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Research Article

EVALUTION OF HEPATOPROTECTIVE EFFECT OF *NIGELLA SATIVA L.*

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ABSTRACT

The Hepatoprotective effect of *Nigella Sativa L.* alcoholic extract (NSE) was investigated on D-Galactosamine (D-GalN)/Lipo polysaccharide induced hepatotoxicity in rats. Twenty four adult wistar albino rats were divided into four groups viz., Control, D-Galatosamine induced hepatotoxicity, *Nigella sativa* alcoholic extract and *Nigella Sativa* alcoholic extract pretreatment prior to the administration of D-Galactosamine. After treatment, the rats were sacrificed, blood sample was collected for the determination of Serum Aspartate transaminase(AST), Alanine transaminase (ALT), and Alkaline phosphatase(ALP). Mean AST, ALT, and ALP levels were found to be significantly higher in D-GalN induced hepatotoxic rats group ($P<0.05$) than in the control group. Levels of these parameters were found to be nearer to control in the NSE pretreated group. Results of the present study indicates that NSE having hepatoprotective efficiency againt D-GalN induced toxicity in rats.

Keywords: *Nigella sativa L.,* Hepatotoxicity, D-Galactosamine, Lipo polysaccharide, Enzymes and Histopathology.

INTRODUCTION

The seeds of *Nigella Sativa L* (NS)(Ranunculaceae) called as Black seed. In the Middle East it has been used as a traditional medicine for a variety of complaints viz, headache, cough, flatulence, as a choleretic, antispasmodic and uricosuric. It has also been used in traditional medicine and for culinary preperations in many countries. *N.sativa* seeds contain 36%-38% fixed oils, proteins, alkaloids, saponin and 0.4%-2.5% essential oil[1]. The C20:2 arachidic and ecosadienoic acids are the main unsaturated fatty acid constituents of NS fixed oil. The essential oil was analysed[2] using GC-MS many components were characterised but the major ones were thymoquinone (27.8%-57.0%), p-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%) t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%).

D-GalN used in this study is an amino sugar normally found *in vivo* only in acetylated form in certain structural polysaccharides. Administration of single dose of this compound results in dose dependent hepatic damage resembling viral hepatitis, with focal necrosis and periportal inflammation. It induces hepatotoxictiy by inhibiting the synthesis of RNA and protein through a decrease in cellular UTP concentration which finally leads to the necrosis of liver cells. Rats intoxicated with D-GalN revealed morphologic featheres closely resembling those seen in viral hepatitis. Acidophilic degeneration, appearance of councilman bodies, single cell necrosis, foci of hepato cellular necrosis, enlarged liver macrophages and periportal inflammatory infiltrations were also found in the rats[3].

Lipopolysaccharide (LPS;endotoxin) is a component of gram-negative bacteria that elicits a potent inflammatory response in mammals. At smaller doses, LPS increases liver sensitivity to galactosamine, ethanol, carbon tetra chloride, aflotoxin B_1, monocrotaline and allyl alcohol[4]. Fulminant hepatitis can be induced in experimental animals by the synergistic action of a small dose of lipopolysaccharide and D-Galactosamine induced an inhibitor of hepatocellular RNA synthesis.The present study looking into the scientific exploration of the alcoholic extract of *Nigella sativa* seed as prospective hepatoprotective agent.

MATERIALS AND METHODS

The powdered *Nigella sativa* seed was subjected to soxlet extraction using 95% ethanol. The solvent was removed *in vacuo* to the extent that there is no smell of ethanol in the oily residue to give an appropriate yield 22%. NSE administration did not produce any abnormalities such as atoxic, circling, lacrimation, laboured breathing etc., in the animals throughout the experimental period. The dose level fixed for the present study was non-toxic and safe. Twenty four male wistar albino rats (180-230 gm), were fed standard rat pellets and drinking tap water *ad libitum*. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of Periyar Maniyammai Pharmaceutical college, Trichy, Tamil Nadu, India. Rats were divided into 4 groups of 6 animals in each group. Control, D-GalN administered, NSE administered and NSE pretreatment prior to D-GalN administration. The control Group (Group I) received only tap water. Group II comprised rats were administrated (i.p) D – Galactosamine (300 mg/kg B.Wt.) and Lipoplolysaccharide (Sero type 0111.B4 extracted by phenol water method from E.Coli 30μg/kg B.Wt.) 18 hrs before the experiment[5], Group III rats received NSE 500 mg/kg orally for 15 days. Group IV rats were given NSE pretreatment for 15 days prior to the administration of D-GalN/LPS. After the scheduled treatments the blood sample was taken form the tail vein and serum was trapped and then used for various enzyme assays. AST, ALT and ALP were determined[6] and then animals were sacrificed by cervical dislocation.

Histopathological studies

Sampling

For histopathological studies liver and kidney tissues of 3mm thickness were fixed in Bouin's fluid, dehydrated in alcohol series, cleared in methyl benzoate and xylol, embedded in paraffin wax and sectioned at 8μ. The sections were stained with Ehrlichae's haematoxylin and counter stained with Ecosine[7].

Microphotography

The stained sections were observed and microphotographed at appropriate magnification using Carl Zeiss microscope model Axiostar Plus (made in Germany).

Statistical significance of differences between the control and treatment groups was determined by ANOVA (Analysis of Variance) followed by Dunnet's t-test using the SPSS 11 version. Data are expressed as mean ± standard deviation (mean ± S.D). The level of significance chosen was($P<0.05$)

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the hepato protective effects of *Nigella sativa* alcoholic extract against D-Galactosamine/Lipopolysaccharide. The D-GalN/LPS injected into rat produced hepatotoxicity manifested as a significant rise in serum AST, ALT, and ALP. The *Nigella sativa* alcoholic extract (NSE) used in the present study seems to offer significant protection and maintained the levels of AST, ALT and ALP near to normal level.

The level of AST, ALT and ALP in serum were significantly increased. The present study elicited a significant increase in the activities of these enzymes in serum with in eighteen hours of exposure of the rats to a

single dose of D-GalN/LPS, indicating the severity of hepatocellular injury. The increased activities of these enzymes indicate cellular leakage and loss of functional integrity of cell membrane in liver[8]. The increased activities of these enzymes reflect a non-specific alteration in the plasma membrane integrity and/or permeability as a response to D-GalN challenge[9 and 10]. The decreased level of AST, ALT, and ALP in NSE pretreated group leads to the inference that *Nigella sativa* alcoholic extract counteracts the abnormal increase in AST, ALT, ALP and lipid peroxides induced by D-GalN. This might be due to anti oxidative nature of the plant. Components isolated from *N sativa* (including thymoquinone, carvacol, t-anethole and 4-terpineol) have appreciable free radical scavenging properties [11].

Table 1: Activies of AST, ALT and ALP in the control and experimental groups of rats

S. No.	Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
1.	Group I (control)	102.30 ± 0.96	41.70 ± 1.90	50.80 ± 1.38
2.	Group II (D-GalN)	410 ± 5.84[a*]	142.20 ± 2.00[a*]	148.00 ± 4.18[a*]
3.	Group III (NSE)	103.70 ± 1.37	42.16 ± 1.90	49.21 ± 1.97
4.	Group IV (NSE and D-GalN)	161.12 ± 1.90[b*]	48.12 ± 2.10[b*]	61.10 ± 2.25[b*]

Data are expressed as mean ± S.D

[a]As compared with Group I; [b]As compared with Group II *(P<0.05)

Active constituent of *Nigella sativa* possesses free radical scavenging activity[12]. In rabbits, experimental liver cirrhosis and fibrosis (induced by carbon tetra chloride) were shown to be prevented by the prior administration of *N.Sativa*. The seed extract improved the histological picture and the indices of oxidative status of the liver [13 &14].

In the present study, *Nigella sativa* alcoholic extract pretreatment significantly reduced D-GalN/LPS induced hepetotoxicity and oxidative stress. The reduced level of D-GalN/LPS toxicity in D-GalN/LPS toxicated animals (Group IV) is manifested by the decreased level in AST, ALT and ALP.

Assessment of the hepato protective potential of a drug is incomplete without histological description of changes in the liver. **Fig. (1-4)** shows the photomicrographs of the histological Studies of the liver of the control and experimental groups of rats. The liver cells of the control animals and NSE only treated animals **Fig. (1 and 3)** revealed a normal architecture. **Fig. (2)** shows the architecture of the liver of the rats toxicated with D-GalN/LPS. Rats given D-GalN/LPS elicited severe hepatic injury as evidenced by the observation of pathological changesin the architecture of the liver viz. infiltration of inflammatory cells, kuffer cell hyperplasia,neutrophil accumulation, focal necrosis, and degenerative changes in the hepatocytes. Thesepathological changes correlated well with the altered enzyme activities. This is in agreement with the observations noted by other previous workers [15,16,17and18] during D-GalN/LPS administration in rats.

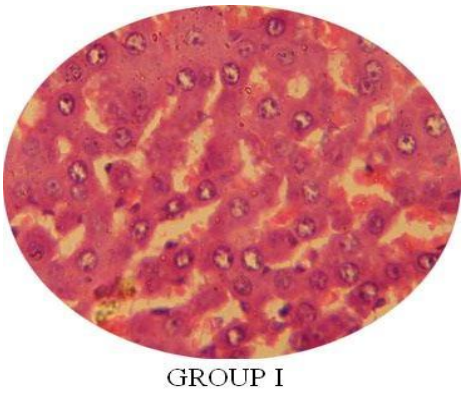

Fig. 1

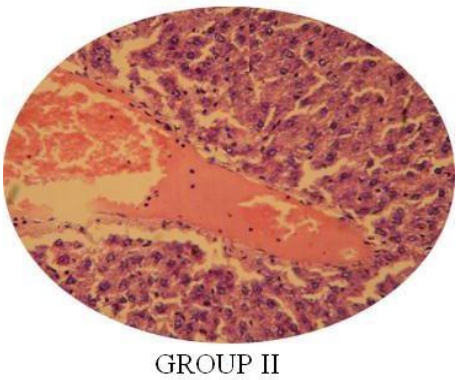

Fig. 2

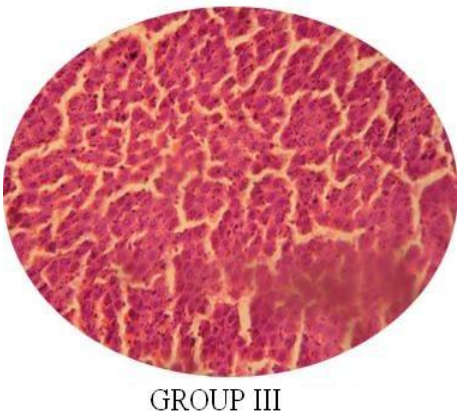

Fig. 3

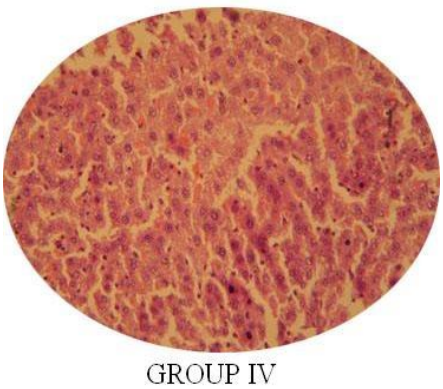

Fig. 4

Rats given *Nigella sativa* extract prior to D-GalN/LPS challenge showed considerable hepatoprotection as observed by the absence of the adverse toxic changes **Fig. (4)** in the liver as compared to the **Fig.(2)**Group II rats. Group III and IV did not show any abnormal change in the architecture of the liver **Fig.(3 and 4)** compared to the control rats. The absence of any adverse toxic effects of *Nigella sativa* extract in the liver is inferred. These findings suggested the possibility of *Nigella sativa* extract being able to protect liver tissues and thus decrease the leakage of the enzymes (AST, ALT and ALP) in to the circulation.

CONCLUSION

Based upon the studies on the toxicity of the D-GalN/LPS on the mammalian system and the hepatoprotective efficiency of alcoholic extract of the *Nigella sativa* L. seed the following conclusions were made, elevation in the diagnostic marker enzymes of hepatic function indicating alterations of membrane permeability and damage during D-GalN/LPS toxication was significantly reduced by the NSE pretreatment, which strongly suggests the protective activity of NSE towards tissues by avoiding the leakage of these enzymes to the serum and histopathological observations of the liver tissue confirmed the hepatoprotective effect of the NSE to maintain the normal architecture of the liver and the results support the use of this plant for the treatment of hepatitis as oriental traditional medicine.

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