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Evaluation of Acute Oral Toxicity of Combined Aqueous Extracts of Nigella sativa Seeds and Hylocereus polyrhizus Fruit

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Abstract:

The safety profile of a combined aqueous extract of *Nigella sativa* (black cumin seed) and *Hylocereus polyrhizus* (dragon fruit) was evaluated through an acute oral toxicity study in female rats. A single dose of 5,000 mg/kg body weight was administered according to OECD guidelines, with observations conducted over a 14-day period. The study revealed no signs of mortality, adverse behavioral effects, or significant changes in body weight. Histopathological analyses of vital organs, including the liver, kidneys, and heart, showed no detectable abnormalities or cellular damage in the treated group compared to the control. These findings corroborate existing evidence regarding the safety of the individual components, which have been previously demonstrated to exhibit negligible toxicity at high doses. Behavioral assessments indicated normal skin, fur, eyes, mucosal membranes, and activity levels, with no symptoms such as tremors, lethargy, diarrhea, or coma observed. The absence of statistically significant body weight differences further suggested that the herbal combination does not interfere with metabolic or nutritional processes. Additionally, histopathological evaluations confirmed the absence of hepatotoxicity, nephrotoxicity, or cardiotoxicity, consistent with prior studies highlighting the antioxidant and protective properties of *Nigella sativa* and *Hylocereus polyrhizus*. The results of this study provide compelling evidence for the non-toxic nature of this herbal mixture, supporting its potential use as a safe therapeutic agent.

Keywords: Acute toxicity; Antihypertensive herbal mixture; nigella sativa seed extract *(kali jiri)*, *Hylocereus polyrhizus* fruit extract (Dragon fruit)

Introduction

In India, herbal drug therapy, particularly Ayurveda, is highly trusted. Many conditions that allopathic medicine can't treat are effectively managed with herbs. *Nigella Sativa* (Black Cumin Seed) is used globally for its medicinal properties, including antihypertensive and liver tonic effects, with thymoquinone as its key active compound.[1] Dragon fruit (*Hylocereus polyrhizus*) is nutrient rich and has significant health benefits like reducing hypertension and diabetes and improving heart health and metabolism. Combining these herbs could enhance their antihypertensive effects.[2]

To ensure the safety of this herbal combination, an acute toxicity study was conducted on female Sprague Dawley rats according to OECD guideline 423. The study classifies the substance based on the Globally Harmonized System for acute toxicity.[3]

Materials and Methods

Test materials

Extracts of *Nigella sativa* (black cumin seed) & *Hylocereus polyrhizus* (Dragon fruit) were sourced from Yucca Enterprises in Mumbai and Vital Herbs in Delhi.

Animals

The study involved six healthy, Sprague-Dawley female young adult rats, weighing between 100 and 120 g and aged 8-12 weeks. The animals, divided into two groups of three, were acclimatized for five days in individual polypropylene cages with clean paddy husk bedding. They were kept under a 12-hour light cycle (6 AM to 6 PM), a room temperature of 25°C (±2°C), and a humidity of 45-55%. They received free access to normal pellet chow (Amrut Feeds, Bangalore) and purified drinking water.

Preparation of the extracts and the herbal mixture:

The aqueous extracts of *Nigella sativa (kali jiri)* and *Hylocereus polyrhizus* (*Dragon fruit*) were mixed in a 2:3 ratio. A fter weighing the extracts separately and triturating them into a uniform mixture,
5000 mg of the mixture (3000mg of *Dragon fruit extract* and 2000 mg of *Nigella sativa seed extract*) was accurately w eighed and dissolved in 20 ml of distilled water. Based on each animal's unique body weight, the required volume was then determined.[4-9]

Administration of herbal mixture (Limit test at 5,000 mg per kg body weight)

The animals spent the entire night fasting and was then weighed. After that, a single oral dose of the herbal mixture was given, with a maximum volume of 2 millilitres per 100 grams of body weight. Each animal's dose was determined using their body weight after a fast. Food was denied for an additional three to four hours after the dosage. Two millilitres of distilled water were given to the control animals. A 5,000 mg/kg limit test was carried out in compliance with OECD regulations 425, Paragraph 33(a).[10-13]

Table 1 Evaluation of Percentage Yield of Hydroalcoholic Extracts from *Nigella sativa* Seeds and *Hylocereus polyrhizus fruit.*

Sr.no	Type of extract	Percentage yield *Nigella sativa* (black cumin seed)	*Hylocereus polyrhizus* (Dragon fruit).
1	Hydroalcoholic extract	25.80±0.2, 23.75±1.2	35.40 ±0.2

Table 2. Toxicological Dose Assessment of an Herbal Blend at 5000 mg/kg Body Weight

Herbal Extract	Vehicle	Mode of Administration	Dosing Schedule
Aqueous Combined Extract of *Hylocereus polyrhizus* (Dragon Fruit) and *Nigella sativa* (Black Cumin Seed)	Water	Oral route	Single dose

Clinical observations: -

Behavioural analysis: For behavioural study, the animals were observed every day for 14 days, intermittently (particularly during the first 4 hours) over the following 24 hours, and constantly for the first 30 minutes after the dose. Alterations in eyes, membranous epithelium producing mucus, and skin and body hair were all noted in detail for every animal. Behavioural alterations were also largely noted. Particular attention was paid to symptoms such as seizures, convulsions, saliva production, bowel movements, fatigue, sedation, a state of coma and mortality.

Body weight analysis: Before the medicine was given on the first day and again on the seventh and fourteenth days, right before blood was drawn, each animal's weight was noted separately. After that, weight changes were computed and contrasted with the control groups.

Histopathology of the kidney, liver, and heart: The tissues were processed, embedded in paraffin, and preserved in 10% buffered formalin. Haematoxylin, eosin, and periodic acid-Schiff reagent were used to stain 3-micrometer sections, they were subsequently examined for any pathological changes using a light microscope.

Results:

Aqueous extracts of *Nigella sativ*a (black cumin seed) & *Hylocereus polyrhizus* (Dragon fruit) have been used medicinally since ancient times, including in Ayurveda. Both internal and external applications of these extracts are reported to be safe. The ratio of the extracts was determined based on their dosages in marketed formulations and available literature.

Assessment of Safety at 5,000 mg/kg Body Weight :

Aimed at determining exposure ranges where lethality is expected rather than calculating a precise LD50. This involves using predefined doses for consistency. Limit test at 5,000 mg per kg body weight is used when the test material is presumed nontoxic. Therefore, the herbal mixture of *Nigella sativa* and *Hylocereus polyrhiz*us was tested for oral toxicity in rats, with detailed behavioural, histopathological, organ, and weight analyses comparing test and control animals. Female rats were chosen due to their generally higher sensitivity in such tests compared to males.

Behavioural observations:

The treated and control animals were found to have Usual skin, body hairs, eyes, membranous epithelium producing mucus, behavioural patterns, production of saliva, sedation and body weight. There were no signs of tremors, lethargy, diarrhoea, or coma. (Tables 3 and 4)

Table 3. Behavioral evaluation of Control Rats for the 5,000 mg/kg Dose Limit Test

Observations	Half an hour	4 hours	24 hours	48 hours	1 week	2 weeks
Behavioural pattern	Usual	Usual	Usual	Usual	Usual	Usual
Skin & Body hairs	Usual	Usual	Usual	Usual	Usual	Usual
Mucus production	Usual	Usual	Usual	Usual	Usual	Usual
Eyes	Usual	Usual	Usual	Usual	Usual	Usual
Production of saliva	Usual	Usual	Usual	Usual	Usual	Usual
Tremors	None	None	None	None	None	None
Sleep	Usual	Usual	Usual	Usual	Usual	Usual
Diarrhea	None	None	None	None	None	None
Coma	None	None	None	None	None	None
Lethargy	None	None	None	None	None	None

Table 4 Behavioral Evaluation of Test Rats during the 5,000 mg/kg Limit Dose Test

Observations	30 min	4 hrs	24 hrs	48 hrs	1 wk	2 wks
Eyes	Usual	Usual	Usual	Usual	Usual	Usual
Skin & Body hairs	Usual	Usual	Usual	Usual	Usual	Usual
Tremors	None	None	None	None	None	None
Mucus production	Usual	Usual	Usual	Usual	Usual	Usual
Production of saliva	Usual	Usual	Usual	Usual	Usual	Usual
Diarrhoea	None	None	None	None	None	None
Behavioural pattern	Usual	Usual	Usual	Usual	Usual	Usual

Sleep	Usual	Usual	Usual	Usual	Usual	Usual
Coma	None	None	None	None	None	None
Lethargy	None	None	None	None	None	None

Table 5 Rats' body weight after receiving a single oral dose of a herbal mixture at a dosage of 5,000 mg/kg b. wt.

Group-Based Therapy	Dose Quantity	Before treatment M1±SD1 (N = 3)	14 days after treatment M2±SD2 (N = 3)
Control	2 ml of distilled water	232.0 ± 2.88	241.0 ± 1.78
Treated	5,000 mg per kg herbal blend	258.5 ± 2.50	267.0 ± 2.04

The sample mean values are M1 and M2, the number of rats is N, NS stands for not significant, and SD1 and SD2 represent the standard deviations of the experimental and control groups, respectively.

Body weight:

Following the oral administration of the herbal blend, all of the rats' body weights increased. Table 5 demonstrates that these changes were statistically insignificant, implying that the animals' development is unaffected by the herbal combination.

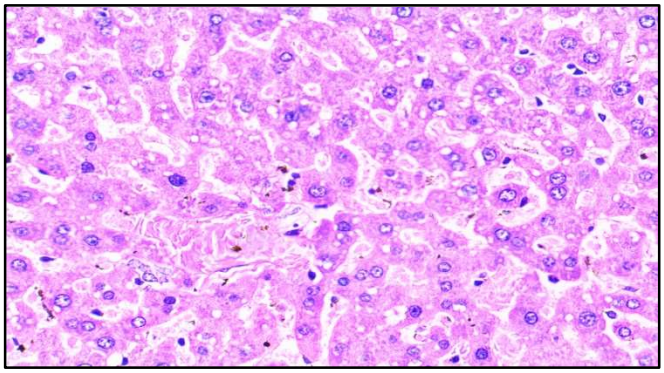
Histopathology of organs:

Following 14 days of oral medication treatment, histopathological examinations of the liver, kidney, and heart were performed on both control and experimental rats. When examined under an oil immersion objective, no detectable differences were observed in the histopatholgy of these organs between the control and treated rats. This indicates that the tested herbal mixture does no t affect cellular structure or cause cell degeneration in these organs (Figures 1-3).

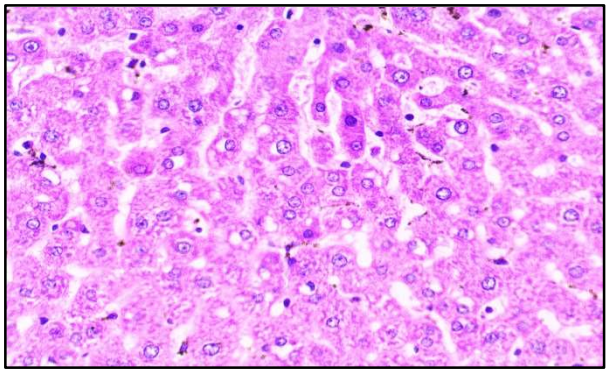
Table 9 The effects of a herbal mixture at a dose of 5,000 mg/kg b. wt. on the histology of the tissue of the rats' heart, kidney, liver, ovaries, and lungs.

Sr. no	Group	Drug	Dose(oral)	Histopathological alterations discovered				
				Kidney	Liver	Lungs	Ovary	Heart
1	Control	Distilled water	2 ml per rat	NA	NA	NA	NA	NA
2	Test	Herbal mixture	5,000 mg per kg	NA	NA	NA	NA	NA

NA – Not affected

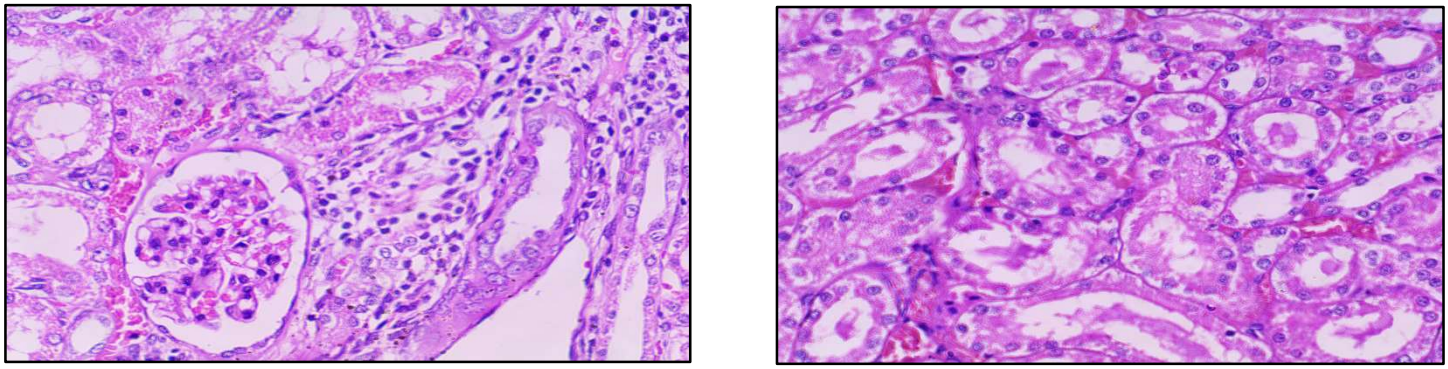


(a)



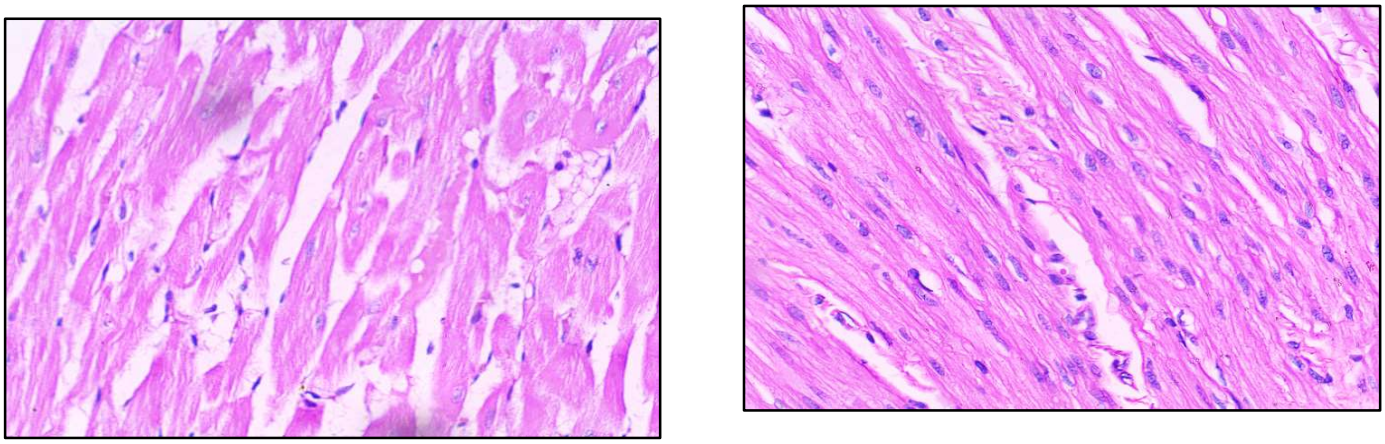
(b)

Figure 1 A photomicrograph compares the liver architecture of a normal rat (a) and a rat treated with a herbal mixture at a dose of 5,000 mg/kg body weight (b). (Magnification: 10X)



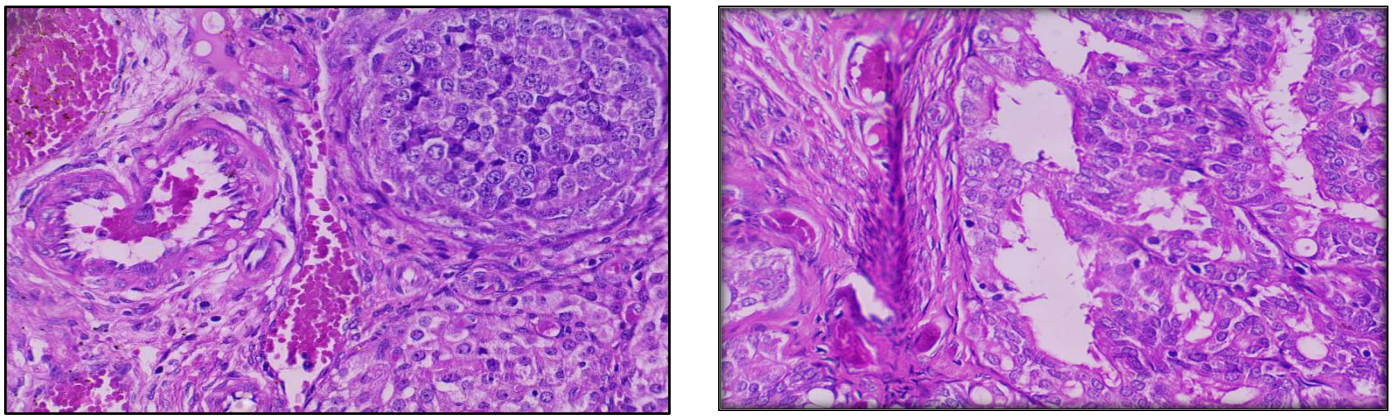
(b)

Figure 2 Microscopic Image Showing Usual Kidney Architecture (a) and Kidney from Rat Treated with 5,000 mg/kg Herbal Blend (b) (10X Magnification)



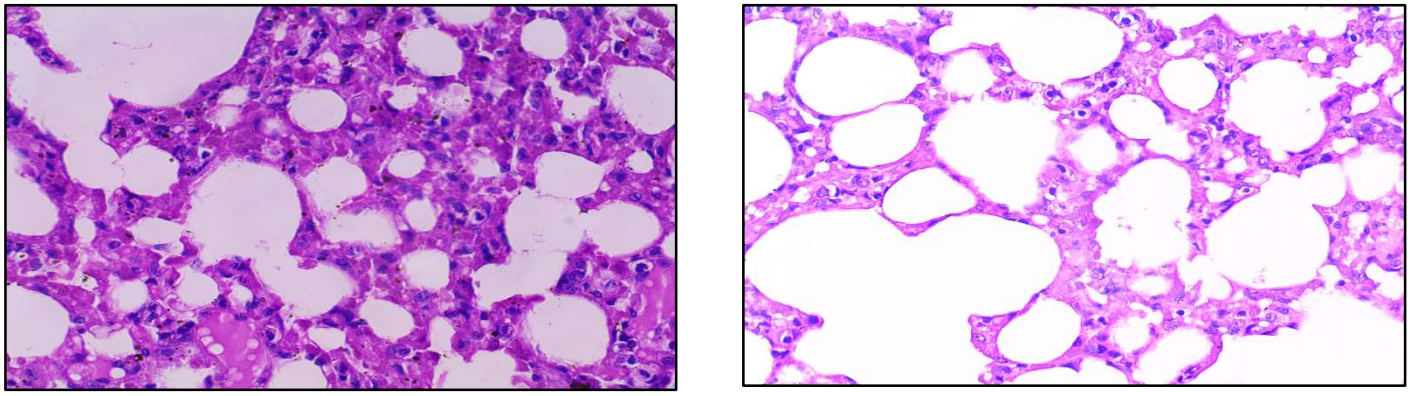
(a) (b)

Figure 3 Photomicrograph showing Usual heart architecture (a) and heart of rat receiving herbal mixture at dose of 5,000 mg/kg b. wt. (b). (Magnification 10X).



(a) (b)

Figure 4: Microscopic Image of Usual Ovary Architecture (a) and Ovary of Rat Receiving 5,000 mg/kg Herbal Mixture (b) at 10X Magnification



(a) (b)

Figure 5: Microscopic View of Usual Lung Architecture (a) and Lung Tissue from Rat Given 5,000 mg/kg Herbal Mixture (b) at 10X Magnification

DISCUSSION:

This study investigated the safety profile of a combined aqueous extract of *Hylocereus polyrhizus (dragon fruit)* and *Nigella sativa (black cumin seed)* through oral administration in rats at a limit test dose of 5,000 mg/kg body weight. The findings provide compelling evidence of the non-toxic nature of this herbal mixture across multiple parameters, including behavioural observations, body weight changes, and histopathological examinations of vital organs. The limit test approach at 5,000 mg/kg body weight is recommended when the test material is presumed to have minimal or no toxicity. The absence of lethality or severe adverse effects in this study corroborates the hypothesis that the combined herbal extract is non-toxic. These behavioural observations further substantiate the claim that the herbal mixture is well-tolerated, providing evidence that the extract does not interfere with the nervous system or other vital functions.

The study observed a consistent increase in body weight among both treated and control groups over 14 days, with no statistically significant differences. This indicates that the herbal mixture does not adversely affect the animals' growth or nutritional status. The absence of weight reduction in treated animals suggests that the herbal mixture does not interfere with metabolism or food intake, further highlighting its safety. Histopathological examinations revealed no detectable differences in the liver, kidney, or heart tissues between the control and treated groups. These findings confirm that the herbal mixture does not induce cellular damage or degeneration in these critical organs, even at high doses. The liver, being a primary organ for drug metabolism, is highly susceptible to toxic insults. However, the absence of hepatotoxicity in this study is consistent with findings by Ahmad et al., who reported that *Nigella sativa* protects against oxidative stress and liver damage due to its rich composition of thymoquinone and other antioxidants. Similarly, *Hylocereus polyrhizus* has been shown to protect liver tissues from oxidative damage, further supporting the non-toxic nature of the combined extract . The kidney and heart, which are also critical targets of systemic toxicity, showed no histopathological abnormalities in this study. Previous studies on *Nigella sativa* and *Hylocereus polyrhizus* have demonstrated similar protective effects on renal and cardiac tissues. These observations highlight the therapeutic potential of the combined extract and its safety for long-term use.

The findings of this study are consistent with prior research on plant-based formulations. For example, a study by Ahmad et al. demonstrated that a combination of herbal extracts, including Nigella sativa, exhibited no histopathological changes in vital organs at high doses. Similarly, Rahmawati et al. reported that Hylocereus polyrhizus extracts were well-tolerated

in rodent models, with no signs of organ damage or toxicity. The results of this study further contribute to the growing body of evidence supporting the safety of plant-based therapeutics. The combined extract of *Hylocereus polyrhizus* and *Nigella sativa* shows promise as a safe and effective therapeutic agent, particularly for applications requiring high-dose administration. The non-toxic nature of the combined extract, demonstrated through behavioural, body weight, and histopathological assessments, provides strong evidence for its safety. These findings align with prior research on the individual components and highlight the potential of this herbal mixture for therapeutic applications. Further studies, including chronic toxicity and clinical trials, will be essential to confirm these results and explore its full therapeutic potential.

Conclusion:

When taken orally, a 5,000 mg/kg body weight dose of the herbal extract of *Nigella sativa* and *Hylocereus polyrhizus* did not exhibit any symptoms of acute toxicity.

This conclusion was based on Usual behavior and histological evaluations, suggesting that the herbal mixture islikely s afe up to this dosage.

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