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Assessment of Acute Toxicity, LD_{50} and Histopathological Evaluation of *Nigella sativa* and *Moringa oleifera* Seed Extracts in Wistar Rats

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ABSTRACT

The study determined the oral LD_{50} value of n-hexane oil extracts of *Nigella sativa* and *Moringa oleifera* and evaluated their histopathological effects. The seeds were extracted using Soxhlet extraction techniques. The following doses were prepared: 1000, 1600, 2900, and 5000 mg/kg for oral route toxicity testing using Wistar rats. The test group rats received a single oral dose of 1000, 1600, 2900, and 5000 mg/kg body weight of n-hexane oil extracts of both *N. sativa* and *M. oleifera* seeds. The control group received an equivalent volume of distilled water. The study showed no mortality in all the rat groups after 24 hours and up to a week after oral administration of the samples. Changes were noted in the body weights of the rats, with slight dullness at the onset of extracts administration. The histology of the liver sections revealed multiple dose-dependent necrosis, histological lesions, and abnormal sinusoids after two weeks of oral administration of both seed oil extracts. The study concluded that the plant's seed oil extracts are relatively safe for nutritional and medicinal uses.

Keywords: *Moringa oleifera, Nigella sativa*, Acute toxicity, Hepatotoxicity, Rats.

Introduction

There is an increase in the discovery of potential medicinal plants and the concurrent screening of their biological activities to provide helpful information to enable patients and physicians to make wise decisions.[1] These medicinal plants can be in the form of plant extract (either as standardized extracts or in pure form) and have paved the way to a wide range of opportunities in drug discoveries due to their unlimited availability and unmatched diversity of their chemical constituents.[2] They are considered to be safer with lesser adverse effects than synthetic drugs[3,4] and have contributed immensely to the management of various diseases.[5,6] Some of the reported plant's therapeutic/bioactive properties, include inflammatory,[7] antioxidant,[8,9] antimicrobial,[10] insecticidal,[11] and antihypertensive[12] activities. However, many of the traditionally used natural products including plants have not been scientifically validated.[13] These scientific investigation are necessary in other to justify the rational for use.[14] *Nigella sativa* and *Moringa oleifera* plants have been mentioned and implicated in ethnomedical practices. Their phytochemistry has been documented.[15-17] There are also reports of their individual uses in treating human ailments. However, despite the growing number of research studies involving the assessment of acute toxicity of *N. sativa* and *M. oleifera* primarily on rodents, the toxicity profile, including the lethal doses reported so far, differed from one another.

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The number of pharmacological substances and chemicals used in the human community today has increased by almost an innumerable amount.[18] These may be presented today as constituents of food substances, medicines, beverages, and other industrial and household products. However, these chemicals/pharmacological substances may result in chronic toxicity in the lining system when used over a long time, or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effects are used.[18] These harmful effects may be mild or severe, depending on the nature of the substance. Acute toxicity is the unwanted effect(s) that occurs immediately or briefly after single or multiple administrations of such substances within 24 hours. The unwanted (or adverse) effects are any effects that produce functional impairments in organs and/or biochemical lesions, which could alter the organism's functioning in general or individual organs.[19] However, a study of acute toxicity of any substance tends to establish the dose-dependent unwanted effect that may occur following administration. It includes all vital information in assessing acute toxicity, including mortality. The assessment of the lethal dose (LD_{50}) (the dose that kills 50 % of the test animal population) has now been used as a significant parameter in measuring acute toxicity and as an initial procedure for general screening of chemical and pharmacological agents for toxicity.[18]

Apart from mortality, other essential parameters in acute toxicity evaluation include other biological effects, the time of onset, duration, and degree of recovery of survived animals. It gives information about the median lethal dose (LD_{50}), therapeutic index, and degree of safety of pharmacological agents.[18] The toxicity assessment and profiling of pharmacological agents is an essential procedure usually carried out before they are allowed into the community. The LD_{50} can be obtained for any route of entry or administration, but dermal (intraperitoneally) and oral administration methods are the most common.[20] The results of oral studies are essential for drugs, food, and accidental domestic poisonings.[20] The lesser the LD_{50} value, the more toxic the chemical is, while the higher the LD_{50} value, the lower the toxicity.[21] Therefore, this research aims to determine the oral LD_{50} value of n-hexane oil seed extracts of *Nigella sativa* and *Moringa oleifera* commonly used in traditional medicine.

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Materials and Methods

Plant material and Preparation of crude methanol extract
The seeds of *N. sativa* and *M. oleifera* were obtained from different locations in Kano LGA, Kano State, Nigeria, in July 2019. The plants were identified and authenticated by a taxonomist Mr. A. O. Ozioko, of the International Centre for Ethnomedicine and Drug production (InterCEED), Nsukka, Nigeria. A voucher specimen of the plants with respective numbers: INTERCEED/056 and INTERCEED/065 for *N. sativa* and *M. oleifera* were deposited at the InterCEED Herbarium. They were cleaned thoroughly to remove dirt, stones, and deteriorated seeds and crushed and ground to fine powders using a mechanical machine. The oils were extracted at room temperature from 500 g of the powdered seeds using the Soxhlet Extraction Process described by Lekgari,[22] with n-hexane serving as the solvent. The extracts were then dried in a Rotary Evaporator apparatus.

Ethical approval
Ethical approval (FPSRE/UNN/19/0021) was obtained from the University of Nigeria Ethics Committee for using experimental animals.

Experimental animals used
A total of 45 rats of both sexes, 7 – 8 weeks old and weighing 18 – 22.5 g, were procured from the animal house, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in standard laboratory conditions and fed with pelletized rodent commercial diet (Vital Feed Nig., Ltd) and water ad libitum throughout the study. These investigations were conducted following laid down procedures by the relevant Ethical Committee on Laboratory animal use and international rules were observed.[23] All animal experiments were conducted following the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, the EEC Directive of 1986 (86/609/EEC).

Acute toxicity (LD_{50}) study of the herbal mixture
The acute toxicity studies of both *Nigella sativa* and *Moringa oliefera* seed oils were investigated by measuring the acute lethal dose (LD_{50}) on rats as described by Lorke.[24]
Briefly, forty (40) rats were randomly divided into two (20 rats for each plant seed oil extract - *N. sativa* and *M. oleifera*), which were further divided into four groups (five rats in a group). The rats were kept in separate cages. Each group (A - E) of 5 rats received, respectively, a single oral dose of 1000, 1600, 2900, and 5000 mg/kg body weight of the corresponding n-hexane extracts of *N. sativa* and *M. oleifera* seeds. The control group F (6^{th} group) of 5 rats received an equivalent volume (10 ml/kg) of distilled water. Administration of all doses was done orally with the help of a syringe. The rats were constantly observed for the first 2 hours, closely for the next 4 hours, and then overnight.
The LD_{50} was calculated using the formula below:

$$LD_{50} = [(X_1)(X_2)]^{1/2}$$

Where: X_1 = Maximal dose that gave no mortality, X_2 = Minimal dose that produces mortality.

Histopathological examinations
The rats were sacrificed through humane killing, and their liver excised from them was fixed in 10 % buffered formalin in labeled bottles and processed for histological examination. They were later processed and embedded into paraffin wax. The tissue block, sectioned into 5 µm, was subsequently stained with hematoxylin and eosin and, after that, mounted on glass slides and examined under a standard light microscope.

Results and Discussion

Oral acute effect of the extracts on the experimental rats.
The acute oral toxicity study's results showed that n-hexane seed oil extracts of *N. sativa* and *M. oliefera* demonstrated a high safety margin, with the animals tolerating up to 5000 mg/kg body weight of the extracts orally (Table 1 and 2).
All concentrations produced low toxicity since no death was recorded in the tested concentrations. It was observed that all the tested doses caused immediate agitation, unstable movement at high speed, and behavioral perturbations with temporary writhing, followed by a quiet attitude period and sedation. Generally, diarrhea was observed. Depending on the dose, the animals quickly recovered their normal activities after some periods ranging from 9 – 14 hours. However, transient restlessness was observed in animals treated with the oil extract doses of 2900 and 5000 mg/kg. The toxicology study revealed that both *N. Sativa* and *M. oleifera* crude oils have low toxicity. The high LD_{50} values of oral doses of both plant seeds oil show their apparent low acute toxicity, suggesting a wide margin of safety for therapeutic doses of *N. sativa* and *M. oleifera* oil extracts. In this study, the calculated LD_{50} of 5000 mg/kg body weight implies that concentrations of the oil extract below 5000 mg/kg poses no threat; however, concentration above it may exhibit toxic effect when administered. In a similar study, no evidence of *N. sativa* seeds oil toxicity was observed; however, when administered in doses up to 10000 mg/kg body weight, p.o., the oil extracts were found toxic.[25] Zaoui *et al.*[26, 27] investigated the toxicity of the fixed oil of *Nigella sativa* seeds in rats by determining the LD_{50} values and examining possible biochemical, hematological, and histopathological changes. LD_{50} values obtained by single doses, orally administered in rats, was 2880 mg/kg body wt. p.o. [26.2 – 31.6]. *N. sativa* crude oil exhibits potent analgesic and sedative properties,[25] which may explain the quiet attitude and sedation following oil administration in rats.

Histology of the liver of the experimental rats
The photomicrograph of the liver showed mild degenerated hepatocytes, as shown in figure 1. The histology of the liver showed dose-dependent necrosis, histopathological lesion, and slight degenerative changes in the liver cells, which may suggest the deleterious effect of higher doses of the extracts and their hepatotoxicity.

Conclusion

The plant oil extracts of *N. sativa* and *M. oleifera* are relatively safe both for nutritional and medicinal uses. The results showed a low acute toxicity effect with a high LD_{50}. Further research is, however, needed to elucidate the precise mechanisms of the different actions of *N. sativa* and *M. oleifera* crude oil extracts.

Table 1: Acute toxicity test of the n-hexane oil extract of *Nigella sativa* on rats

Animal groups	Extract doses (mg)	Average				Time of death (Hr)
		Weight (g)	Mg = wxD/1000	Vol = mg/Conc.	Conc. (mg/mL)	
1	1000	24.362	24.362	0.238	100	-
2	1600	22.754	36.406	0.364	100	-
3	2900	22.786	66.08	0.662	100	-
4	5000	22.974	114.87	0.57	200	-

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Table 2: Acute toxicity test of the n-hexane oil extract of *Moringa oleifera* on rats

Animal groups	Extract doses (mg)	Average				Time of death (Hr)
		Weight (g)	Mg = wxD/1000	Vol = mg/Conc.	Conc. (mg/mL)	
1	1000	21.532	21.532	0.214	100	-
2	1600	22.342	35.748	0.358	100	-
3	2900	20.638	59.852	0.6	100	-
4	5000	20.092	100.46	0.814	200	-

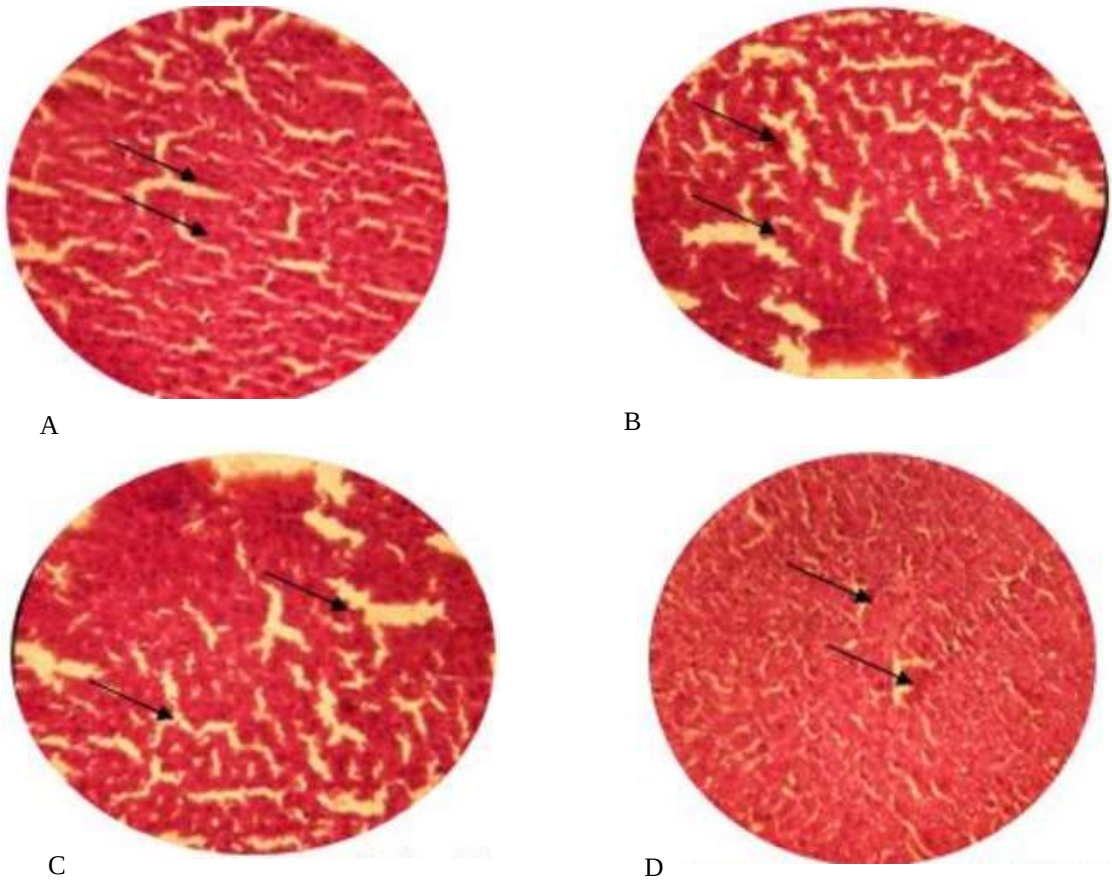

Figure 1: Photomicrograph showing the liver of the experimental rats: A: 1000 mg/kg body weight dose; B: 1600 mg/kg body weight dose; C: 2900 mg/kg body weight dose; D: 5000 mg/kg body weight dose; Arrows show inflammatory responses: distorted and necrotic cells.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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