

EVALUATION OF HEPATOPROTECTIVE EFFECT OF *NIGELLA SATIVA* OIL IN CYCLOSPORINE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: The main objective of our study is to see the hepatoprotective effect of *Nigella sativa* in Cyclosporine induced hepatotoxicity in rats.

Methods: the effect of *Nigella sativa* on cyclosporine induced hepatotoxicity was studied in albino rats. The animals were divided into 3 groups with 6 rats in each group. Group I served as vehicle control, Group II animals were given Cyclosporine 25mg/Kg body weight orally for 21 days and Group III were given Cyclosporine 25mg/Kg + *Nigella sativa* 1ml/Kg body weight orally for 21 days. The blood samples were collected for liver enzymes estimation before and after the experiment. The animals were sacrificed on 22nd day and liver was subjected to histopathological examination.

Results: Cyclosporine provoked hepatotoxicity was evident from increase of hepatic enzyme levels and histopathological changes. Addition of *Nigella sativa* has resulted in the decrease of elevated hepatic enzyme levels and improvement in histopathological changes in liver.

Conclusion: The present study showed that *Nigella sativa* oil has protective effect on Cyclosporine induced hepatotoxicity in albino rats.

Keywords: *Nigella sativa*; Cyclosporine; hepatotoxicity.

INTRODUCTION

The liver is considered the major "Metabolic clearing house" for both endogenous chemicals and xenobiotics.¹ It regulates several important functions including protein synthesis, storage and metabolism of fat and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin.²

The incidence of drug induced liver disease has continued to rise steadily since the late 1960s. It is estimated that 15-40% of acute liver failure cases may be attributable to drugs.³ Toxic liver injury produced by drugs and chemicals may virtually mimic any form of naturally occurring liver disease.⁴

Treatment of drug induced hepatotoxicity relies on correct diagnosis, prompt withdrawal of the causative agent and supportive therapy.³

Cyclosporine is a cyclic polypeptide with 11 amino acids, obtained from a fungus and was introduced in 1977 as a highly selective immunosuppressant which has markedly increased the success of organ transplantations and also useful in various autoimmune disorders. The adverse effects of Cyclosporine therapy include nephrotoxicity, liver function impairment, rise in BP, precipitation of diabetes, anorexia, lethargy, hyperkalemia, opportunistic infections, hirsutism, gum hyperplasia, tremors and seizures.⁵

Cyclosporine induced hepatotoxicity is characterized by cholestasis, hyperbilirubinemia, hypoproteinemia, increased alkaline phosphatase and transaminase activities, bile salts in the blood, inhibition of protein synthesis and disturbed lipid secretion in both human and experimental animals.⁶ The exact mechanism by which Cyclosporine induces hepatotoxicity is not clearly known. Various mechanisms of Cyclosporine induced hepatotoxicity have been proposed, one among them is production of reactive oxygen species (ROS), oxidative stress, depletion of hepatic antioxidant system and increase in malondialdehyde (MDA).⁷ There are evidences suggesting that antioxidants could play a beneficial role in Cyclosporine induced hepatotoxicity.⁸ In the absence of reliable liver protective drugs, herbal drugs play a role in the management of various liver disorders one of them is *Nigella sativa*.

Nigella sativa is one of the common herbal drugs used in various medical disorders. It belongs to family "Ranunculaceae" is a spice which is well known for its medicinal properties. Recently its hepatoprotective activity has been enlightened.⁹

It seems that most of the pharmacological actions of *Nigella sativa* are due to antioxidant activity which is mainly due to its ability to scavenge free radicals and/or inhibit lipid peroxidation.¹⁰ Thus the objective of the

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study was to determine whether the administration of *Nigella sativa* oil will protect Cyclosporine induced hepatotoxicity in rats.

MATERIALS AND METHODS

The protocol for the study was approved by Institutional Animal Ethics Committee (IAEC/JAN/2013 dated 19/1/2013). The experiment was carried out in Post Graduate lab of Pharmacology department.

Albino rats of either sex weighing 230 to 250g were obtained from Central animal house of CAIMS, Karimnagar.

Cyclosporine (Sandimmun Neoral – Novartis India Limited (Biochemie), Sandoz House, 4th Floor, Dr. Annie Besant Road, Worli, Mumbai – 400018.) was purchased from Pharmacy and used for the study.

Nigella sativa oil was obtained from Mohammadi Company, Karimnagar, AP – 505001, a GMP product having the strength of 91mg/100ml.

Albino rats were divided into 3 groups of 6 animals each group.

Group I: Control group (No treatment)

Group II: Each animal was given Cyclosporine 25mg/Kg body weight orally for 21 days.

Group III: Each animal was given Cyclosporine 25mg/Kg + *Nigella sativa* oil 1ml/Kg body weight orally for 21 days.

The blood samples of each rat were collected separately before and after the experiment. On the 22nd day the animals were sacrificed and the livers were subjected to histopathological examination.

RESULTS

In general the extent of liver damage is assessed by increase of hepatic enzymes and histopathological evaluation.

The effect on liver enzymes

The mean values of serum alanine transaminase (ALT/SGPT), aspartate transaminase (AST/SGOT) and alkaline phosphatase (ALP) were significantly higher ($P < 0.001$) in Cyclosporine treated group (Group II) when compared to Control group (Group I) of animals (Table - 1).

Table 1: Effect of Cyclosporine and *Nigella sativa* oil on serum hepatic enzymes, glucose and albumin in albino rats (Mean $\pm$ SD)

Group	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)	Serum Glucose mg/dl	Serum Albumin g/dl
Group I (Control)	79 $\pm$ 1.25	65 $\pm$ 1.2	23.7 $\pm$ 1.2	130 $\pm$ 2	5 $\pm$ 0.4
Group II (Cyclosporine)	305 $\pm$ 3.1 ^{***}	83 $\pm$ 2.2 ^{***}	92.9 $\pm$ 1.4 ^{***}	160 $\pm$ 4	4.1 $\pm$ 0.2
Group III (Cyclosporine + <i>Nigella sativa</i>)	85 $\pm$ 2.3 ^{***}	72 $\pm$ 4.1 ^{***}	30 $\pm$ 1.5 ^{***}	148 $\pm$ 3	4.5 $\pm$ 0.4

^{***} Highly significant, group II verses group I

^{***} Highly significant, group III verses group II

When *Nigella sativa* oil was given with Cyclosporine for 21 days (Group III) the liver enzymes mean values were significantly reduced ($P < 0.001$) when compared to Cyclosporine treated group (Group II).

Histopathological examination of Liver

There were no histopathological changes in all liver samples obtained from Control group (Group I) (Fig. 1) of the rats.

In Cyclosporine treated animals (Group II), there were considerable histopathological changes showing moderate hepatotoxicity, there is loss of normal architecture of liver, congestion, edema and proliferation of inflammatory cells (Fig. 2).

In Cyclosporine + *Nigella sativa* treated rats (Group III), *Nigella sativa* oil showed protective effect against Cyclosporine induced hepatotoxicity by retaining liver architecture almost to the normal and also by decreasing congestion, edema and inflammatory cell proliferation (Fig. 3).

HISTOPATHOLOGY OF RAT LIVER

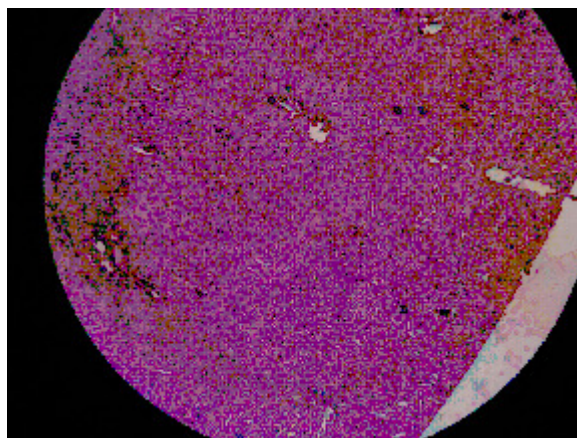


Fig. 1 : Group I (control Group) showed normal liver architecture.

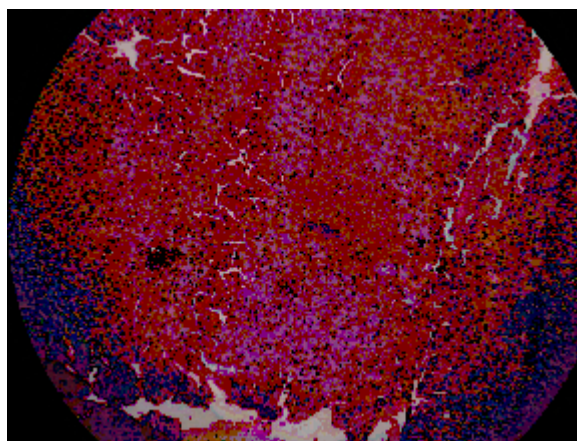


Fig. 2 : Group II (Cyclosporine 25mg/Kg orally for 21 days) revealed altered architecture of liver showing congestion, mild edema and proliferation of inflammatory cells.

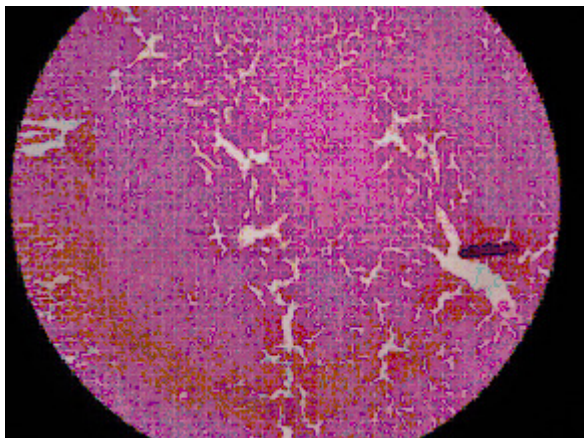


Fig. 3 : Group III (Cyclosporine 25mg/Kg + *Nigella sativa* oil 1ml/Kg orally for 21 days) indicated liver showing almost normal architecture and there was decrease in congestion, edema and inflammatory cell proliferation.

DISCUSSION

Every drug has side effects. While giving therapy, we always consider risk benefit ratio.

Cyclosporine revolutionized the field of transplantation and also has got beneficial effect in some autoimmune disorders.

There are some drugs whose toxic effects can be minimized by supplementation of other drugs eg. methotrexate toxicity can be reduced by supplementation with L-Leucovorin.¹¹

There are number of side effects of Cyclosporine, one among them is hepatotoxicity. Numerous evidence suggest that Cyclosporine induced oxidative stress is associated with biochemical changes that contribute to liver toxicity. Cyclosporine increase reactive oxygen species (ROS) and malondialdehyde (MDA) production that hall marks of oxidative stress.¹²

There are some studies which proved hepatoprotective effect of *Nigella sativa*.¹³ *Nigella sativa* possesses antioxidant property which has been demonstrated both *in vivo* and *in vitro* studies.^{14, 15}

Some studies have been done on normal rats to see the effect of *Nigella sativa* on the liver with different doses ranging from 0.1 – 1g/Kg body weight and have found no changes in serum hepatic enzymes and histopathology of liver.¹⁶

Estimation of liver enzymes like AST, ALT and ALP can make the assessment of the liver function. When the liver cells are damaged then the enzymes are released into the blood. Therefore, the estimation of these enzymes can predict hepatocellular damage. Any chemical that decreases these increased levels of enzymes have hepatoprotective activity. Therefore, in our study the hepatoprotective effect of *Nigella sativa* oil in Cyclosporine induced hepatotoxicity in rats has been studied.

In the present study increased activity of AST, ALT and ALP was seen in Cyclosporine treated rats compared to control group of rats indicating its hepatotoxic effect. When *Nigella sativa* was given along with Cyclosporine there was reduction in the enzyme levels compared to Cyclosporine treated rats offering protection of hepatic cells. Serum albumin levels reflects liver synthetic function which has been decreased by Cyclosporine, the hypoglycaemic effect of *Nigella sativa* has been well documented.¹⁷

Histopathological changes in Cyclosporine treated rats showed altered architecture of liver and congestion, mild edema, proliferation of inflammatory cells indicating toxicity to liver, which are changed to near normal architecture and decrease in congestion, edema and proliferation of inflammatory cells in liver when *Nigella sativa* oil was coadministered indicating protective action.

CONCLUSION

It can be concluded that *Nigella sativa* oil given orally has hepatoprotective effect against Cyclosporine induced hepatotoxicity in albino rats.

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