VARIABLE DOSES OF NIGELLA SATIVA IN ISONIAZID INDUCED LIVER TOXICITY IN RABBITS

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Abstract

Introduction: Nigella sativa (black seeds) is an herbal product belongs to ranunculaceae family which is an annual herbaceous medicinal plant has oldest historical and religious background.

Objective: To evaluate the effect of various doses of Nigella sativa in Isoniazid-induced liver biochemical changes in Rabbits.

Study design: Animal Study (randomized case-control).

Place of study: Department of Pharmacology and Therapeutics in collaboration with the Animal House BMSI, JPMC Karachi.

Methods: In this animal study, 48 healthy rabbits (n=8) of either sex were randomly selected and equally divided into four groups (n=12 in each group). Group I was given healthy diet only. Group II was given Isoniazid at 50mg/kg body weight per day orally. Group III was given Isoniazid 50mg/kg plus Nigella sativa 500 mg/kg body weight per day orally and Group IV was given Isoniazid 50mg/kg plus Nigella sativa 1gm/kg body weight per day orally. The study duration was 20 days. Liver biochemical changes, i.e. Serum Total Bilirubin, Alanine Transaminase (ALT) and Aspartate Transaminase (AST), were investigated on 00 and 21 days. The data was collected using a design proforma and assessed statistically with SPSS version 16.0. Paired T-Test was applied.

Results: In Group I, the liver biochemical changes were non-significant on day 00 and 21 (S. Total Bilirubin, p =0.06; ALT, p=0.09 & AST, p=0.74). In Group II, the parameters were significantly raised, when compared on day 00 and 21 (S. Total Bilirubin, p=0.00; ALT, p=0.00 & AST, p=0.00). In Group III, the liver biochemical changes noted were both significant and non-significant when compared on day 00 and 21 (S. Total Bilirubin, p=0.06; ALT, p=0.00 & AST, p=0.74). In Group IV, the liver biochemical changes were non-significant when compared on day 00 and 21 (S. Total Bilirubin, p=0.06; ALT, p=0.09 & AST, p=0.74).

Conclusion: Nigella sativa showed a protective role in isoniazid-induced liver toxicity in rabbits.

Keywords: Anti-tuberculosis therapy (ATT), Alanine transaminase (ALT), Aspartate transaminase (AST), Isoniazid (INH), Nigella sativa (NS), Serum total bilirubin, Tuberculosis (TB)

INTRODUCTION

Medicinal plants are used in allopathic field to cure various medical conditions in different part of the world. Nigella sativa herbal product belongs to ranunculaceae family which is an annual herbaceous plant (1), is a medicinal plant has oldest historical and religious background. It is used for researches to evaluate its pharmacological efficacy. Nigella sativa have therapeutic potential and broad spectrum activities i-e anti-diabetic, anti-diuretic, anti-hypertensive,anti-inflammatory, anti-microbial, anti-cancer, hapato &...
gastro-protective effects. Nigella sativa grows 20 cm to 90 cm tall, composed of leaves, leaf, soft flower white, yellow, pink, pale blue/purple color have 5-10 petals and encapsulated fruit have 3 to 7 united follicles and contain multiple seeds (2).

Nigella sativa also known as black seeds used in traditional medicine in various countries in the form of essential oil, paste and powder in different medical conditions like headache, body ache, joints pain, bronchial asthma, bronchitis, amenorrhea, hypertension, skin lesions, mental disease and so many others. Nigella sativa used in different type of researches including pharmacology, medicine and biochemistry. The Prophet Muhammad (PBUH) says that Nigella sativa is curative for all disease except death. IBN-Sina father of early modern medicine highlight different beneficial effects of Nigella sativa on various health issue like to increased energy, recovery from illnesses and protective effects on different organs (3).

Nigella sativa black seed contains fat (28.5%), protein (26.7%), carbohydrate (24.9%), fatty oil (35.6%-41.5%), cellulose (6.8%-7.4%), crude fiber (8.4%), total ash (4.8%), vitamin B1, B2, B3, A, C and minerals Zn, Ca, Fe, Se, P, K in various quantity. Nigella sativa have potent ant-bacterial effects on gram negative & gram positive species, anti-fungal, anti-viral, anti-parasitic and anti-oxidants effects (4).

Liver is a vital and inevitable organ in the human body for metabolism of carbohydrates, proteins and fats (5). It has a significant role in homeostatic regulation, bile production and storage of vitamins and minerals. It has a major role in the metabolism of drugs, other chemicals, and regularly detoxifying them (6-8). The latter function renders liver susceptible to drug-induced injury. Generally the lesions related to liver are categorized as hepatitis (acute or chronic), cirrhosis and malignancy (9, 10).

Tuberculosis (TB) causes high rates of morbidity & mortality worldwide, the ratio being higher in Africa and Asia. In 2006, around 1.7 million deaths were recorded from TB, and 0.2 million of these cases were also HIV/AIDS positive (11). The prevalence of Koch’s was higher in the HIV positive community. According to the World Health Organization (WHO), globally in 2014, the prevalence of total TB cases was 9.6 million, among these cases, 5.4 million were males, 3.2 million were females and one million were children. In Pakistan, according to 2006 survey, 263 cases of TB were reported in per million populations, and the ratio of spread was 181 per million populations per year. In 2014, there were 370 to 650 cases of TB reported per 0.2 million population, which increased from 530 to 740 cases of TB per 0.2 million population (11, 12).

The spread of TB is predominantly controlled by the effective use of anti-tuberculosis therapy (ATT). But these drugs are known to be a frequent cause of liver damages that may progress to chronic liver disease (13). The drugs that are used as first-line ATT include a two monthly regimen of isoniazid, rifamycin, ethambutol and pyrazinamide, and a successive four months of isoniazid, rifamycin and ethambutol. Hepatic toxicity resulting from ATT is a major health problem caused by prolonged duration of therapy as all these drugs, except ethambutol, are mainly metabolized and detoxified in the liver. For most of these drugs, the mechanism of the injury is not clear (14).

Isoniazid (INH) produces severe hepatic damage which increases with increased age i.e. 1.2 % below 50 years and 2.3 % above 50 years of age groups. The most common form of injury is drug-induced hepatitis (15). Among the different proposed mechanisms for hepatotoxicity induced by INH, the genetic basis of drug metabolism may provide a plausible explanation (16). Acetylator phenotypes are known to influence the serum concentration of several drugs, including INH, but phenotyping for acetylator status is generally done when drug interactions are suspected. Slow acetylator status may be a risk factor for developing hepatotoxicity as it may influence the metabolism of isoniazid and increase its serum levels (17).

Generally, liver injury induced by ATT is variable and includes simple changes in liver enzymes to acute hepatitis, chronic hepatitis, chronic liver failure or chronic liver disease (CLD) with an increased mortality rate that may require liver transplantation (11). Approximately 5% of total hospitalizations and 50% of acute hepatic failures have been reported to be from drug-induced injuries (18).

Nigella sativa (black seeds) active ingredient thymoquinone has hepato-protective activity against tert-butyl hydroperoxide (TBHP) toxicity by controlling the depletion of glutathione (GSH), decreasing leakage of liver enzymes ALT, AST and Serum total Bilirubin in isolated rabbit hepatocytes (19, 20).
Thymoquinone inhibits the presence of inducible nitric oxide synthase and lipogenesis in the hepatocytes and accelerates the activities of quinine reductase, catalase, superoxide dismutase (SOD) and glutathione transferase. The protective property may be attributed to the preservation of intracellular glutathione or may be related to the inhibition of thromboxane B2 production. Thymoquinone is involved in immunomodulation, membrane stabilization and neutralization of free radicals (21, 22).

Therapeutically, Nigella sativa has been used as a bronchodilator, anti-bacterial, hypotensive, hepatic & gastro protective agent, anti-diabetic, anti-histamine, anti-oxidative and diuretic. It has been noted to have anti-neoplastic activity as well (23, 24).

METHODS

The current 20 days animal study was conducted at Basic Medical Science Institute (BMSI) with the collaboration of the animal house Jinnah Postgraduate Medical Center (JPMC) Karachi, after review and approval by the Institutional Ethics Committee. Locally bred and sexually mature Oryctolagus cuniculus rabbits of both sexes were taken, with body weights ranging from 01 and 1.5 kg. A total of 48 (N) healthy rabbits of either sex were randomly divided into four equal groups (n=12) and kept in the animal house. A general examination was done to evaluate their general health conditions. All the animals were kept on a standard laboratory diet (containing wheat flour, vitamins, etc) and water.

Isoniazid (isonicotinoyl hydrazine) 100 mg tablets were purchased from the local market manufactured by UNEXOLABS (PVT) Limited. Nigella sativa (herbal product) was purchased from the local market in solid form and then grinded to convert it into powdered form.

Liver biochemical parameters were assessed on day 00. These included serum total Bilirubin (mg/dl), serum Alanine Transaminase (ALT) (units/litre) formerly called Glutamic Pyruvic Transaminase (SGPT) and serum Aspartate Transaminase (AST) (units/litre) formerly called Glutamic Oxaloacetic Transaminase (SGOT). Of the four randomized groups, Group I was given a healthy diet only. Group II was given only Isoniazid 50mg/kg body weight per day orally. Group III was given Isoniazid 50mg/kg plus Nigella sativa in the dose of 500 mg/kg body weight per day orally. Group IV was given Isoniazid 50mg/kg plus Nigella sativa 1000 mg/kg body weight per day orally. These treatment regimens were given for 20 days. On day 21, the liver biochemical parameters were reassessed in all groups. The data (expressed as mean ±SEM) was statistically analyzed by paired T-test and a p-value of < 0.05 was considered statistically significant. SPSS version 16.0 was used in compiling the results.

RESULTS

The changes observed in mean serum Total Bilirubin in all four groups are shown in Fig. 1. Serum Total Bilirubin in Group I noted on day 00 (0.10± 0.02) and day 21 (0.11± 0.03) had a p-value of 0.20. In Group II, the values noted on day 00 (0.10± 0.03) and day 21 (1.33± 0.35) had a statistically significant difference with a p-value of 0.00. In Group III, the values noted on day 00 (0.11±0.03) and day 21 (0.14±0.03) had a significant difference with a p-value of 0.005. In Group IV, the values noted on day 00 (0.12±0.02) and day 21 (0.16± 0.06) had a p-value of 0.10.

Fig. 1. Comparison of serum total bilirubin of each group (n=12) from a total of 48 rabbits
The changes observed in mean serum Alanine Transaminase (ALT) in all four groups are shown in Fig. 2. Serum ALT noted in Group I on day 0 (25.53 ± 05.25) and day 21 (26.80 ± 04.84) had a p-value of 0.15. In Group II, the values noted on day 0 (31.18 ± 08.68) and day 21 (90.25 ± 17.82) had a significant difference with a p-value of 0.00. In Group III, the values noted on 00 day (33.61 ± 05.22) and day 21(36.08 ± 05.53) had a significant difference with a p-value of 0.00. In Group IV, the values noted on day 0 (32.68 ± 06.75) and day 21 (33.08 ± 05.86) had a p-value of 0.37.

![Fig. 2. Changes in mean serum alanine transaminase (ALT) of each group (n=12) from a total of 48 rabbits](image1)

The changes observed in mean serum Aspartate Transaminase (AST) in all four groups are shown in Fig. 3. Serum AST noted in Group I on day 0 (27.66 ± 03.11) and day 21 (28.00 ± 03.24) had a p-value of 0.58. In Group II, the values noted on 00 day (39.66 ± 04.19) and day 21 (99.45 ± 06.39) had a significant difference with a p-value of 0.00. In Group III, the values noted on day 0 (40.83 ± 05.22) and day 21 (41.25 ± 06.81) had a p-value of 0.74. In Group IV, the values noted on day 0 (39.58 ± 04.95) and day 21 (39.65 ± 05.13) had a p-value of 0.92.

![Fig. 3. Changes in mean serum asparate transaminase (AST) of each group (n=12) from a total of 48 rabbits.](image2)

**DISCUSSION**

Tuberculosis (TB) is contagious, chronic infectious condition caused by mycobacterium tuberculosis, associated with high mortality rate over the centuries and nowadays. Initially its pathological and anatomical presence discovered by Francis Sylvius in 1679, who described tubercles, abscesses formation, lead to cavitations and empyma of lung. In 1699 the republic of Lucca declared as infectious disease. In 1735 the health board ordered the isolation of consumptives’ admission in public health centers, which were established at specific places for treatment. In 18th century TB become epidemics in Western Europe with a rate of 900 deaths per 100,000 population and give the name of “the robber of youth” 2017 (25).
Hepatotoxicity induced by routine multi drug ATT regimens has been reported to be 2% to 28%, 2006 (26). The incidence varies with the population to population and depends on how the hepatic insult is defined 2008 (27).

The results in Group II showed an increased serum Total Bilirubin ranging from 0.10 to 1.33 mg/dl, ALT from 31.18 to 90.25 u/l and AST from 39.66 to 99.45 u/l. These values were comparable to the albino rat Indian study by Jadhav et al, 2013(13) who reported that with ATT the serum Total Bilirubin increased from 0.89 to 2.35 mg/dl, ALT increased from 37.54 to 172.26 u/l and AST increased from 34.0 to 74.38u/l. The Basic Medical Sciences Institute’s study by Sarwat et al, (2015) observed that serum Total Bilirubin increased from 0.24 to 0.46 mg/dl and ALT decreased from 47.09 u/l to 22.15u/l (28). A human-based study conducted by Khorharo et al, 2010(11) reported that patients on ATT showed an increase in serum Total Bilirubin from 01 mg% to 03g%. A minor to severe increase in ALT from a baseline value of 37 u/l was observed in 58.24% patients taking INH. These discrepancies could be due to differences in methodology, sample size and study duration.

In the current study, serum Total Bilirubin decreased from 1.33 mg/dl in Group II to 0.14 mg/dl & 0.16 mg/dl in Group III & IV respectively, ALT decreased from 90.25 u/l in Group II to 36.08 u/l & 33.08 u/l in Group III& IV respectively, and AST decreased from 99.45 u/l in Group II to 41.25 u/l & 39.65 u/l in Group III & IV respectively. These results were in agreement with a study by Prakash et al, 2008(6) who showed that serum Total Bilirubin decreased from 2.35 mg/dl to 1.2 mg/dl & 0.97 mg/dl, ALT decreased from 172.26u/l to 84.61 & 36.45 u/l and AST decreased from 74.38 u/l to 38.24 u/l and 32.25 u/l, respectively. Another study conducted by Trivedi et al, 2013(15) noted that serum Total Bilirubin decreased from 0.25 mg/dl to 0.18 mg/dl & 0.14 mg/dl, ALT decreased from 316.5 u/l to 124 u/l & 72.75 u/l and AST was decreased from 374.75 u/l to 149.88 u/l & 92 u/l respectively. These differences can be attributed to environmental factors (including climate & humidity) and the study animals.

Isoniazid and other ATT drugs that are metabolized by liver also have potential hepatotoxic effects, but low serum levels by reducing the dose can result in inadequate therapeutic efficacy and treatment failure. Our findings suggest that for a constant serum concentration of isoniazid, increasing the dose of Nigella sativa decreases liver enzymes but further increase did not decrease serum Total Bilirubin. Similar findings were observed in a study where only one of the total four groups was exposed to INH (40 mg/ml) and Nigella sativa (1 g/kg/day) 2012 (29).

High serum levels of INH have been identified in slow acetylator phenotypes 2010 (30). Furthermore, there is limited data available on the effects of individual ATT drugs. A suitable adjunct therapy to reduce the adverse effects in potential slow acetylators can be considered when initiating the six months treatment. A previous study has explored the potential benefits of silymarin (or milk thistle, belonging to the Carduus marianum family) in this regard, reporting its beneficial role when administered concurrently with INH (28). Our study provides an insight into the use of oral Nigella sativa in different doses as a suitable adjunct therapy that may address concerns related to INH dosage and provide the desired hepatic protection.

CONCLUSION

Isoniazid (INH), a key drug in the recommended two and four month’s treatment regimen for tuberculosis, which produces liver toxicity and contributes to overall hepatotoxicity caused by ATT drugs. Our result favors that INH-induced liver biochemical changes are well treated with concurrent administration of Nigella sativa. In low doses, it is less effective compared to high doses. Proper dosage of Nigella sativa should be recommended for Kochs’ patients to mitigate hepatotoxicity. A similar study with human subjects in our set up will prove beneficial to our population.

Conflict of interest:
The authors declare no financial or non-financial competing interests.
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