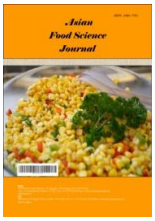

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Effects of *Nigella sativa* Linn. Oil on Gut Bacteria and Liver Function Status of Albino Wistar Rats

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Authors' contributions

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

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ABSTRACT

Background: *Nigella sativa* oil (NSO) has been suggested for use in several food and pharmaceutical applications due to its bioactive contents.
Objectives: The present study investigated the effects of 14 µl/g body weight dosage of NSO on body weight, gut microflora and liver function status (LFS) of albino wistar rats. Phytochemical analysis of NSO extract was done.
Materials and Methods: Sixty male Wistar rats were randomly assigned into two groups: 14 µl/g body weight of NSO was administered to group A while group B was given an equal volume of distilled water. Five rats provided baseline data for weight, the microbial counts and LFS in a 12-weeks experiment. At two weeks interval, five rats were sacrificed from each group and their intestinal contents were used for the microbial count (Viable, Coliform, *E. coli*, *Staphylococci* and Lactic acid bacteria (LAB)) assessment and the blood samples for LFS study.
Results: *Nigella sativa* oil showed the presence of alkaloids (0.083 mg/g), flavonoids (0.302 mg/g), saponins (0.325 mg/g), terpenes (0.138 mg/g), steroids (0.152 mg/g), tannins (0.008 mg/g) and

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terpenoid (0.138 mg/g). In both groups, the weight of rats continued to increase from the onset of the study, but between 10^{th} and 12^{th} week, non-significant ($p>0.05$) weight reduction was observed from 191.72±3.23 g to 189.30±4.71 g in the treatment group. Baseline Viable, Coliform, *E. coli*, *Staphylococci* and LAB counts (x 10^6 CFU/g) were 160, 146, 55, 23, and 154 respectively. Sequel to intake of NSO for twelve weeks, the microbial counts (x 10^6 CFU/g) were respectively 49, 38, 27, 11, and 318. Blood samples also showed a significant ($p<0.05$) reduction in LFS for Aspartate aminotransferase (78.48 to 60.06U/L), Alanine aminotransferase (30.80 to 18.54 U/L), Alkaline phosphatase (97.00 to 79.34U/L), and Bilirubin (0.52 to 0.20 U/L).

Conclusion: Beneficial effects of NSO at the investigated dosage of 14 µl/g body weight has been demonstrated as no toxicological effect was observed.

Keywords: Nigella sativa oil; gut bacteria; liver enzymes; phytochemicals; wistar rats.

1. INTRODUCTION

Herbal remedies have been in use by people as a medicine or treatment that relieves or is intended to relieve a disease or disorder. *Nigella sativa* which belongs to the buttercup family Ranunculaceae is commonly known as black seeds. Nigella seeds have many pharmaceutical uses. The seeds have occupied a special place for their medicinal value for centuries in the Middle East and Southeast Asia [1]. They have been traditionally used in the treatment of several ailments including respiratory, stomach and intestinal diseases, kidney, hypertension, bladder and liver function, circulatory and immune system support and for general overall well-being [2-4].

The seeds of *Nigella sativa* contain more than 30% fixed oil and 0.4 - 0.45% wt/wt volatile oil including 18.4 - 24% thymoquinone and 46% monoterpenes such as *p*-cymene and α-piene [5]. The protective ability of the oil against toxicity belongs to its radical scavenging (anti-oxidative) activity [6], inhibition of 5-lipoxygenase products during inflammation [7], as well as to its suppression of cell proliferation [8].

Nigella sativa oil is known to possess many nutritional and therapeutic properties due to the presence of many bioactive compounds such as essential fatty acids, polar lipids, galactolipids, sterols, aroma compounds and many other constituents [9]. Consequently, *Nigella sativa* oil is being used as a supplement for the treatment of mastalgia and sperm dysfunctionality [10]. Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement the normal diet [11]. They can be vitamins, minerals, herbs or other plants, amino acids or parts of these substances. They can be in the form of a pill, capsule, tablet, or liquid form. They supplement the diet and should not be considered a substitute for food [11].

Traditional people believe that herbal medicines are safe and have no side effects. Also, they do not believe that they are slow to cure diseases leading to high morbidity and mortality which is usually blamed on evil forces. Herbal drugs also lack appropriate measurement which can result in under dose or overdose. The most disadvantageous aspect of the use of herbal drugs is that they may contain active ingredients that are detrimental. Wood et al. [12] reported an active ingredient called chlorpropamide in a diabetic patient who abandoned orthodox drug for the herbal treatment. It was concluded that that particular herbal drug had a hazardous active ingredient.

Though *Nigella sativa* oil is already being used as a food supplement and as a local herbal extract in many parts of Nigeria, its toxicological safety must be ascertained. The present study, therefore, investigated the effects of *Nigella sativa* oil on gut bacteria and liver function status (LFS) of albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Source of *N. sativa* Oil and Phytochemical Analyses

N. sativa oil was purchased from off the shelf in a local herb store in Abeokuta, Ogun State, Nigeria. Both qualitative and quantitative phytochemical analyses of the *N. sativa* oil were carried using standard methods [13].

2.2 Experimental Animal

Sixty-five male Wister albino rats, weighing 60 - 110 g, averagely 15 days old were obtained. The

animals were kept in individual propylene cages of the dimension of 30×50×25 cm under standard laboratory conditions. Rats were maintained on a 12 hour light/dark cycle at 27°C and 60-70% humidity during the dry season of 2016. The animals were kept in standard room conditions and fed with standard ration (Vital Feed Limited, Abeokuta) and clean water. All animals received human care according to standard criteria outlined for Laboratory Animals [14].

2.3 Experimental Design

Sixty rats were divided into two groups (30 rats for group A and 30 rats for group B). Daily, a dose of 14 µl/g of *Nigella sativa* oil was administered to group A orally and group B was given an equal volume of distilled water. Five rats were used to obtain base-line data. The weight of the individual rat was obtained using the electronic weighing balance at two weeks interval. The experiment was carried out for twelve weeks, during which 5 rats from each group were sacrificed at the end of two weeks. The rats were sedated with diethyl-ether soaked cotton wool swabs in a desiccator and sacrificed by a jugular puncture. The blood was collected in an anticoagulant free bottle and centrifuged at 3500 rpm for 15 min using refrigerated centrifuge RC650s, and the serum obtained was preserved at −8°C until required for use. The sacrificed rats were then immediately transferred to a dissecting board, cleaned with ethanol. Their limbs were pinned to the board with thumb pins. The rats were dissected and the intestinal samples were collected into sterile plain tubes containing normal saline pending microbiological analyses.

2.4 Intestinal Microflora Analysis

The gastrointestinal tract (GIT) samples of dissected rats were collected for isolation and enumeration of bacteria. The GIT samples (tissues plus contents) were washed with sterile 0.9% NaCl solution and placed in peptone water and kept in a refrigerator (2 - 8°C) until they were ready for microbiological examination. Ten-fold serial dilutions were made and appropriate dilution was plated out on respective agar for viable, coliform, *E.coli*, *Staphylococci* and Lactic acid bacteria counts and the plates incubated under appropriate conditions.

2.5 Liver Function Status Assays

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and Alkaline phosphatase in the serum of experimental animals were determined using Randox kit manufactured by Randox Laboratories UK following the manufacturer instructions.

2.6 Statistical Analysis

Data obtained were analyzed using SPSS 17.0 for windows. One-way analysis of variance (ANOVA) and Duncan tests was carried out to test significant differences between their means. Differences between means at 5% significant level (P value<0.05) were considered.

3. RESULTS AND DISCUSSION

The phytochemical constituents of *N. sativa* oil sample are shown in Table 1. The sample contained phenol, alkaloid, flavonoids, saponin, terpenes, steroids, tannin and glycoside with phenolic compounds constituting the most abundant phytochemicals.

Table 1. Phytochemical contents of *N. sativa* oil sample

Phytochemicals	Quantity/100 g
Flavonoids	0.302 ± 0.003
Saponin	0.325 ± 0.0007
Tannin	0.008 ± 0.0005
Steroids	0.152 ± 0.0002
Alkaloids	0.0831 ± 0.0069
Phenol	1.0887 ± 0.0151
Terpenoids	0.1384 ± 0.0004

The weight of albino rats fed with 14 µl/g body weight of *N. sativa* oil for 12 weeks is shown in Table 2. The baseline weight was 82.01 ± 1.19 g. In both the treatment and control groups, there was a consistent significant ($p<0.05$) increase in the weight of the rats until the 10^{th} week of the experiment. However, the rats in the treatment group lost weight in the last two weeks of the experiment while the control group continued to gain weight. Studies have shown that some lactobacilli could lower total cholesterol and low-density lipoprotein (LDL) [15-17]. The reduction in body weights in the treatment group toward the end of the study could then be attributed to the proliferation of Lactic acid bacteria in rats intestine which have cholesterol reduction effects.

The microbial counts (x 10^6 CFU/g) of the gastro-intestinal tract of albino rat fed with *N. sativa* oil in a 12-weeks study are shown in Table 3. Baseline viable, coliform, *E. coli*, *Staphylococci*

Table 2. Weight (g) of albino rats fed with *N. sativa* oil in a 12-weeks study

Weeks	Control	Treated
Baseline value	82.21 ± 1.19[a]	82.21 ± 1.19[a]
2	126.38 ±3.62 [b]	153.24 ± 5.99 [b]
4	155.92 ± 6.37 [c]	162.32 ± 6.85 [c]
6	180.49 ± 4.97 [d]	177.21 ± 7.53 [d]
8	192.30 ± 8.88 [e]	185.49 ± 5.14 [e]
10	203.05 ± 5.54 [f]	191.72 ± 3.23 [f]
12	207.43 ± 8.89 [fg]	189.30 ± 4.71 [f]

Values are means ± standard deviation (n=5). Within a column, values with different superscripts are significantly p<0.05) different

and LAB counts (x 10^6 CFU/g) were 160, 146, 55, 23 and 154 respectively. Sequel to intake of *N. sativa* oil for twelve weeks, the microbial counts (x 10^6 CFU/g) were respectively 49, 38, 27, 11 and 318. In all the different microbial counts, there was no significant difference ($p>0.05$) between the baseline and 12th-week values in the control group. However, the ingestion of *N. sativa* oil significantly ($p<0.05$) reduced the viable, coliform, *E. coli* and *Staphylococci* counts while the LAB count increased significantly ($p<0.05$).

The observed result on microbial counts indicates that *N. sativa* oil exhibited antimicrobial activity against both Gram-positive and negative bacteria as demonstrated in the significant reduction of viable, coliform, *E. coli* and *Staphylococci* populations. This is in agreement with the previous study which had reported on the antibacterial properties of *N. sativa* oil. For example, Salman et al. [18] reported on the antibacterial activity of *N. sativa* oil against many multidrug-resistant clinical isolates which include coagulase-negative *Staphylococcus* and *Pseudomonas.* Previous reports have shown that phytochemicals such as phenol, saponins, flavonoids, steroids, and terpenoids are responsible for antibacterial activities of many medicinal plants. Incidentally, these phyto-chemicals are also detected in sufficient amount in the oil used for the present investigation.

It is worthy to note that data from the present study indicate that *N. sativa* oil did not lower the LAB population, a trend that was contrary to other intestinal bacteria. The LAB population gradually and steadily increased from the onset of the experiment until the 12th week of the study. The resistance of the LAB species in the present study might be an acquired antibiotic resistance as LAB species living in human and animal intestines are found to contain resistant genes located on conjugative or mobilizable plasmids and transposons [19]. The gradual but steady increase in the LAB population of the treatment group indicates that favourable conditions for the proliferation of these bacteria groups were elicited by the ingestion of *N. sativa* oil. The significant reduction of other bacteria population might have been consequent upon the synergistic antibacterial activity of *N. sativa* oil and the LAB microflora as the LAB are known to exhibit antibacterial activity through mechanisms including bacteriocin production [15,16,20]. The rapid reduction in the other intestinal microflora and simultaneous proliferation of the LAB population observed in the present study might be lending credence to the use of *N. sativa* in the treatment of digestive problems including intestinal gas and diarrhoea [10].

The liver function status of albino rats fed with *N. sativa* oil in a 12-weeks study is shown in Table 4. There was a significant ($p<0.05$) reduction in LFS for Aspartate aminotransferase (AST) (78.48 to 60.06U/L), Alanine aminotransferase (ALT) (30.80 to 18.54U/L), Alkaline phosphate (ALP) (97.00 to 79.34U/L) and Bilirubin (0.52 to 0.20U/L). In all the indices of LFS, there was no significant difference ($p>0.05$) between the baseline and those values obtained throughout the twelve weeks study in the control group. However, feeding of *N. sativa* oil to the albino rats resulted in significant ($p<0.05$) and consistent reduction of AST, ALT, ALP, and bilirubin till the end of the 12th week of the study.

Normal levels of AST and ALT may slightly vary depending on the individual laboratory's reference values. Typically the range for normal AST is reported between 10 to 40 units per litre and ALT between 7 to 56 units per litre. Mild elevations are generally considered to be 2-3 times higher than the normal range. They can

Table 3. Microbial counts (x 10^6 CFU/g) of the gastrointestinal tract of albino rat fed with *N. sativa* oil in a 12-weeks study

Organism	Group	Baseline	2 week	4 week	6 week	8 week	10 week	12 week
Viable count	control	160.40±23.37^{d}	158.40±32.58^{d}	156.00±32.58^{d}	161.60±25.83^{d}	158.80±33.54^{d}	158.80±33.54^{d}	162.80±30.25^{d}
	treated		105.40±11.24^{c}	95.80±13.65^{bc}	96.00±13.23^{bc}	85.20±13.23^{bc}	68.00±13.19^{a}	49.60±6.84^{a}
Coliform count	control	146.40±22.70^{f}	146.40±23,14^{f}	146.40±13.28^{f}	146.00±21.56^{f}	147.80±16.02^{f}	146.40±14.56^{f}	141.60±11.52^{f}
	treated		102.20±10.49^{de}	106.20±10.45^{e}	93.80±8.95^{cd}	86.00±8.97^{c}	75.60±14.31^{b}	38.20±4.91^{a}
Escherichia coli	control	55.60±11.80^{b}	54.00±8,70^{b}	52.80±7.23^{b}	55.60±9.35^{b}	57.20±6.58^{b}	57.20±7.12^{b}	55.20±7.65^{b}
	treated		58.60±7,33^{b}	57.00±5.52^{b}	51.60±4.92^{b}	46.00±4.69^{ab}	41.60±7.92^{ab}	27.80±4.81^{a}
Staphylococcus species	control	23.20±4.14^{c}	25.00±7.07^{dc}	25.80±7.08^{c}	25.40±6.76^{de}	24.80±4.45^{de}	26.20±7.08^{c}	24.20±5.67^{de}
	treated		21.00±3.93^{bcde}	17.80±4.14^{abcd}	16.20±5.49^{abc}	15.60±3.84^{ab}	12.80±3.34^{a}	11.40±2.79^{a}
Lactic acid bacteria	control	154.80±13.08^{a}	154.00±45.51^{a}	151.00±44.49^{a}	153.20±39.43^{a}	149.20±45.88^{a}	158.80±41.31^{a}	161.20±32.82^{a}
	treated		153.80±46.94^{a}	163.80±38.43^{a}	192.60±32.90^{a}	209.20±22.11^{a}	271.60±56.85^{b}	318.40±54.12^{a}

Values are means ± standard deviation (n=5). Within a row, values with different superscripts are significantly p<0.05) different

Table 4. Liver function status of albino rats fed with *N. sativa* oil in a 12-weeks study

Liver function status parameter	Baseline	2 week	4 week	6 week	8 week	10 week	12 week
Control		79.16±2.93^{a}	78.72±2.16^{a}	79.10±0.96^{a}	78.81±0.93^{a}	79.16±0.81^{a}	78.64±1.19^{a}
AST	78.48±2.59^{c}	73.29±3.62^{c}	71.36±0.82^{bc}	70.22±0.90^{bc}	69.51±0.65^{bc}	64.52±0.44^{b}	60.66±0.50^{a}
Control		32.82±11.47^{b}	28.36±10.76^{a}	30.02±9.33^{b}	29.24±5.37^{b}	32.24±1.58^{b}	32.72±9.33^{b}
ALT	30.80±10.59^{b}	28.45±1.50^{c}	26.48±0.50^{bc}	25.34±3.22^{bc}	22.35±2.12^{b}	20.34±0.45^{b}	18.54±4.24^{a}
Control		97.32±2.49^{a}	97.66±4.43^{a}	97.98±2.16^{a}	96.72±2.07^{a}	96.02±2.76^{a}	95.20±2.06^{a}
ALP	97.00±2.51^{d}	92.51±1.50^{b}	89.31±4.34^{b}	86.03±0.38^{ab}	85.43±0.38^{ab}	81.34±1.12^{a}	79.34±1.04^{a}
Control		0.51±0.11^{a}	0.52±0.14^{ab}	0.52±0.20^{ab}	0.52±0.08^{ab}	0.54±0.11^{ab}	0.51±0.15^{a}
Bilirubin	0.52±0.083^{a}	0.40±0.08^{bc}	0.38±0.34^{bc}	0.30±0.15^{b}	0.28±0.07^{b}	0.24±0.07^{a}	0.20±0.11^{a}

Values are means ± standard deviation (n=5). Within a row, values with different superscripts are significantly p<0.05) different

also be severely elevated, possibly up to 10 to 20 times the normal values, suggesting more significant damage to the liver [21]. Data from the present study are indicating the liver-protective properties of *N. sativa* oil as previously reported by El-DakhaKhani et al. [7].

4. CONCLUSION

Data from the present study are suggesting the liver-protective properties of *N. sativa* oil. In conclusion, the beneficial effects of NSO at the investigated dosage of 14 µl/g body weight have been demonstrated as no toxicological effect was observed.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gilani A, Jaben Q, Khan MAU. A review of medicinal uses and pharmacological activities of *Nigella sativa*. Pakistan Journal of Biological Sciences. 2004;7(4):441-451.
2. Deliorman D, Calis T, Ergun F, Dogan BS, Buharalioghu CK, Kanzik K. Studies on the vascular effects of the fractions and phenolic compounds isolated from *Viscum album.* J Ethnopharm. 2002;72:323-339.
3. Malhotra SK, Peter KV. Handbook of Herbs and Spices. Woodhead Publishing Ltd., Cambridge. 2006;2:206-214.
4. Tasawar Z, Siraj Z, Ahmad N, Lashari MH. The effects of *Nigella sativa* (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. Pakistan Journal of Nutrition. 2011;10:162-167.
5. El-Kadi A, Kandil O. The black seed (*Nigella sativa*) and immunity: Its effect on human T cell subset. Federation Proceedings. 1987;46:12-22.
6. Kanter M, Akpolat M, Aktas C. Protective effects of the volatile oil of *Nigella sativa* seeds on beta-cell damage in streptozotocin-induced diabetic rats: A light and electron microscopic study. Journal of Molecular Histology. 2009;40:379-85.
7. El-DakhaKhani M, Mady NL, Halim MA. *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittel-forschung. 2000;50:832-836.
8. Salim ET, Fukushima F. Chemopreventive potential of volatile oil from black cumin (*Nigella sativa* L.) seeds against rat colon carcinogenesis. Nutr Cancer. 2003;45(2): 195-202.
9. Ibtissem H, Kchouk ME, Marzouk B. Lipid and aroma composition of Black Cumin (*Nigella sativa* L.) seeds from Tunisia. Journal of Food Biochemistry. 2008;32: 335-352.
10. WebMD. Black seed: Uses, side effects, interactions and dosage; 2019a.
    Available:https://www.webmd.com/vitamins/ai/ingredientmono-901/black-seed (Accessed 26/09/2019)
11. FAO/WHO. Statistical pocket book of the food and agricultural organization for the United Nations. 2014;249.
12. Wood DM, Athwal S, Panahloo A. The advantages and disadvantages of herbal medicine in a patient with diabetes mellitus: A case report. Diabetes Medicine. 2004;21(6):625–627.
13. Okwu DE, Edeoga HO, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685-688.
14. Larry W, Clark MS. User protocol for evaluation of qualitative test performance. Approved Guideline, Second Edition, EP122A; 2008.
15. Sanders ME. Considerations for the use of probiotic bacteria to modulate human health. J. Nutr. 2000;130:384S-390S.
16. Agerholm Larsen L, Bell ML, Grunwald GK. The effect of a probiotic milk product on plasma cholesterol: A meta-analysis of short-term intervention studies. Eur. J. Clin. Nutr. 2000;54:856–860.
17. Prakash S, Jones ML. Artificial cell therapy: New strategies for the therapeutic delivery of live bacteria. Bio Med Research International. 2005;1:44-56.
18. Salman MT, Khan RA, Shukla I. Antimicrobial activity of *Nigella sativa* Linn.

seed oil against multi-drug resistant bacteria from clinical isolates. Natural Product Radiance. 2008;7(1):10-14.
19. Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie van Leeuwenhoek. 1999;76:115-137.
20. Abo-Amer. Characterization of a bacteriocin-like inhibitory substance produced by *Lactobacillus plantarum* isolated from Egyptian home-made yoghurt. J Microbiol. 2007;33:313-319.
21. Web MD; 2019b. What_should_i_know_about_liver_blood_tests_why_are_they_used Available:https://www.emedicinehealth.com/liver_blood_tests/article_em.htm#what_should_i_know_about_liver_blood_tests_why_are_they_used (Accessed 30/09/2019)

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