liver for elimination (Lawn, 1992).

Table 1: Lipid profile of albino rats fed with differently processed *Nigella sativa*-based diets

Experimental group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal rat diet	49.50±5.89	59.14±8.32	25.58±3.29	12.44±1.65
Raw <i>N. sativa</i> -based diet	56.08±1.72	72.66±3.33	28.15±0.83	12.60±0.75
Parboiled <i>N. sativa</i> -based diet	50.75±3.59	58.48±1.07	24.92±2.11	13.60±0.94
Boiled <i>N. sativa</i> -based diet	45.33±1.90	63.05±8.29	22.83±0.95	10.03±0.90
Roasted <i>N. sativa</i> -base diet	50.15±1.93	62.05±3.11	24.65±0.92	12.48±0.68

HDL=High Density Lipoprotein; LDL= Low Density Lipoprotein

Table 2: Ratios of lipid fractions

Experimental group	TC/HDL	LDL/HDL
Normal rat diet	0.15	0.48
Raw <i>N. sativa</i> -based diet	1.99	0.44
Parboiled <i>N. sativa</i> -based diet	2.03	0.54
Boiled <i>N. sativa</i> -based diet	1.98	0.43
Roasted <i>N. sativa</i> -base diet	2.03	0.51

TC = Total cholesterol; HDL=High Density Lipoprotein; LDL= Low Density Lipoprotein

Conclusion

The findings of the this study with differently processed *N. sativa*-based diets showed that *Nigella sativa* has no appreciable toxicity, but beneficial effects on the predisposition of sustained intake to the plasma lipid profile as it relates to TC levels; meaning that the consumption of *N. sativa* seeds, in whatever methods as considered by this work does not contribute to the aetiology of cardiovascular disease, arising from alterations in plasma TC concentrations and TC/HDL ratio. The consumption of *N. sativa* seed is, therefore, good for the body.

Acknowledgements

We would like to thank the Tertiary Education Trust Fund (TETFUND) that provided the fund for the study as well as the publication of the paper and the Management of Oyo State College of Agriculture and Technology, Igboora (our place of work)

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.

Funding

This research was funded by Tertiary Education Trust Fund (TETFUND)

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria.

²Department of Animal Health Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria.

References

- Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Tharakan, S. T., Sung, B. and Anand, P. (2008). Potential of spice-derived phytochemicals for cancer prevention. *Journal of Planta Medica*, 74 (13): 1560-1569.
- Arunkumar, S. and Muthuselvan, M. (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* (L.) against clinical pathogens. *World Journal of Agricultural Science*. 5 (5): 572-576.
- Friedwald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low – density lipoprotein in plasma without the use of the ultracentrifuge. *Clin. Chem.*, 18: 499-502.
- Hodis, H. N., Mack, W. J., Krauss, R. M. (1999). Pathophysiology of triglyceride rich lipoproteins in atherosclerosis: clinical aspects. *Clin. Cardiol.*, 22: 1115-1120.
- Ihedioha, J. I., Noel-Uneke, O. A. and Ihedioha, T. E. (2013) Reference values for the serum lipid profile of albino rats (*Rattus norvegicus*) of varied ages and sexes. *Comparative Clinical Pathology*, 22 (1): 93 - 99.
- Kamal, A., Arif, J. M. and Ahmad, I. Z. (2010). Potential of *Nigella sativa* L. seed during different phases of germination on inhibition of bacterial growth. *Journal of Biotechnology Pharmaceutical Research*, 1(1): 09-13.
- Kamisiah, Y., Adam, A., Wan-Ngah, W. Z., Gapor, M. T., Azizah, O., Marzuki, A. (2005). Chronic intake of red palm oil and palm olein produce beneficial effects on plasma lipid profile in rats. *Pakistan J. Nutr.*, 4 (2): 89-96.
- Kartesz, J. T. (2019). Classification for kingdom plantae down to species *Nigella sativa* L. In *Natural Resource Conservation Service*. United States Department of Agriculture. plants.usda.gov/java/ClassificationServlet?source=display&classid=NISA2
- Kulloli, S. K. (2016). *Redefining black cumins* (Issue May). Spice India English. <https://www.researchgate.net/publication/303550909>
- Lawn, R. M. (1992). Lipoprotein(a) in heart disease. *Sci. Am.*, 266: 26-32.
- Mahr, S. (2009). *Love-in-a-mist*. University of Wisconsin Garden Facts. <https://pddc.wisc.edu>.
- Mansour, M. A., Nagi, M. N., El-Khatib, A. S. and Al-Bekairi, A. M. (2002). Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. *Cell Biochemistry Function*, 20(2): 143-151.
- Oladiipo, A., Regina, N. H., Florence, I., Olugbenga, A. (2005). Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria. *Lipid Health Dis.*, 4: 19.
- Siedel, J., Haagele, E. O., Ziegnhorn, J., Wahlefed, A. (1983). Reagents for determination of serum total cholesterol with improved lipolytic efficiency. *Clin. Chem.*, 29:1075-1080.

- Sultan, M. T., Butt, M. S., Anjum, F. M., Jamil, A., Akhtar, S., and Nasir, M. (2009). Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pakistan Journal of Botany*, 41(3), 1321-1330.
- Sultana, S., Asif, H. ., Akhtar, N., Iqbal, A., Nazar, H., and Rehman, R. . (2015). *Nigella sativa*: Monograph. *Journal of Pharmacognosy and Phytochemistry*, 4 (4), 103-106.
- Tietz, N. W. (1990). *Clinical guide to Laboratory Tests*, 2nd edn. WB Saunders Company: Philadelphia, USA; 554-556.