

**Original Research Article**

Hepatoprotective activity of *Nigella sativa* oil against Antitubercular drug-induced Hepatotoxicity in Rats

**ABSTRACT**

**AIM:** Drug induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazid (INH) and rifampicin. The aim of this study is to check the hepatoprotective action of *Nigella sativa* against the antitubercular drugs (Rifampicin and Isoniazid)-induced hepatotoxicity in rats.

**PLACE AND DURATION OF STUDY:** This study was carried out in the Department of Pharmacy, Integral University, Lucknow, UP, India in the year 2013.

**METHODOLOGY:** *Nigella Sativa* oil was purchased from local market of Lucknow India with assured quality in the month of January, 2013 and study was performed in same year. Powder form of drugs like Rifampicin and Isoniazid were gifted by Cadila Pharmaceuticals limited, Ahmedabad, Gujarat, India whereas 24 Albino rats (Wistar strain) female, weighing 120–150 g, were procured from the Animal House Facility, Integral University, Lucknow. Rats were divided into four groups; first group untreated control was given only standard diet. Second group was treated with *Nigella sativa*, which served as drug control. Third group were treated with 100 mg of each Isoniazid and Rifampicin/kg of body weight. In fourth group rats were treated with *Nigella sativa* oil and 100 mg of Isoniazid and Rifampicin/kg of body weight. At the end of dosing, serum was used for estimation of marker enzymes like aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total and direct bilirubin. Grading of treated and untreated liver was also done.

**RESULTS:** This study showed that *Nigella sativa* acted as antiinflammatory and antinecrotic in isoniazid and rifampicin administered drugs in rats. *Nigella sativa* oil significantly maintained marker enzymes like AST, ALT, LDH, ALP and bilirubin in serum of antitubercular drugs-administered rats compared to control but drug administered rats.

**CONCLUSIONS:**

Due to above properties of *Nigella sativa* can be used as hepatoprotective against antitubercular drugs.

**Keywords:** *Nigella sativa*, hepatoprotective, antioxidant activity, Rifampicin, Isoniazid, grading of liver, Rats

## 1. Introduction

Liver is the only organ in the body that can easily replace damaged cells, but if enough cells are lost, the liver may not be able to meet the needs of the body. Liver is considered as a factory whose functions include 1) production of bile which is required for the digestion of food particularly fats 2) conversion of the extra glucose into glycogen in the liver cells and then converting it back into glucose when the need arises 3) production of blood clotting factors and amino acids including those used to fight infection 4) processing and storage of iron necessary for the production of red blood cells 5) manufacture of cholesterol and other chemicals required for fat transport 6) conversion of waste products produced by the metabolism of the body into urea that is excreted in the urine and 7) metabolization medications into their active ingredient in the body.

The causes of liver disease include infection, injury, exposure to drugs or toxic compounds, an autoimmune process, or a genetic defect that leads to the deposition and build-up of damaging substances such as iron or copper which may result in inflammation, scarring, obstructions, clotting abnormalities, and liver damage [1].

Most antituberculous drugs, with the notable exception of streptomycin, are prone to cause liver injury. The hepatotoxic potential of isoniazid given alone is well established, while data on the hepatotoxicity of rifampicin, pyrazinamide, and ethambutol are difficult to interpret since these drugs are almost always used in different combinations. The evidence supporting possible hepatotoxic interaction between rifampicin and isoniazid is circumstantial [2]. Drug induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazid (INH) and rifampicin. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimen. Thus, though INH and rifampicin each in itself are potentially hepatotoxic, when given in combination their toxic effect is enhanced. The conversion of monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incident of hepatitis [3]. This

has been postulated to be due to rifampicin-induced cytochrome P450 enzyme-induction, causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). Other investigators demonstrated that rifampicin increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination. Rifampicin induction of the hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine was reported [2].

The seed of *Nigella sativa* L (NS), an annual *Ranunculaceae* herbaceous plant, known to have many properties in traditional medicine and used as a natural remedy for a variety of complications including liver diseases (include some reference) and also for the treatment of asthma<sup>[4]</sup> from many centuries in middle East, Northern America, Far East and Asia. NS contains more than 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% thymoquinone and 46% many monoterpenes such as p-cymene, and  $\alpha$ -pinene. Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, anti-inflammatory [4, 5] and anti-tumour agents [6]. It has been reported that *Nigella Sativa* Oil (NSO) could diminish the CCl<sub>4</sub>-induced hepatotoxicity, the doxorubicin-induced cardiotoxicity and the harmful effects of some chemicals [7, 8]. Many studies have shown that *Nigella sativa* plays a protective and antioxidant role [9]. Based on the above facts, the present study was designed to investigate the hepatoprotective action of *Nigella sativa* oil against the antitubercular drug-induced hepatotoxicity in rats.

## **1. Materials and Methods**

### **1.1. Collection of *Nigella sativa* oil, drugs and rats**

*Nigella Sativa* oil was purchased from local market of Lucknow India with assured quality in the month of January, 2011 and study was performed in the year 2011. The powder form of

drugs like Rifampicin and Isoniazid were gifted by Cadila Pharmaceuticals limited, Ahmedabad, Gujarat, India whereas 24 Albino rats (Wistar strain) female, weighing 120–150 g, were procured from the Animal House Facility, Integral University, Lucknow. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hr light and 12 hr dark at day and night cycle) and had a free access to appropriate diet what was the appropriate diet please mention and tap water *adlibitum*. The temperature of animal house was maintained at  $25 \pm 2^{\circ}\text{C}$  & relative humidity at  $(50 \pm 15\%)$ .

### **1.2. Treatment of animals**

The rats were divided into four groups. Each group consisted of 6 animals. The first group is the untreated control was given only the standard diet. The second group of animals was treated with *Nigella Sativa* oil (1 ml/kg-body weight), orally for 30 days served as drug control. In the third group the animals were treated with 100 mg of each Isoniazid and Rifampicin/kg of body weight for 30 days. In group fourth, the animals were treated simultaneous with *Nigella Sativa* oil and the two antitubercular drugs (Isoniazid and Rifampicin 100 mg/kg body weight) orally for 30 days. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC), IU/Pharm./M.Pharm./CPCSEA/10/27 Faculty of pharmacy Integral University, Dasauli, P.O. Bas-ha Kursi Road; Lucknow – 226026 (U.P), India.

### **1.3. Biochemical determination**

At the end of the dosing, i.e. 24 hour after the last dose of treatment, rats were sacrificed and blood was collected by retro orbital puncture from all the animals without using any anticoagulant using a 2 ml syringe under light ether anesthesia and the blood was allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at  $30^{\circ}\text{C}$  for 15 minutes and used for the estimation of biochemical enzymes like aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase

(ALT), alkaline phosphatase (ALP) by the method of Reitman and Frankel [10] and Kind and King [11] and total and direct bilirubin by the method of Mallory and Evelyn [12] using the diagnostic kits (Oscar Diagnostic Services Pvt. Ltd., New Delhi India).

#### **1.4. Grading of liver**

The Liver with and with out treatment were dissected out immediately, chilled and perfused with Ice-cold saline. The tissue was kept for 48 hours in 10% formalin. The specimens were dehydrated in ascending grades of ethanol and cleared in xylene and then embedded in paraffin wax. Sections of about 5  $\mu$ m were cut and then stained with hematoxylin and eosin for examination by light microscopy.

#### **1.5. Statistical Analysis**

Data of the measured parameters were subjected to analysis of variance (ANOVA) and significant partial difference (LSD) was calculated at 5% probability level. Values indicate mean  $\pm$  S.D of replicates.

### **2. Results**

#### **2.1. Body Weight and Grading of Liver**

In the present study the authors found the harmful effect of Rifampicin and Isoniazid drugs on rat liver and the possible ameliorating effect of *N. sativa* treatment in those animals. The body weight of control and treated animals varied among treatments. The body weight of Rifampicin+Isoniazid treated rats significantly increased compared to control animals. The body weight of *N. sativa* and Rifampicin+Isoniazid treated rats did not increase significantly compared to the only drug (Rifampicin+Isoniazid) treated rats (Table 1). The results also showed that the drug (Rifampicin+Isoniazid) treated rats showed scar formation and necrotic lesions in the liver compared to the *Nigella sativa* and Rifampicin+Isoniazid treated rats which showed less inflammation, yellowish colour and necrotic lesions (Fig. 1).

Table 1. Weight of rats (g) before and after treatment with the drugs and *Nigella sativa*

| Weight of Rats | Treatment of Rats |                                    |                           |                                                 |
|----------------|-------------------|------------------------------------|---------------------------|-------------------------------------------------|
|                | Control           | <i>N. sativa</i><br>(drug control) | Rifampicin +<br>Isoniazid | <i>N. Sativa</i> +<br>Rifampicin<br>+ Isoniazid |
| Initial Weight | 125± 3.2          | 127 ± 2.9                          | 122± 2.4                  | 129± 2.7                                        |
| Final Weight   | 127± 3.1          | 128 ±3.3                           | 145* ± 3.5                | 132± 3.1                                        |
| <b>LSD</b>     | 10.2              | 11.4                               | 11.1                      | 9.7                                             |

\* Significantly different from control at  $p \leq 0.05$ ; Values indicate mean  $\pm$  S.D of replicates



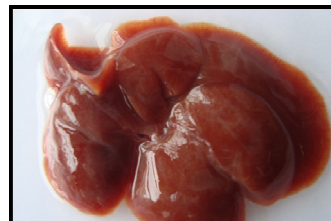
Control Normal, No lesions



Rifamycin+Isoniazid. Scar formation,  
Necrosis and inflammation



Drug control *Nigella Sativa* No lesions.



*Nigella Sativa*+Rifamycin+Isoniazid.  
Inflammation and Yellowish in portal and  
lobular area, Necrosis

**Fig. 1.** Effect of treatment with *N. sativa*, Rifampicin and Isoniazid either alone or in combination on the liver of rats

## 2.2. Biochemical Determination

The results of this study showed that the marker enzymes like AST, ALT, LDH, ALP and bilirubin significantly increased in the serum Rifampicin + Isoniazid antitubercular drugs-administered rats as compared to normal controls rats (Table 2 ). But when *nigella sativa*

(Table 1) was coadministered with Rifampicin + Isoniazid, it resulted in the decrease of these marker enzymes and maintained these enzymes at normal levels in the serum of rats compared to the only Rifampicin + Isoniazid administered rats thus indicates the hepatoprotective action of *Nigella sativa*.

Table 2. Effect of *Nigella. sativa* on marker enzymes and total bilirubin in the serum of control and drug administered rats.

| Treatment of Rats                         | Production of Marker Enzymes    |                                   |                              |                             |                           |
|-------------------------------------------|---------------------------------|-----------------------------------|------------------------------|-----------------------------|---------------------------|
|                                           | Alanine aminotransferase (IU/L) | Aspartate aminotransferase (IU/L) | lactate dehydrogenase (IU/L) | alkaline phosphatase (IU/L) | Bilirubin (Total) (mg/dl) |
| Control                                   | 9.18 ± 0.4                      | 76.1 ± 0.4                        | 1089 ± 14.3                  | 106.8 ± 0.6                 | 0.48 ± 0.1                |
| <i>N. sativa</i> (drug control)           | 19.40* ± 0.5                    | 53.01* ± 0.3                      | 1227* ± 16.6                 | 119.1* ± 0.4                | 0.65 ± 0.1                |
| Rifampicin + Isoniazid                    | 28.59* ± 0.3                    | 105.7* ± 0.5                      | 2113* ± 20.9                 | 219.8* ± 0.3                | 1.1* ± 0.1                |
| <i>N. Sativa</i> + Rifampicin + Isoniazid | 23.02* ± 0.6                    | 65.58* ± 0.3                      | 1584* ± 19.5                 | 156.1* ± 0.3                | 0.87* ± 0.1               |
| LSD                                       | 4.3                             | 8.5                               | 55.6                         | 11.4                        | 0.33                      |

\* Significantly different from control at  $p \leq 0.05$ ; Values indicate mean ± S.D of replicates

### 3. Discussion

Liver is the primary target of toxic chemicals (including pesticides, food additives, pharmaceuticals, and industrial intermediates) tested in long-term toxicity safety assessment assays [13]. Some of them are Rifampicin and Isoniazid which induces multiple cellular, molecular, and biochemical changes [3, 2]. It is the main organ involved in the metabolism of biological toxins medical agents. Such metabolism always associated with the disturbance of hepatocyte biochemistry and generation of reactive oxygen species (ROS) [14]. Lots of liver damages ranging from subclinical icteric hepatitis to necroinflammatory hepatitis, cirrhosis and carcinoma have been proved to associate with the redox imbalance and oxidative stress [15].

Among the toxic chemicals, Rifampicin and Isoniazid are well known liver carcinogen, induces multiple cellular, molecular, and biochemical changes [3, 2]. In this study we found

the harmful effect of Rifampicin and Isoniazid on rat liver and the possible ameliorating effect of *N. sativa* treatment in those rats. The body weight of control and treated animals varied among treatments. The body weight of Rifampicin+Isoniazid treated rats significantly increased compared to control animals. The body weight of *N. sativa* and Rifampicin+Isoniazid treated rats did not increase significantly compared to the only drug (Rifampicin+Isoniazid) treated rats (Table 2). The results also showed that the drug (Rifampicin+Isoniazid) treated rats showed scar formation and necrotic lesions in the liver compared to the *Nigella sativa* and Rifampicin+Isoniazid treated rats which showed less inflammation, yellowish colour and necrotic lesions (Fig 1). The similar results were shown by [16] who observed a decrease in the weight of liver of the animals treated with a hepatoprotective agent and drugs and also found less necrotic lesions compared to control animals. Our results are also in agreement with the results of Desmet and Fevery [17] and Bedossa and Poynard [18] who also found the same results as in our study.

Antibiotic therapy favors the production of free radicals in excess of basal rates. Many antibiotics that depend on bound metals for their activity are able to generate free radicals and cause cellular damage [19]. The combination of isoniazid and rifampicin treatment in experimental animals enhanced lipid peroxidation, indicating increased oxidative stress in liver [20]. Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins [21]. It is reported that compounds isolated from *N. Sativa* (including thymoquinone, carvacol, tanethole and 4- terpineol) have good free radical scavenging properties [8] which could be the reason in our study that the *N. Sativa* and honey reduced the toxicity of these harmful drugs. Generation of free radicals may be the basis of many human diseases and conditions. Therefore, the antioxidant action of *N. sativa* may explain its claimed usefulness in folk medicine. This antioxidant property would explain its action against hepatotoxicity [9], liver fibrosis and cirrhosis [22], and hepatic damage induced by *Schistosoma Mansoni* infection [23]. There are many reports that support the use of

antioxidant supplementation for reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [24]. Recently there has been an interest towards the use of natural antioxidants to prevent oxidative damage [24]. In the same time, flavonoids and other phenolic compounds of plants origin have been reported as scavengers and inhibitors of lipid peroxidation [25]. Fruits and vegetables which are reported to contain natural antioxidants can provide a sufficient protection thus can slow down the process of oxidative damage caused by reactive oxygen species (ROS) [26]. Among the promising medicinal plants, *N. sativa*, an amazing herb with rich historical and religious background are the source of the active ingredients of this plant [27]. A majority of the studies on *N. sativa* have confirmed its antitoxic properties both *in vitro* and *in vivo* [27]. *Nigella Sativa* is hepatoprotective probably due to the antioxidant nature by blocking isoniazid- and rifampicin-induced lipid peroxidation. The results of this study showed that the marker enzymes like AST, ALT, LDH, ALP and bilirubin significantly increased in the serum of Rifampicin + Isoniazid antitubercular drugs-administered rats as compared to normal controls rats (Table 1). But when *Nigella sativa* (Table 1) was coadministered with Rifampicin + Isoniazid, it resulted in the decrease of these marker enzymes and maintained these enzymes at normal levels in the serum of rats compared to the only Rifampicin + Isoniazid administered rats thus indicates the hepatoprotective action of *Nigella sativa* [28]. Similar results were also found by Mahesh *et al.*, [29], who found protective effect of Indian honey on acetaminophen produced oxidative stress and liver toxicity in rat. The protective action of *nigella sativa* against these antitubercular drugs-induced necrotic damage could be probably due to the membrane stabilizing action [30]. The findings of this study are in agreement with the study of El-Dakhakhany *et al.*, [31] who reported that the daily administration of *N. sativa* oil (800 mg/kg orally for 4 weeks) did not adversely effect the serum transaminases, alkaline phosphatase or bilirubin. In another study Turkdogan *et al.*, [32] found that the *N. sativa* resulted in successful prevention of liver fibrosis in rabbits and that its oil may play a role against liver damage induced by *Schistosoma mansoni* infection in mice [23].

#### 4. Conclusions

Due to the multifeous properties shown by *Nigella sativa* can be used as hepatoprotective against antitubercular drugs.

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