Int.j.pathol.2022;20(3): 99-103

Original Article

Protective Effect of Nigella Sativa on Isoniazid Induced Hepatic Toxicity in Rabbits

Abdullah Jan Panezai[1], Syed Muhammad Ishaque[2], Ahmed Ali Khan[3], Shahista Gul[4] Shahid Zafer[5] and Moosa Khan[6]

[1] Department of Pharmacology, Loralai Medical College Loralai, [2,4] Department of Pathology, Bolan Medical College, [3]Department of Anatomy, Bolan Medical College, [5] Department of Pathology, Liaquat College of Medicine and Dentistry, Karachi, [6] Department of Pharmacology & Therapeutics Basic Medical Sciences Institute, JPMC Karachi.

ABSTRACT

Introduction: The potential of ATT treatment regimens to induce hepatotoxicity necessitates a suitable adjunct therapy to diminish such effects. Nigella sativa is a herbal product. It has also been used as an anti-spasmodic, anti-oxidant, anti-asthmatic, anti-inflammatory, analgesic and an immune modulatory agent.

Objective: To evaluate the protective effect of Nigella sativa in Isoniazid (INH)-induced hepatic toxicity in rabbits

Material and Methods: This Randomized case-control study was conducted in the Department of Pharmacology and Therapeutics, BMSI, Karachi, in collaboration with the Animal house of BMSI, JPMC Karachi. In the current animal study 48 (N) healthy rabbits of either sex were randomly selected and divided into four groups comprising of 12 (n) rabbits in each group. Group-I was given a healthy diet only, Group-II was given only Isoniazid (INH) 50mg/kg body weight per day orally, Group-III was given INH 50mg/kg plus Nigella sativa 500 mg/kg body weight per day orally and Group-IV was given INH 50mg/kg plus Nigella sativa 1g/kg body weight per day orally. The treatment duration lasted for 20 days. Tissue sections were stained with Hematoxylin & Eosin stains, and observed under a light microscope using scanner (04x), low (10x) and high power (40x). The data was analyzed with using SPSS version 16. Paired T-Test and Chi-square were applied.

Results: Hepatic tissue was observed with a variable degree of histopathological changes induced by INH-associated hepatic toxicity. The changes were either prevented or mitigated by giving Nigella sativa in combination therapy.

Conclusion: Nigella sativa showed protective effects in INH-induced hepatic tissue injuries in rabbits.

Key Words: Anti-tuberculosis Therapy (ATT), Fatty changes, Isoniazid (INH), Nigella sativa (N-S), Piecemeal necrosis, Portal inflammation, Tuberculosis (TB)

Introduction

Liver plays an important role in several biochemical functions that are responsible for the adjustment of internal body environment and preventing disease. It provides energy, nutrition and has a role in sexual activity as well. [1] A normal functioning liver ensures harmony between various important metabolic processes, and thus maintains a normal or disease-free internal environment of a person's body.[2] Besides metabolism and production of bile, the major functions of liver included synthesis of blood clotting factors, storage of vitamins & minerals and detoxification of drugs & other chemicals.[3,4,5]

CORRESPONDENCE AUTHOR
Syed Muhammad Ishaque,
Ishaqsyed784@gmail.com. 03003801784
Adress: Pathologist Dow Laboratory,
Faiz Muhammad Road, Quetta, Pakistan.

Annually, about 1.4 million deaths globally are attributed to tuberculosis (TB). According to the National TB Control Program (NTBCP), 413 to 450 TB cases are reported every year in Pakistan with a ratio of 231/100,000 population, the existing cases of TB are 630,000 cases at a rate of 364/100,000 population, and a mortality rate accounting for 60,000 deaths (34/100,000 person).[6]

Hepatic toxicity leading to liver injury is a recognized side-effect of anti-tuberculosis therapy (ATT) which can present as serious health problem.[7]ATT induced liver injury is believed to be through oxidative stress and damage by free radicals to the liver cells. Hepatotoxicity also results from the lack of enzymes due to some alterations in genetics, environmental factors, slow acetylator status and cytochrome-2E1 (CYP).[3, 7]

The potential of ATT treatment regimens to induce hepatotoxicity necessitates a suitable adjunct therapy

to diminish such effects. Scientists have turned to herbal products such as Silybummarianum (Milk thistle), Ocimumsanctum (Tulsi), Rhododendron arboreum (Burans), Coriandrumsativum (Coriander),[11] and Zingiberofficinale (Ginger) to see their protective effects and possible use as adjunct therapy with ATT[8,9,10,11].

Nigella sativa is a herbal product that contains 38% carbohydrates, 35% plant fats and 21% proteins. Other constituents include 38% fixed oil, near to 2.5% essential oils, calcium, potassium, iron, zinc, magnesium, selenium, vitamins A, B_1 & B_2, niacin, arginine, methionine, lysine, glycine and leucine. [7, 12]

Nigella sativa is therapeutically used in different parts of the world for various purposes, including complaints like headache & cough, decreased dietary cholesterol absorption, hepatic injury, and CNS ischemia. It has also been used as an anti-spasmodic, anti-oxidant, anti-asthmatic, anti-inflammatory, analgesic and an immune modulatory agent. [7,13,14,15]

We also tried to evaluate the protective effect of Nigella sativa in Isoniazid (INH)-induced hepatic toxicity in rabbits.

Methods

The current study lasted for twenty days and was conducted at the Basic Medical Science Institute (BMSI), Department of Pharmacology and Therapeutics in collaboration of the animal house and Department of Pathology Jinnah Postgraduate Medical Centre (JPMC), Karachi. A total 48 (N) of locally bred sexually mature Oryctolagus-cuniculus rabbits of both gender were taken. Their body weights ranged between 1.0 -1.5 kg. They were randomly divided into four equal groups (n=12 in each), and kept in the BMSI animal house. They were routinely examined to evaluate their general health condition. A standard laboratory diet (containing wheat, flour, vitamins, etc.) and water were given to all the animals. Locally purchased Isoniazid (isonicotinylhydrazine, INH) 100 mg tablets (manufactured by UNEXOLABS (PVT) limited) and Nigella sativa (available in solid form) were used. Nigella sativa was ground to convert it into powder form.

The four random groups were given their respective specific treatment regimen for twenty days. Group I was given healthy diet only, Group II was given only Isoniazid 50 mg/kg body weight per day orally, Group III was given Isoniazid 50 mg/kg plus Nigella sativa 500 mg/kg body weight per day orally and Group IV was given Isoniazid 50 mg/kg plus Nigella sativa 1 g/kg body weight per day orally. On day 21, hepatic tissue was obtained from these animals for processing, formalin-fixation and embedding in paraffin blocks. Thin slices of five microns were made to be stained with Hematoxylin & Eosin. All slides were studied under a light microscope using scanner (04 x), low (10 x) and followed by high power (40 x) by two different histopathologists. The changes were observed in hepatic zone I, II and III (anatomical). The study parameters included five sub-types of histopathological changes, i.e. fatty change, portal inflammation, piecemeal necrosis, spotty necrosis and swollen hepatocytes.

Fatty change (steatosis) was identified as appearance of empty vacuoles of variable sizes within the hepatocyte cytoplasm. The percentage of liver parenchyma involved was assessed through the acinar architecture as 0 to 33% - mild, 33 to 66% - moderate, and > 66% - severe steatosis. Portal inflammation was identified by few mononuclear cells in more than one portal tract when mild, and by moderate to marked density of inflammation or presence of lymphoid aggregates in at least one portal tract when more than mild. Peacemeal necrosis was observed as inflammation extending from the portal tract into the peripheral zone, and necrosis of periportal hepatocytes. Spotty necrosis was observed as necrosis of very small clusters of hepatocytes associated with lymphocytes. Several adjacent hepatocytes were observed to be absent and replaced by inflammatory cells. Swollen (or ballooned) hepatocytes were identified as enlarged cells with a swollen pale cytoplasm, a large nucleus and a prominent nucleolus. The degree or severity of these histopathological changes were classified as absent when no changes were observed under the light microscope, mild when minimum changes were observed, moderate when the changes were more than mild, and severe when the changes were observed throughout the three zones. The data (expressed as mean ± SEM) was statistically analyzed by SPSS version16. Paired T-Test and Chi-square were applied in compiling the results.

Results

The histopathological changes (parameters) were observed with the following features:

i. *Fatty change*: Group II (INH 50 mg/kg only) showed 58% moderate & 25% severe changes. Group III (INH 50mg/kg plus Nigella sativa 500 mg/kg) showed 25% mild & moderate changes

(each), 08% severe and 42% absent or no morphological changes. Group IV (INH 50 mg/kg plus Nigella sativa 1g/kg) showed 17% mild morphological changes and 83% absent or no morphological changes.

ii. *Portal inflammation*: Group II showed 58% moderate and 42% mild lymphoplasmacytic cells infiltrates, but both Group III and Group IV showed similar changes i.e. 67% mild changes and 33% absent or no morphological changes.

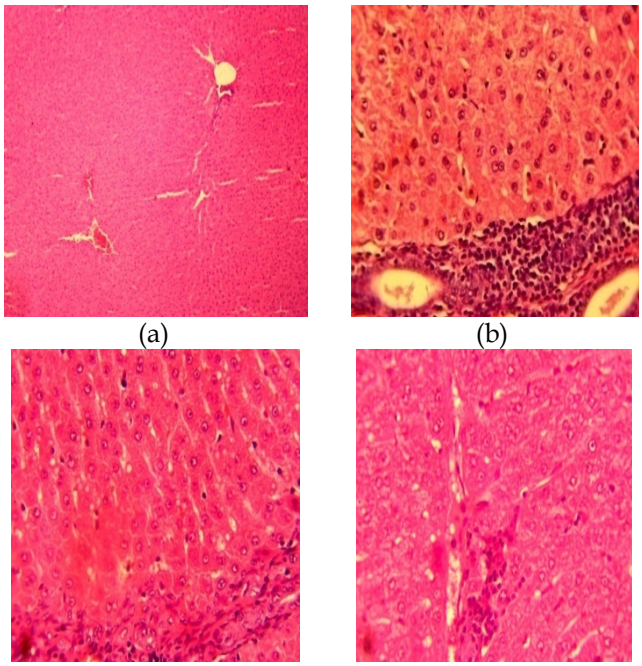
iii. *Piecemeal necrosis* (Peri-Portal or Peri-Septal Hepatitis)*:* Group II showed 67% moderate & 33% mild changes. Group III and Group IV showed 58% mild and 42% absent or no morphological changes.

iv. *Spotty necrosis* (*Apoptosis or Lytic Necrosis*)*:* Group II showed 83% mild changes. Group III showed 92% mild & 08% moderate changes, reflecting an increase in the changes observed. Group IV showed 08% mild changes only.

v. *Swollen hepatocytes:* The results showed that all the animals receiving INH alone or in combination with Nigella sativa, i.e. Groups II, III and IV, showed 50% severe and 50% moderate changes respectively.

Table-1: Comparison of different histopathological parameters of hepatic changes between various groups (N=48)

Parameters	Severity	Control and Treatment groups (N=48)			
		Group I n=12	Group II n=12	Group III n=12	Group IV n=12
Fatty change	Absent	100 (100.0%)	02 (16.7%)	05(41.7%)	10 (83.3%)
	Mild	00	00	03(25%)	02 (16.7%)
	Moderate	00	07 (58.3%)	03(25%)	00
	Severe	00	03 (25.0%)	01(08.3%)	00
Portal inflammation	Absent	99 (91.7%)	00	04(33.3%)	04 (33.3%)
	Mild	01(08.3%)	05 (41.7%)	08(66.7%)	08 (66.7%)
	Moderate	00	07 (58.3%)	00	00
	Severe	00	00	00	00
Piecemeal necrosis	Absent	100 (100.0%)	00	05(41.7%)	05 (41.7%)
	Mild	00	04 (33.3%)	07(58.3%)	07 (58.3%)
	Moderate	00	08 (66.7%)	00	00-
	Severe	00	00	00	00
Spotty necrosis	Absent	100 (100.0%)	02 (16.7%)	00	11 (91.7%)
	Mild	00	10 (83.3%)	11(91.7%)	01 (08.3%)
	Moderate	00	00	01(08.3%)	00
	Severe	00	00	00	00
Swollen hepatocyte	Absent	100 (100.0%)	00	00	00
	Mild	00	00	00	00
	Moderate	00	06 (50.0%)	06 (50.0%)	06 (50.0%)
	Severe	00	06 (50.0%)	06 (50.0%)	06 (50.0%)



(a) (b) (c) (d)

***Figure Ia*: Control (10x). Normal hepatic tissue (*Group I*), *Figure Ib*: H&E (40x).Effects of INH: Swollen hepatocytes, fatty changes, portal inflammation (Lymphoplasmacytic infiltrates), spotty necrosis & piecemeal necrosis (*Group II*) *Figure Ic*: H&E (40x). Effects of N-S & INH: Hepatic parenchyma with portal inflammation and spotty necrosis (*Group III*) *Figure Id*: H&E (40x) Effects of N-S and INH: Hepatic parenchyma with mild portal inflammation and spotty necrosis (*Group IV*)**

Discussion

The current study result showed when INH given alone had increased incidence of moderate to severe fatty changes, but in combination with low dose of Nigella sativa, the incidence decreased. However, in higher dose of Nigella sativa, the changes were absent and only few cases showed mild fatty changes. Almost similar finding as protective role in concomitant administration with high dose of Nigella sativa were also noted by Tiwari A et al (2022).[16]

Similarly alone INH showed mild to moderate, portal inflammation but in combination with Nigella sativa, in majority of cases observed mild changes and less number of cases with no changes. In peri-portal or peri-septal hepatitis (piecemeal necrosis) with only INH, observed moderate severity in majority of cases but with Nigella sativa therapy the changes frequently noted to be mild category, our results are in agreement with Yasmin F et al (2013) with same finding.[13]

Our results are also comparable to several animal studies investigating protective effect of Nigella sativa in ATT-induced hepatotoxicity.[7, 17] There are studies that have investigated drug-induced hepatotoxicity through biochemical (including serum liver enzymes, serum albumin, total protein and serum bilirubin) and histopathological changes.[4, 7, 18,19] The histopathological findings were observed to corroborate with the biochemical findings. It was found that Nigella sativa extracts not only detoxified the toxicity but also reversed the biochemical changes by restoring the levels of liver enzymes (i.e. alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT)), serum proteins and serum bilirubin back to normal.[20] Higher doses were observed to have better results compared to lower doses in almost all of these studies.

Recent studies have emphasized that histopathological changes observed in the liver results from oxidative stress and the anti-oxidant properties of Nigella sativa can counteract these changes or even reverse them. Furthermore, beside its therapeutic effects, the prophylactic effects of Nigella sativa has also been investigated with promising results.[21] Besides the return of raised serum liver enzymes or decreased serum protein levels to normal levels, the histopathological changes have also been reported to have been mitigated and reversed, which compares favorably to our findings.[22]

Conclusion and Recommendations

Increasing dose of Nigella sativa (a herbal product) delivered promising results in our study regarding its protective effects on the hepatotoxicity induced by isoniazid.

We recommend that a specific and proper dosage of Nigella sativa should be given to patients receiving ATT for 6 months or more, e.g. in patients with Koch's disease, to mitigate the hepatotoxic effects of the therapy.

Acknowledgment:

We acknowledge Professor Dr. Naseer Khan, Department of Pharmacology BMC and Professor Dr. Sanaullah Gazozai Department of Pathology BMC Quetta for their cooperation in reviewing the work and help in statistical analysis

Conflict of Interest: Authors declare no conflict of interest.

Funding: No funding was received for this project

References

1. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: A Review. J Appl Pharm Sci. 2012; 02 (05): 233–243.
2. Saleem M, Naseer F. Medicinal plants in the protection and treatment of liver diseases. Bangladesh J Pharmacol. 2014; 9: 511-526.
3. Grattagliano I, Bonfrate L, Diogo CV, Wang HH, Wang DQH, Portincasa P. Biochemical mechanisms in drug-induced liver injury: certainties and doubts. World J Gastroenterol. 2009; 15 (39): 4865–4876.
4. Mudie K, Seifu D, Debella A, Challa F, Abebe A, Gemeda N. Hepatoprotective activity of aqueous seed extract of Nigella sativa against highly active antiretroviral therapy induced hepatotoxicity in rats. J Bioanal Biomed. 2017; 9 (4): 64.
5. Abdul Bari MAM. The protective effect of Nigella Sativa in Paracetamol induced liver toxicity in mice. Al Mustansiriyah J Pharm Sci. 2020; 20 (2): 11–8.
6. 249Khan AH. Tuberculosis control in Sindh, Pakistan: Critical analysis of its implementation. J Infect Public Health. 2017; 10 (1): 1–7.
7. Paul J, Nasiruddin M, Khan I, Khan R, Arif S. Therapeutic Effect of Nigella sativa Oil in Hepatotoxicity Induced by Isoniazid in Rats. Indian J Pharm Educ. 2019; 53: 242–8.
   Tao L, Qu X, Zhang Y, Song Y, Zhang SX. Prophylactic Therapy of Silymarin (Milk Thistle) on Antituberculosis Drug-Induced Liver Injury: A Meta-Analysis of Randomized Controlled Trials. Can J Gastroenterol Hepatol. 2019; 19: 1–11.
8. Lahon K, Das S. Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. Pharmacogn Res. 2011; 3 (1): 13–8.
9. Prakash T, Dayalal Fadadu S, Raj Sharma U, Surendra V, Goli D, Stamina P, et al. Hepatoprotective activity of leaves of Rhododendron arboreum in CCl4 induced hepatotoxicity in rats. J Med Plants Res. 2008; 2 (11): 315–320.
10. Zein N, Elghani E, Talat E. Effect of coriandrumsativum on experimentally induced hepatotoxicity of carbon tetrachloride in rats. Biochem Lett. 2014; 9 (11):135–55.
11. Ahmed M. The Protective Effect of Ginger (Zingiber Officinale) Against Adriamycin- Induced Hepatotoxicity in Rats: Histological Study. Life Sci J. 2013; 10 (1): 1412–1422.
12. Sayeed R, Khan A, Tazneem B, Ali S. To study the effect of Nigella sativa on various biochemical parameters on stress induced in Albino Rats. Int J Pharm Pharm Sci. 2010; 2: 185–189.
13. Yesmin F, Rahman Z, Dewan J, Helali A, Islam Z, Rahman A, et al. Hepatoprotective effect of aqueous and N-hexane extract of Nigella sativa in paracetamol (acetaminophen) induced liver disease of rats: a histopathological evaluation. J Pharm. 2013; 4: 90–94.
14. Salman MT, Khan R, Shukla I. Antibacterial activity of Nigella Sativa linn. Seeds against multiple antibiotics resistant clinical strains of Staphylococcus aureus. Int Arch Biomed Clin Res. 2016; 2:96–99.
15. Trivedi M, Chansoria AK, Dixit RK. An experimental study to see the protective effect of thymoquinone against anti tubercular drug induced hepatic toxicity in rats. Int J Univ Pharm Bio Sci. 2013; 2(1): 1–8
16. Tiwari A, G S, Meka S, Varghese B, Vishwakarma G, Adela R. The effect of Nigella sativa on non-alcoholic fatty liver disease: A systematic review and meta-analysis. Hum Nutr Metab. 2022; 28: 1–9.
17. Mollazadeh H, Hosseinzadeh H. The protective effect of Nigella sativa against liver injury: a review. Iran J Basic Med Sci. 2014; 17(12): 958–966.
18. Trivedi M, Chansoria AK, Dixit RK. An experimental study to see the protective effect of thymoquinone against antitubercular drug induced hepatic toxicity in rats. Int J Univers Pharm Bio Sci. 2013; 2(1): 1–8.
19. Jaswal A, Shukla S. Therapeutic efficacy of Nigella sativa Linn. seed extract against CCl4 induced hepatic injury in Wistar rats. Indian J Exp Biol. 2015; 53 (1): 44–50.
20. Mahdi N. protective effect of crude oil of Nigella sativa on liver in male albino mice treated with low toxic dose of paracetamol. Med J Babylon. 2013; 10 (4): 929–936.
21. Kushwah DS, Salman MT, Singh P, Verma VK, Ahmad A. Protective effects of ethanolic extract of Nigella sativa seed in paracetamol induced acute hepatotoxicity in vivo. Pak J Biol Sci. 2014; 17 (4): 517–522.
22. Ghadlinge M, Jaju J, Chandane R, Jadhav R, Bhosle R. A study of effect of Nigella sativa oil in paracetamol induced hepatotoxicity in albino rats. Int J Basic Clin Pharmacol. 2014; 3 (3): 539–545.

HISTORY	
Date received:	27-7-2022
Date sent for review:	25-8-2022
Date received reviewers comments:	23-9-2022
Date received revised manuscript:	10-12-2022
Date accepted:	12-12-2022

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

CONTRIBUTION OF AUTHORS	
Author	**Contribution**
Abdullah Jan Panezai	A
Syed Muhammad Ishaque	A
Ahmed Ali Khan	C
Shahista Gul	B
Shahid Zafer	B
Moosa Khan	C