ORIGINAL ARTICLE

Role of Nigella Sativa Seeds on Pyrazinamide Induced Hepatotoxicity

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ABSTRACT

Objective: To determine the hepatoprotective effect of *Nigella sativa* seeds in Pyrazinamide induced hepatic damage in albino mice.

Study Design: An experimental study.

Place and Duration of Study: The study was conducted in pharmacology department of Islamic International Medical College, Riphah International University, in collaboration with National Institute of Health, Armed Force Institute of Pathology and Pakistan Institute of Medical Sciences Islamabad, Pakistan.

Materials and Methods: A total of 68 male albino mice were included in the study. They were divided into 4 group's i.e, seventeen mice in each group. Group A (control group), Group B (Pyrazinamide only treated), Group C (Pyrazinamide + Low dose *Nigella sativa* treated) and Group D (Pyrazinamide+ High dose *Nigella sativa* treated). Group B, C and D received Pyrazinamide through gavage needle once daily for six weeks. Group C and D received *Nigella sativa* along with Pyrazinamide through gavage needle once daily for six weeks. Blood sampling (baseline, end of 3 and 6 weeks) was done to analyze serum ALT and AST levels. Data was analyzed by using SPSS version 21.

Results: Pyrazinamide treatment for 6 weeks increased serum ALT and AST levels. *Nigella sativa* administration along with Pyrazinamide for six weeks decreased the elevated levels of ALT and AST in C and D groups.

Conclusion: *Nigella sativa* seeds exerted hepatoprotective effect by decreasing the raised levels of ALT and AST in Pyrazinamide treated albino mice.

Key Words: *ALT, AST, Hepatotoxicity, Nigella Sativa Seeds, Pyrazinamide.*

Introduction

Tuberculosis is an infectious bacterial ailment caused by several species of mycobacterium.[1] One-third of the global population is infected with tuberculosis, out of whom 5%–10% may develop active tuberculosis in rest of their lives. Tuberculosis is a disease that causes 1.4 million deaths each year.[2] All three major drugs for the treatment of tuberculosis i.e., Pyrazinamide(PZA), Isoniazid(INH) and Rifampicin(RIF) have hepatotoxic effects.[3] The occurrence of liver injury due to antituberculosis treatment (ATT) varies from 2.0% to 28.0%.[4] An important first line and sterilizing tuberculosis drug is Pyrazinamide which aids in condensing the duration of present chemotherapy regimens for tuberculosis.[5] Pyrazinamide causes more hepatotoxicity compared to Isoniazid and Rifampin.[6,7] Hepatotoxicity by Pyrazinamide is either dose dependent or idiosyncratic. Free radical species are produced as result of alteration of nicotinamide acetyl dehydrogenase levels by PZA.INH and PZA may have similar mechanism of injury because of similarity in their molecular structure. In Patients with latent tuberculosis infection, more toxic effects came into observation with RIF and PZA. PZA may cause hypersensitivity reactions and liver damage.[8] *Nigella sativa* has emerged as a miraculous herb with its wide range of pharmacological effects.[9] Hassan et al, (2012) has done research on hepatoprotective effect of *Nigella Sativa* seeds on Isoniazid induced hepatotoxicity and that research was conducted on rabbits. We have conducted the research on male albino mice.

Danladi et al, (2013) has conducted his research on

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protective effects of *Nigella Sativa* in CCl_4 induced hepatotoxicity in rats, while in our research PZA was used.

Nigella sativa (Black cumin) was found to be protective against various hepatotoxic chemicals and drugs.[10] The antioxidant, anti-angiogenesis and anti-inflammatory properties of *Nigella sativa* may play a part in its hepatoprotective role.[11] Thymoquinone (TQ) is an active constituent and is proven to bear hepatoprotective qualities.[9] TQ has immunomodulatory effects on the antioxidant defense mechanisms of the body that underwent toxicity.[12] The hepatoprotective activity was revealed by a reduced release of alanine aminotransferase (ALT), aspartate aminotransferase (AST)and reduced uptake of trypan blue.[9] Many studies have been conducted on hepatotoxic effect of ATT and PZA is a component of this therapy. Its hepatotoxic effect is a main factor in compromising the treatment of TB patients. Therefore to search for a drug that can reduce the effects of PZA toxicity is a challenge for pharmacologists.

This research was aimed to determine the hepatoprotective activity of *Nigella sativa* seeds on PZA induced hepatic damage in male albino mice.

Materials and Methods

An experimental study was conducted at Pharmacology Laboratory, Department of Pharmacology and Therapeutics and Multidisciplinary Research Laboratory, Islamic International Medical College, Rawalpindi with access to Animal House for mice at National Institute of Health (NIH). The project was accepted by the Ethics Review Committee of Islamic International Medical College, Riphah International University,Islamabad. The study took 6 weeks (10^{th} November, 2015 to 22^{nd} December, 2015). Sixty eight adult male Albino mice 2 months of age weighing 25-50gm, purchased from the animal house of NIH were chosen for research project and were then divided into 4 groups i.e. Group A as control group and Groups B, C and D were experimental groups each comprising 17 male albino mice.

Standard nutrition consisting eating regimen and clean tap water was started *ad libitum*. A specific room was assigned for experimental mice at NIH. Mice were retained in a well-ventilated place, humidity 50-70 %, room temperature 24±2°C and 12 hours light/dark cycle was maintained. [12, 13] Study was initiated after one week acclimatization of mice under the standard research facility settings. *Nigella Sativa* (kalonji) seeds were obtained from National Agriculture Research Centre, Islamabad. Plant material was certified by National Agriculture Research Centre, Islamabad through proper taxonomical rules. *Nigella Sativa* seeds were grounded into fine powder in electrical grinder. Suspension was made by adding glucose water into the fine powder. 500mg seeds powder was added to 5ml glucose water equivalent to 100mg of seed powder per ml for group C mice (A dose of 500mg/kg was administered to each mouse in group C). One gram seed powder was added to 5ml glucose water equivalent to 200mg of seed powder per ml for group D mice (A dose of 1000mg/kg was administered to each mouse in group D). [14] 500mg (research grade salt of Pyrazinamide) was dissolved in 5ml glucose water equivalent to 100mg of drug per ml (A dose of 500mg/kg was administered to each mouse in group B, C and D). The powder of Pyrazinamide was dissolved completely to obtain a homogenized solution. The dose of Pyrazinamide was calculated for body weight of mice.[14, 15] Glucose water suspension of Pyrazinamide was prepared in an amount of 500 mg/kg body weight (i.e 20 mg of research grade salt of Pyrazinamide was dissolved in 0.5ml glucose water) and was administered once daily to mice in group B for 6 weeks through a gavage tube and syringe. Glucose water suspension of *Nigella sativa* was used in a dosage of 500 mg/kg body weight in the group C mice (20 mg *NS* seeds powder *was* dissolved in 0.5ml glucose water) along with 500mg/kg body weight of Pyrazinamide(i.e 20 mg of research grade salt of Pyrazinamide was dissolved in 0.5ml glucose water) and in dosage of 1000mg/kg body weight in group D(40 mg *NS* seeds powder *was* dissolved in 0.5ml glucose water) along with 500mg/kg body weight of Pyrazinamide(i.e 20 mg of research grade salt of pyrazinamide was dissolved in 0.5ml glucose water) given once daily to mice for 6 weeks by means of a gavage tube and syringe.[14] Blood samples of mice from all groups were taken three times (baseline, at the end of 3 and 6 weeks). Blood samples of 2 mice from each group were collected at day 0 for baseline liver enzymes (ALT, and AST) and of 5 mice from each group at day

21 for evaluation of progress of research. Finally a blood sample of 10 mice from each group was taken at day 42 for final evaluation of liver enzymes.[16] Intracardiac blood sampling technique was utilized under Chloroform anesthesia to acquire the blood and further biochemical assay measurement. After centrifugation at 3000 rev/min for 10min serum was separated from blood. Bench top centrifuge was used for this purpose. Serum was kept at -20°C until biochemical assay measurement took place.[17] Serum ALT and AST were determined by using ALT and AST kit by (Merck) on Chemistry Analyzer, Micro lab 300 (Merck) by IFCC method.

Statistical analysis was done using SPSS version 21 to analyze the data. Mean ± S.D was calculated for ALT and AST. One way ANOVA was used to evaluate mean difference between control and experimental groups. Post Hoc Tuckey test was applied for the comparison of mean difference between groups. $p<0.05$ was considered significant.

Results

The levels of serum ALT and AST in various animal groups and values of their mean, S.D and Post Hoc comparisons between the groups is given in tables I - VI. Pyrazinamide treatment for 6 weeks has significantly increased levels of ALT and AST in group B. Nigella sativa seeds administration at low (500mg/kg body weight) and high dose (1000mg/kg body weight) along with pyrazinamide for 6 weeks was reduced (P<0.05) the elevated levels of ALT and AST in group C and D respectively.

Table I: Description of Mean and Std. Deviation of ALT in all Groups at day 42

Groups	Minimum	Maximum	Mean	Std. Deviation
A	14	38	27.40	7.291
B	56	124	82.80	21.883
C	29	55	38.40	7.891
D	23	51	36.70	8.179

Table II: Comparison of serum ALT amongst different Groups at the end of experiment by one way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	18440.275	3	6146.758	37.187	.000
Within Groups	5950.500	36	165.292		
Total	24390.775	39			

Table III: Post-hoc Comparison of Total ALT between the Groups Tukey HSD Dependent Variable: ALT (U/L)

(I) Experimental Group	(J) Experimental Group	Mean Difference (I-J)	Sig.
1 Group A	2 Group B(Z)	-55.400(*)	.000
	3 Group C	-11.000	.241
	4 Group D(High dose herb+ Z)	-9.300	.382
2 Group B(Z)	1 Group A	55.400(*)	.000
	3 Group C	44.400(*)	.000
	4 Group D(High dose herb+ Z)	46.100(*)	.000
3 Group C	1 Group A	11.000	.241
	2 Group B(Z)	-44.400(*)	.000
	4 Group D(High dose herb+ Z)	1.700	.991
4 Group D(High dose herb+ Z)	1 Group A	9.300	.382
	2 Group B(Z)	-46.100(*)	.000
	3 Group C	-1.700	.991

* The mean difference is significant at the .05 level.

Table IV: Description of mean and std. deviation of AST in all Groups at day 42

Groups	Minimum	Maximum	Mean	Std. Deviation
A	28	70	48.30	15.011
B	74	159	103.00	28.063
C	44	87	67.40	12.721
D	32	71	54.40	14.683

Table V: Comparison of serum AST amongst different Groups at the end of experiment by one way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17981.075	3	5993.692	17.244	.000
Within Groups	12512.900	36	347.581		
Total	30493.975	39			

Discussion

In our study we observed that oral administration of Pyrazinamide for 06 weeks has significantly elevated the levels of serum ALT and AST in male albino mice. Drug induced hepatotoxicity is a leading health problem with far reaching implications and is the

TableVI: Post-Hoc Comparison of Total AST between the Groups Tukey HSD
Dependent Variable: AST (U/L)

(I) Experimental Group	(J) Experimental Group	Mean Difference (I-J)	Sig.
1 Group A	2 Group B(Z)	-54.700(*)	.000
	3 Group C	-19.100	.119
	4 Group D(High dose herb+ Z)	-6.100	.884
2 Group B(Z)	1 Group A	54.700(*)	.000
	3 Group C	35.600(*)	.001
	4 Group D(High dose herb+ Z)	48.600(*)	.000
3 Group C	1 Group A	19.100	.119
	2 Group B(Z)	-35.600(*)	.001
	4 Group D(High dose herb+ Z)	13.000	.414
4 Group D(High dose herb+ Z)	1 Group A	6.100	.884
	2 Group B(Z)	-48.600(*)	.000
	3 Group C	-13.000	.414

***** The mean difference is significant at the .05 level.

most common cause of termination of drug development programs.[18] More than 900 drugs have been implicated with this side effect.[19] Liver is pivotal to all detoxification processes and adversely targeted by administration of Pyrazinamide.[20] Present study was channeled in order to explore the hepatoprotective effect of seeds of *Nigella sativa* in Pyrazinamide induced hepatotoxicity. Our study revealed that glucose solution of Nigella sativa has marked hepatoprotective activity ($p < 0.05$) in a dose dependent manner. High dose of *Nigella sativa* (1000mg/kg) lowered level of ALT and AST in group D to a greater extent as compared to low dose (500mg/kg) given in group C. Combined administration of 1000mg/kg of Nigella sativa with PZA significantly prevented the elevation of ALT and AST as compared to drug treated group B. Current study is in accordance with multiple studies which proved hepatoprotective effect of *Nigella sativa* oil or the *Nigella sativa* seeds aqueous suspension against several hepatotoxins.[21,22,23] Thymoquinone is an active constituent of *Nigella sativa* that is proven for bearing hepatoprotective activity.[9]

The antioxidant, anti-angiogenesis and anti-inflammatory properties of *Nigella sativa* also play a part in its hepatoprotective role.[11] It is proven that harmful effects of ischemia reperfusion injury of liver are relieved by Nigella sativa.[24] Also its administration protects liver tissue from harmful properties of toxic chemicals and metals.[25]

Our results clearly show that Nigella sativa is hepatoprotective which is indicated by changes in hepatic enzymes. This result is greatly supported by data given in literature.[14,26] Our study shows the hepatoprotective effects of Nigella Sativa. However we recommend search for the hepatoprotective effect of different components of this herb to find the most active component which can further provide a lead for the development of a hepatoprotective drug.

Conclusion

Concurrent administration of *Nigella sativa* seeds with pyrazinamide produce hepatoprotective effect thereby preventing hepatotoxicity caused by pyrazinamide.

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