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Nigella sativa seeds attenuate the hepatic histoarchitectural changes induced by Pyrazinamide

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ABSTRACT

Introduction: Though highly effective as an anti-tuberculosis drug, Pyrazinamide has a notable side effect of causing hepatic toxicity and damaging the liver architecture. *Nigella sativa* has been shown to have hepatoprotective effects and should be tested against Pyrazinamide induced liver damage.

Objective: To determine the protective role of *Nigella sativa* (NS) seeds in preserving the hepatic histoarchitectural changes induced by Pyrazinamide (PZA) in mice.

Materials & Methods: It was a laboratory based randomized control trial conducted on forty male albino mice divided into 4 groups (n = 10). Group-I served as control. Group-II was given 500mg/kg PZA. Group-III and Group-IV was given *Nigella sativa* seeds powder 500 mg/kg and 1000 mg/kg respectively along with 500mg/kg PZA. All the animals were treated once daily for six weeks. Animals were then dissected after 24 hours of the last dose and liver samples were obtained for histopathological evaluation. Assessment of liver damage (histological grading) was done by using the Batts-Ludwig system. Data were analyzed by SPSS 23 for descriptive and comparative statistics, keeping $p \leq 0.05$ as significant.

Results: PZA caused moderate to severe liver damage in mice characterized by presence of parenchymal inflammation, slight edema of hepatocytes, vascular congestion, vascular dilatation, periportal and portal inflammation and few scattered areas of necrosis. NS seeds preserve normal hepatic histoarchitecture in dose dependent fashion.

Conclusion: Concomitant administration of *Nigella sativa* seeds attenuate the hepatic histoarchitectural changes induced by pyrazinamide in dose dependent manner.

Keywords: *Nigella sativa*; Pyrazinamide; Tuberculosis; Drug Toxicity; Hepatitis, Toxic; Drug-Induced Liver Injury.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Tuberculosis (TB) is the second-leading fatal infectious disease worldwide, despite the availability of all the preventive and curative measures, with a global mortality rate of 1.3 million during the year 2022. The WHO recommended first line management of TB involves isoniazid, rifampin, ethambutol, pyrazinamide and streptomycin for initial rigorous 2 months followed by isoniazid, rifampin and/or ethambutol for next 4 months. Most of these anti-TB drugs are hepatotoxic though drug induced liver injury (DILI) indued by pyrazinamide (PZA) has higher incidence as compared to other first line anti-TB drugs.[1]

PZA is a unique bactericidal that works against both active and static TB. However, the incidence of adverse drug reactions with PZA is 49.9% as compared to 11.1% and 16.6% for isoniazid and rifampicin respectively, thereby limiting the clinical use and patient adherence to PZA.[2] The most frequent adverse effect of PZA is hepatotoxicity with the incidence rate of 44.5%, followed by gastrointestinal intolerance which occurs in 23.8% of TB patients.[3] PZA induced DILI is dose dependent and mostly seen with daily dose more than 40mg/kg.[4] The exact mechanism by which PZA causes liver damage is still uncertain, though studies have shown that two of its metabolites, pyrazinoic acid, and 5-hydroxy-pyrazinoic acid are hepatotoxic.[4,5] The PZA induced DILI is a direct toxic effect on the liver instead of an immune-mediated or hypersensitive reaction.[4]

Nigella sativa (NS) seeds (Black Cumin) are known to have therapeutic potential according to the Old Testament and Islam.[6] These seeds have abundance of beneficial essential fatty acids, vitamins and minerals. The main active principal of NS is Thymoquinone (TQ). This phytonutrient has potent antioxidant, anti-inflammatory, antifibrotic, immunomodulatory and anticancer properties.[7,8] Thymoquinone and two other natural metabolites of NS; thymol and alpha-hederin are known to have incredible hepatoprotective effects.[9] Moreover, research has shown NS and its components may have antibacterial, antiviral, antifungal, antitussive, anti-asthmatic, analgesic and gastroprotective potentials.[9–12]

The objective of the current study was to determine the protective role of *Nigella sativa* seeds in preserving the hepatic histoarchitectural changes induced by Pyrazinamide in mice.

MATERIALS & METHODS

Forty male albino mice, of age 2 months, weighing 25-50g were utilized in this experiment. Sample size was selected on the basis of previous studies. The controlled and standard laboratory conditions (temperature: 20±2ºC; humidity 50-70%; light/dark cycle 12hr/12hr, fresh air) were provided to animals in National Institute of Health (NIH), Islamabad. Mice were given free access to their diet and water for the entire experiment.

Ethics Review Committee of Islamic International Medical College, Riphah International University, Islamabad, had granted approval of the current study. Biosafety, guidelines for working and collection of data was closely monitored during the study.

Animals were selected by non-probability convenience sampling method and divided into 4 groups (n=10 each) randomly. Group-I (control) was given glucose water. Group-II (toxic) was given 500mg/kg PZA added in glucose water. Group-III (low dose NS + PZA) and Group-IV (high dose NS + PZA) was given *Nigella sativa* seeds powder 500mg/kg and 1000mg/kg dissolved in glucose water respectively along with 500mg/kg PZA. All the drugs and glucose water were administered once daily for six weeks. Animals were dissected after 24 hours of the last dose and liver samples were obtained. Liver was then fixed in 10% buffered formalin, processed, and imbued with paraffin wax and paraffin blocks were made. Sections were cut by rotatory microtome at 4-5 microns for staining purposes. Tissue sections were then stained with Eosin and Hematoxylin dyes and examined thoroughly under light microscopy for histopathological changes. The Batts-Ludwig system was used for histological grading (Table 1).

SPSS 23 was used to calculate the frequencies and percentages of histopathological changes which were then further explored by Chi square test and Fisher's exact test as applicable. In all comparisons, p value≤0.05 was taken as significant.

Table 1: Batts-Ludwig system for histological grading & staging.

Batts-Ludwig system	
Portal / periportal activity	**Grade**
None or confined to portal tracts	0
Minimal, patchy interface hepatitis	1
Mild interface hepatitis involving some or all portal tracts	2
Moderate interface hepatitis involving all portal tracts	3
Severe interface hepatitis / bridging necrosis	4
Lobular activity	**Grade**
None	0
Minimal - rare spotty necrosis (can find 1 - 2 dead hepatocytes in biopsy)	1
Mild - scattered necrotic hepatocytes	2
Moderate - confluent necrosis (clusters of dead hepatocytes)	3
Severe - bridging necrosis	4
Amount of fibrosis	**Stage**
No increased fibrous tissue	0
Fibrous portal expansion	1
Periportal fibrosis with periportal septa	2
Bridging fibrosis with portal - portal fibrous septa and distorted architecture	3
Probable or definite cirrhosis with nodule formation	4

RESULTS

The gross examination of liver showed normal fresh appearance i.e., dark maroon color with smooth surface in group-I, group-III and group-IV; however, in group-II, color of liver varied from light maroon to pale yellow. Liver texture was firm in all groups.

Histopathological assessment of severity of toxic changes in liver parenchyma are listed in Table 2. Morphology of group-II was significantly ($p<0.001$ to $p=0.007$) changed from the rest of the groups.

The most striking morphological changes were seen in the categories of vascular dilatation (80% in group-II) and vascular congestion (90% of group-II) with $p<0.001$ each; this was followed by portal inflammation in 80% of group-II ($p=0.001$), parenchymal inflammation in 70% of group-II ($p=0.002$), and periportal inflammation in 70% of group-II ($p=0.007$). The best results were seen in group-IV, which gave results fairly close to the control group-I.

Table 3 depicts the comparison of the four groups based on the Batts-Ludwig system of histological grading and staging. Significant differences were seen between group-II and the other groups in the domains of portal/periportal activity (group-II 50% in grades 2 & 3, $p<0.001$), and Lobular activity (group-II 40% in grade 2, $p<0.001$). Once again, the group-IV showed grades identical to the control group-I.

These results are corroborated by the microscopic examination of the liver architecture in all groups, as shown in Figures 1 to 8.

Microscopic examination of group-I (control) mice liver revealed normal liver cells, intact hepatic lobules and normal portal tract that indicated normal liver parenchyma as also shown in Figure 1. Mice in group-II (toxic) presented moderate to severe liver

damage characterized by presence of parenchymal inflammation (presence of great number of lymphocytes and disarray of normal architecture of liver), vascular congestion, vascular dilatation, peri portal and portal inflammation and diffuse areas of necrosis as shown in figures 2-6 and are graded according to Batts-Ludwig system. Liver cells of mice in group-III (Low dose NS + PZA) were normal and showed a significant reduction in the characteristic features of hepatic injury observed in the liver of pyrazinamide treated group-II (PZA). Signs of minimal toxicity were seen as the normal architecture of hepatic tissue was preserved, structure of hepatocytes, nuclei and portal triad was approximately similar to normal liver as shown in figure 7. These morphologies indicate that NS seeds have significantly ($p<0.001$) reduced the hepatotoxic effects of PZA. In liver tissue of group-IV (High dose NS + PZA), even the mild architectural changes were not observed showing high dose of NS totally preserves the hepatic architectural changes indued by PZA as shown in figure 8.

Table 2: Frequencies and Percentages of Hepatic Histopathological parameters of all groups (n=10 per group).

Histopathological parameters		Groups				Fisher's Exact Test* (Value)	p value
		G-I (Control)	G-II (Toxic)	G-III (Low dose NS + PZA)	G-IV (High dose NS + PZA)		
Parenchymal Inflammation	Present	0 (0.0%)	7 (70%)	2 (20%)	1 (10%)	13.3	0.002
	Absent	10 (100%)	3 (30%)	8 (80%)	9 (90%)		
Vascular Dilatation	Present	0 (0.0%)	8 (80%)	3 (30%)	0 (0.0%)	19.7	<0.001
	Absent	10 (100%)	2 (20%)	7 (70%)	10 (100%)		
Vascular congestion	Present	0 (0.0%)	9 (90%)	3 (30%)	2 (20%)	19.3	<0.001
	Absent	10 (100%)	1 (10%)	7 (70%)	8 (80%)		
Periportal Inflammation	Present	0 (0.0%)	7 (70%)	3 (30%)	2 (20%)	11.77	0.007
	Absent	10 (100%)	3 (30%)	7 (70%)	8 (80%)		
Portal Inflammation	Present	0 (0.0%)	8 (80%)	4 (40%)	2 (20%)	15.22	0.001
	Absent	10 (100%)	2 (20%)	6 (60%)	8 (80%)		

*Fisher's exact test was done instead of chi square test as 50% of cells have expected count less than 5.

Table-3: Comparison of hepatic histopathological grading according to Batts-Ludwig system between all groups.

Histological Parameter	Grades	Groups				Fisher's exact test* (value)	p value
		G-I (Control)	G-II (Toxic)	G-III (Low dose NS + PZA)	G-IV (High dose NS + PZA)		
Portal / Periportal activity	0 (None)	10 (100%)	0 (0.0%)	8 (80%)	10 (100%)	30.43	<0.001
	1 (Minimal)	0 (0.0%)	2 (20%)	2 (20%)	0 (0.0%)		
	2 (Mild)	0 (0.0%)	3 (30%)	0 (0.0%)	0 (0.0%)		
	3 (Moderate)	0 (0.0%)	3 (30%)	0 (0.0%)	0 (0.0%)		
	4 (Severe)	0 (0.0%)	2 (20%)	0 (0.0%)	0 (0.0%)		
Lobular Activity	0 (None)	10 (100%)	0 (0.0%)	10 (100%)	10 (100%)	32.50	<0.001
	1 (Minimal)	0 (0.0%)	6 (60%)	0 (0.0%)	0 (0.0%)		
	2 (Mild)	0 (0.0%)	4 (40%)	0 (0.0%)	0 (0.0%)		

*Fisher's exact test was done instead of chi square test as 80% of cells have expected count less than 5

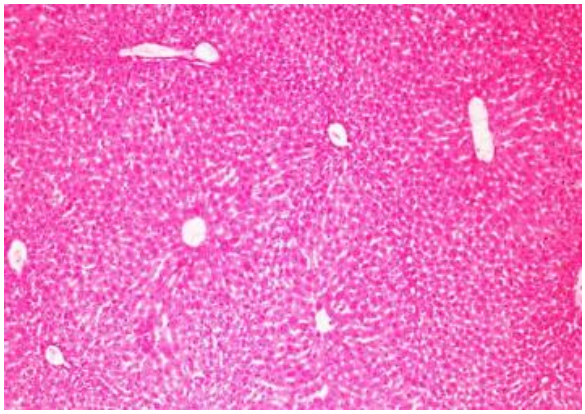

Figure 1: Hepatic tissue photomicrograph of group-I (control) showing normal hepatocytes, intact hepatic lobules and normal parenchyma. (H&E 10X)

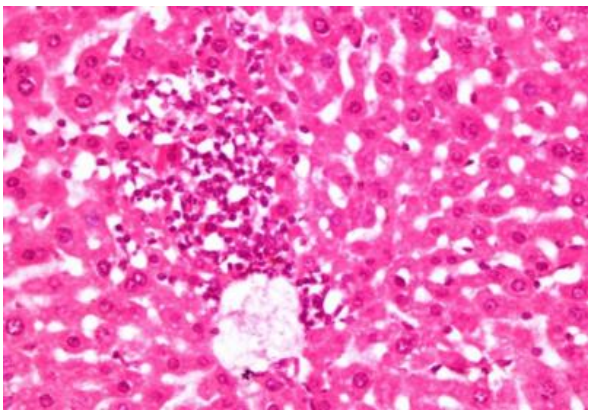

Figure 2: Hepatic tissue photomicrograph of group-II (PZA) showing grade 2 mild interface hepatitis involving portal tract. (H&E 40X)

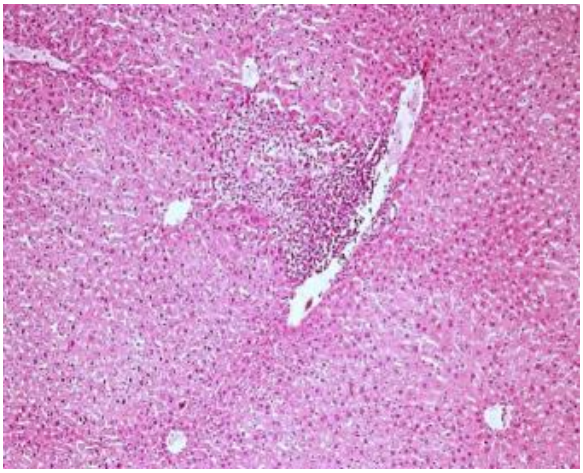

Figure 3: Hepatic tissue photomicrograph of group-II (PZA) showing grade 3 moderate interface hepatitis involving portal tracts. (H&E 10X)

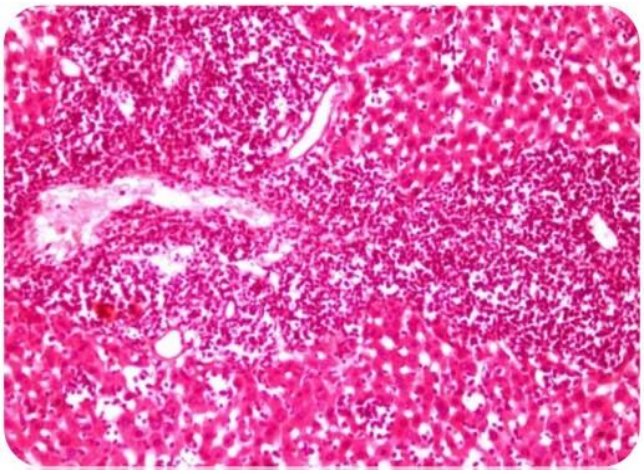

Figure 4: Hepatic tissue photomicrograph of group-II (PZA) showing grade 4 severe interface hepatitis. (H&E 40X)

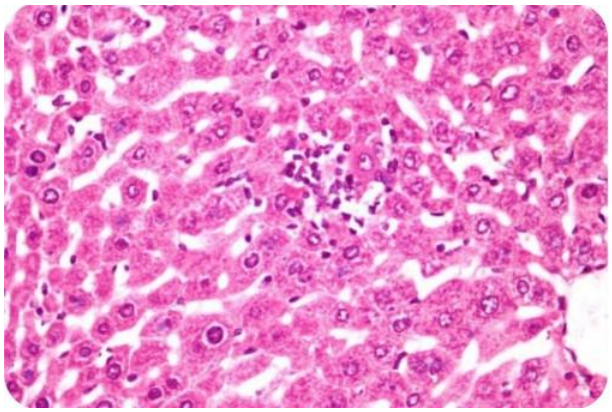

Figure 5: Hepatic tissue photomicrograph of group-II (PZA) showing mild interface hepatitis involving portal tract with scattered necrotic hepatocytes. (H&E 40X)

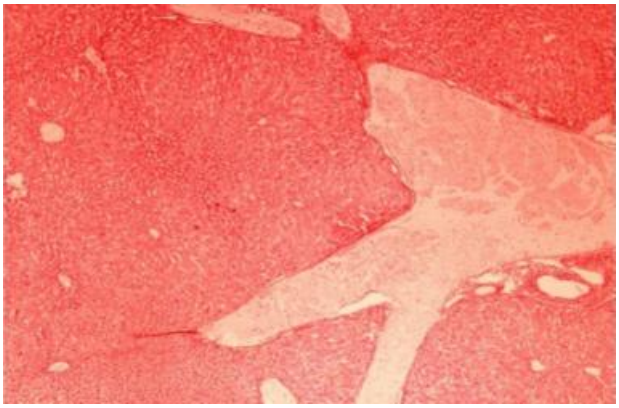

Figure 6: Hepatic tissue photomicrograph of group-II (PZA) showing vascular congestion. (H&E 10X)

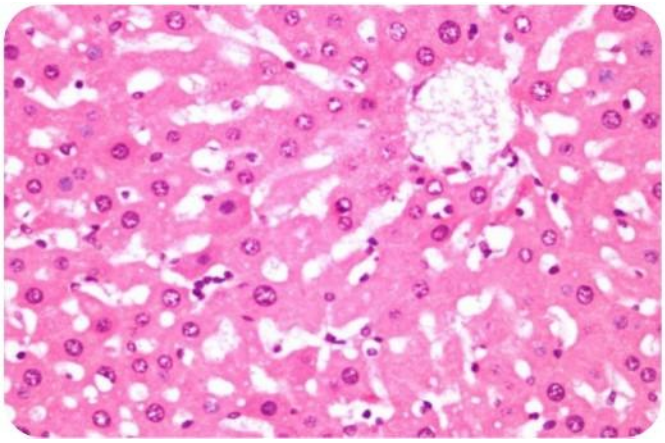

Figure 7: Hepatic tissue photomicrograph of Group-III (low dose NS + PZA) showing mild parenchymal inflammation with spotty necrosis manifested as few drop off hepatocytes (H&E 40X)

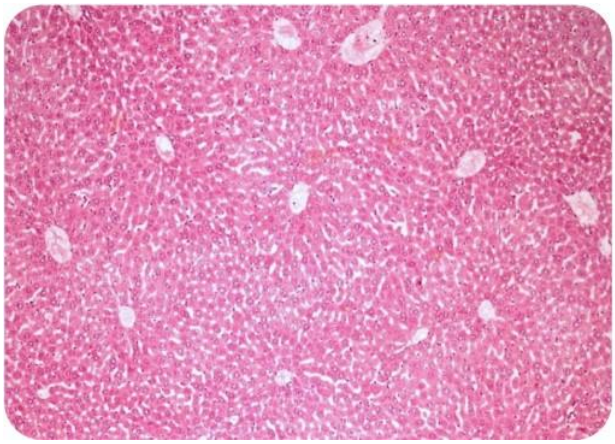

Figure 8: Hepatic tissue photomicrograph of Group-III (high dose NS + PZA) showing preserved architecture of parenchyma and portal triads. (H&E 10X)

DISCUSSION

Pyrazinamide (PZA) is a crucial antibiotic in the treatment of Tuberculosis (TB), that has sputum sterilizing and treatment shortening capability. Its ability to eradicate M*ycobacterium tuberculosis* in the acidic pH of phagosome and inflammatory tuberculous lesions renders it an indispensable component of anti-TB regimens.[13] Although benefits of pyrazinamide in treating TB outweigh the associated risks, its potential to cause liver damage cannot be disregarded. Accordingly, ongoing research is still focused on identifying an agent to mitigate the deleterious adverse effect of anti TB drugs. The current study is, hence, conducted in a quest to evaluate the hepatoprotective capacity of a multiorgan protective herb against hepatic injury induced by PZA.

In current study, PZA in 500mg/kg daily for six weeks caused moderate to severe liver damage in mice characterized by presence of parenchymal inflammation, slight edema of hepatocytes, vascular congestion, vascular dilatation, periportal and portal inflammation and diffuse areas of necrosis. Parenchymal inflammation was characterized by presence of great number of lymphocytes and disarray of normal architecture of liver. Hepatic necrosis and inflammation were also seen in other studies,[2,14-17] though hepatic steatosis found in those studies was surprisingly not detected in PZA treated hepatic tissues of our study.

The liver is highly susceptible to damage by PZA which is manifested in humans by typical acute hepatitis, hepatocellular necrosis, portal and lobular inflammation and variable degree of cholestasis.[4,16] The multitude of mechanisms underlying this hepatic insult includes enhanced lipid peroxidation, mitochondrial dysfunction, apoptosis, and inhibition of antioxidant capacity of hepatocytes in dose dependent manner, though the exact mechanism of PZA induced hepatic insult remains unknown.[2]

In group-III and group-IV, 500 mg/kg and 1000 mg/kg *Nigella sativa* seeds powder were administered respectively along with PZA. In both groups all the histological findings observed in group-II were reduced significantly, with high dose of NS exhibiting more pronounced protective effect as compared to low dose. This indicates dose dependent response to NS supplementation. Several studies have documented this hepatoprotective potential of NS seeds and oil against other hepatotoxins including anti tuberculous drugs.[18–21] This anti-inflammatory and hepatoprotective effects of NS seeds may be attributed to the presence of Thymoquinone (TQ), a major bioactive component in the seeds. TQ suppresses the production of pro-inflammatory cytokines (e.g., IL-1b, IL-6, TNF-α, IFN-γ, and PGE2) and inhibits the activation of inflammatory signaling pathways such as NF-Kβ by reducing cytochrome c production and PGE2 formation by inhibiting both cyclooxygenase and lipoxygenase.[9] This modulation of inflammatory responses reduces liver inflammation and prevents tissue injury in pyrazinamide-induced hepatic damage. Moreover, TQ has been shown to possess antioxidant properties, which assist in perseverance of hepatic tissue structural integrity by reducing oxidative stress. TQ reduces oxidative stress by scavenging oxygen free radicals and preventing lipid peroxidation in dose dependent fashion. NS suppresses oxidative stress mediated hepatic damage by increasing the activities of various enzymes and also by upregulating glutathione-S-transferase which is involved in detoxification and biotransformation of chemotherapeutic agents.[9,12]

Additionally, *Nigella sativa* constituents have been shown to influence drug-metabolizing enzymes, particularly cytochrome P450 (CYP) enzymes, involved in drug metabolism. By modulating the activity of these enzymes, *Nigella sativa* may alter the metabolism of pyrazinamide and its toxic metabolites, thereby reducing hepatotoxicity. Moreover, TQ inhibits the activity of hepatic CYP1A1/A2 isozymes which are involved in biotransformation of xenobiotics into genotoxic reactive derivatives, leading to genetic mutation.[9,10]

LIMITATIONS

Whereas the study's findings could contribute significantly to the field, it is important to address its limitations and validate results through additional research to ensure their applicability and efficacy in human clinical settings. To observe long-term effects of PZA and the potential protective benefits of *Nigella sativa* over extended periods, the study's duration should be prolonged as chronic exposure studies might provide more comprehensive insights. Histopathological examination provides valuable insights but do not cover all aspects of liver damage or recovery. Additional biomarkers and functional assays could complement histological findings.

The study focuses on preclinical models; to look for the efficacy and safety of *Nigella sativa* in human subjects, particularly in conjunction with PZA, further investigations are needed. Positive results from this study could pave the way for clinical trials in humans to verify the hepatoprotective effects of *Nigella sativa* and determine appropriate dosing, safety, and efficacy in TB patients.

CONCLUSION

Concomitant administration of *Nigella sativa* seeds is concluded to be beneficial to counter the hepatotoxicity induced by pyrazinamide and these seeds attenuate the hepatic histoarchitectural changes induced by pyrazinamide in dose dependent manner.

RECOMMENDATIONS

A varied range of doses should be evaluated, and longer duration of research should be conducted with larger sample sizes. Studies should be conducted on humans and effective therapeutic dosages should be found. Other parts of NS plant should also be evaluated for hepatoprotective roles.

Biochemical exploration and electron microscopy should be done in centers of excellence for its therapeutic potential.

Further research could explore the precise mechanisms through which *Nigella sativa* exerts its hepatoprotective effects and assess its interactions with other TB medications.

If proven effective, *Nigella sativa* could influence treatment guidelines and protocols, leading to the development of new

standards for managing TB-related hepatotoxicity and improving overall treatment strategies.

Nigella sativa demonstrates significant hepatoprotective effects; it could be used as an adjunct therapy to mitigate liver damage associated with PZA, potentially enhancing the safety and tolerability of TB treatment regimens. The study could promote the integration of natural products into conventional medical treatments, offering complementary options for managing drug-induced side effects and improving patient outcomes contributing to better adherence to TB treatment crucial for effective disease management and control.

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