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RESEARCH ARTICLE

Hepatoprotective Effect of *Nigella sativa* Seed Oil on Rat Model of Alcoholic Liver Disease

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ABSTRACT

The present study was conducted to investigate the hepatoprotective effect of *Nigella sativa* seed oil in ethanol- induced male albino rat. Ethanol was administered to animals for eight weeks to induce liver injury. Ethanol induced rat were treated with *N. sativa* seed oil at a dose of 5 mL and 10 mL/kg body weight. After eight weeks of treatment, liver function markers, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin level were studied. Antioxidants like, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidise (GPx) and lipid peroxidation were also studied. A significant decrease ($p<0.05$) in the level of ALT, AST, ALP, GGT and malondialdehyde (MDA) were observed after treatment with 10 mL/kg body weight as compared to ethanol induced. A significant increase (p<,0.05) in the level of CAT, SOD, GPx and albumin level was also observed after the treatment with 10 mL/kg body weight *N. sativa* oil, in comparison to ethanol intoxicated rat. These results suggest the ameliorating effect of *N. Sativa* on alcohol induced liver injury by preventing cell membrane disturbances, reduction of oxidative stress by free radical scavenging and antioxidative activity. Thus *N. sativa* oil can be useful in the treatment of liver damage caused by alcohol.

KEYWORDS

Hepatoprotective, *Nigella sativa*, Ethanol-induced, Antioxidative

INTRODUCTION

Chronic alcoholism produces a wide spectrum of liver and other organ diseases. Alcoholic liver disease (ALAD) is one of the serious consequences of chronic alcohol abuse which causes morbidity and mortality.[1] It is one of the serious problem of the western country which still remains a challenge for the scientific community. The toxicity of alcohol is linked to its metabolism via alcohol dehydrogenase enzyme which convert ethanol to cytotoxic acetaldehyde in liver which is further oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to reactive oxygen species (ROS) via cytp 450[2,3], which causes lipid peroxidation[4] and membrane damage.[5] This causes lowering of body's normal defence mechanism, altered enzyme activity, decreased DNA repair which eventually leads to hepatitis, necrosis and liver cirrhosis. In recent years, the popularity of native medicine has increased for various reasons. Since there is no reliable hepatoprotective drug available in modern science, alcohol researchers have focussed on developing phytotherapeutic medicine which can provide many individual drugs to treat alcoholic liver disease. Thus the research conducted on several natural plant products used as hepatoprotective agent is well documented.[6]

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Nigella sativa (family : Rununculaceae.) commonly known as 'kalongi' or black seed has been used for centuries as a natural remedy to promote health and treat diseases. The seeds of *N. sativa* has been extensively investigated both phytochemically and pharmacologically. It has been reported to strengthen the immune system, purify the blood, protect against irritants and support healthy living.[7] The seed of *N. sativa* have been used as a natural remedy for a number of illness like bronchial asthma[8], rheumatism, hypertension[9], diabetes[10] inflammation[11], cough, headache, eczema, fever[11] and influenza. The seeds are also used as carminative, diuretic, lactagouge and vermifuge.[12] Most properties of the whole seeds are mainly attributed to the quinone constituents present in the volatile oil, of which thymoquinone is the most abundant component.[12] Other pharmacologically active constituents, identified by HPLC and HPTLC, include dithymoquinone, Nigellone and thymol.[13,14] These active principles are mainly responsible for protecting tissue damage and various disorders and have been studied extensively for their high antioxidant content and health protective potential. However the inhibition of lipid peroxidation[15] and free radical scavenging activity has been suggested as possible mechanism of hepatoprotective action.

Therefore the present study was undertaken to establish the hepatoprotective effect of oil of *N sativa* seed in ethanol induced albino rat.

MATERIALS AND METHOD

Animals

Male albino rats *(Rattus norvegicus*) weighing 125-175g and 12-14 weeks old were used for study. They were acclimatized in the laboratory condition at a constant temperature of 22˚±3˚ and 12: 12 hr. Light: dark for fifteen days. They were provided with pelleted rat feed (M/S Amrut Feed, Pranav Agro Industries ltd. Sangli, India) and water *ad libitum*. All the animals receive humane care during the study and the protocol was approved by institutional animal ethics committee.

Dehydrated ethanol was purchased from Mecrk (India) and *Nigella sativa* oil was purchased from Mohammedia Pruducts, Hyderabad, India. All other the chemicals used were of laboratory standard.

Experimental Design

Albino rats (40) were divided into four groups each containing ten rats.

Group 1

Normal control, receiving equal amount of vehicle (distilled water) for eight weeks.

Group 2

Ethanol induced, receiving ethanol at a dose of 6g/kg/day for first week and 8 g/kg/day for seven weeks.

Groups 3 and 4

N sativa treated, receiving 5mL and 10 mL/kg body weight respectively. Both the group received same amount of ethanol, after one hour administration of *N sativa* oil for the same period of time.

After eight weeks of experiment, blood samples were collected from the reteroorbital plexus and the serum obtained was stored in eppendrof tube at -20˚C for further analysis. The liver tissue was excised, rinsed in ice-cold 9% normal saline, cut into small pieces and homogenized with Potter-Elveghan glass- Teflon homogenizer in ice-cold, 1.5M KCl. The homogenate was centrifuged at 1000 rpm for 10 min. Supernatant was collected for various analysis. Remaining portion of liver was fixed in alcoholic buoin solution and processed for histopathological evaluation using haematoxyline (H) and eosin (E).

Biochemical Analysis

Estimation of Liver Marker Enzymes

The activities of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were analysed by method by Reitman and Frankel.[16] Serum alkaline phosphatase (ALP) was estimated by using Kind and King's method.[17] The serum

gamma-glutamyl transferase (GGT) was assayed according to the method of Rosalki and Race.[18] Serum albumin was measured with bromocresol green.[19]

Lipid Peroxidation and Enzyme Assay

Lipid peroxidation was measured using thiobarbituric acid reaction (TBARS). TBARS was estimated by the method of Okhawa *et al* (1979).[20] The activities of enzymatic antioxidants superoxide dismutase (SOD) by the method of Marklund and Marhlund (1974).[21] The activity of catalase (CAT) was assayed by Abei (1974).[22] The activity of glutathione peroxidise (GPx) was assayed by the method of Lawrence (1976).[23]

Statistical Analysis

The values obtained were expressed as mean ± SD. Statistical evaluation was done using one way analysis of variance (ANOVA). The level of statistical significance was set as $p<0.05$.

Histopathological Examination

Liver tissue specimen of different groups were fixed in Bouins' solution and embedded in paraffin and 5µm thick sections were stained with Eosin and Haematoxyline.

RESULTS

Table 1 show that intoxication with ethanol significantly increased the serum ALT, AST, ALP and GGT as compared to normal rats. However, administration of *N. Sativa* oil at 10mL and 20mL/kg b.w. orally, decreased the activity of these enzymes as compared to ethanol induced rats. The observed changes were found to be more significant at 20ml/kg b.w. dose of *N.sativa*. The level of albumin was decreased in alcohol induced rat which on treatment with *N.sativa* oil returned back to near normal, however it was more significant at 20ml/kg b.w. dose

Table 2 shows that the level of lipid peroxidation was significantly high as indicated by increase in the level of malondialdehyde in ethanol induced group. A significant reversal was observed in the level of malondialdehyde following treatment with 20ml/kg b.w. of *N sativa* oil. The value of SOD, CAT and GPx were significantly ($p<0.05$) lower in alcohol administered rat as compared to normal one. A significant ($p<0.05$) increase were observed in all the three parameters following treatment with 20ml/kg b.w. of *N sativa* oil.

Table 1: Effect of *N.sativa* seed oil on hepatic marker enzyme and serum protein (alanine) in ethanol induced albino rat

Parameters	**Group 1 Normal**	**Group 2 Ethanol Induced**	**Group 3 Ethanol + *N.sativa* (10ml/kg bw)**	**Group 4 Ethanol + *N.sativa* (20ml/kg bw)**
ALT (U/l)	65.67±2.5	86.45±30*	69.75±4.5	66.43±3$^{\#}$
AST (U/l)	105.60±6.97	145.70±7.45*	120.50±6.07	109.50±5.01$^{\#}$
ALP (U/l)	205.20±8.9	290.65±9.7*	250.53±10.12	231.56±8.7$^{\#}$
GGT (U/Ll)	50.67±5.8	75.11±5.30*	65.75±7.7	54.86±6.5$^{\#}$
Albumin (g/dl)	3.35±.22	2.13±.26*	2.67±.25	2.87±.65$^{\#}$

Low dose = 5 ml/kg body weight; High dose= 10 ml/kg body weight.

Results are expressed as ± SD from six animals in each group.

*Significant difference from control group.

$^{\#}$Significant difference from alcohol group.

$p<0.05$.

Table 2: Effect of *N.sativa* seed oil on lipid peroxidation and antioxidant enzymes in ethanol induced albino rat

Parameters	**Group 1 Normal**	**Group 2 Ethanol Induced**	**Group 3 Ethanol + *N.Sativa* 10ml/kg.bw**	**Group 4 Ethanol + *N.sativa* (20ml/kg.bw)**
MDA (n moles/g protein)	122.33±11.4	210.43±11.5*	140.50±10.6	133.60±12.6#
SOD (U/g protein)	45.50±5.6	19.75±3.05*	54.45±4.60	50.30±5.5#
CAT ((U/g protein)	58.6±3.30	48.40±4.0*	55.65±5.6	56.95±5.5#
GPx (U/g protein)	4.56±.56	2.10±.45*	3.95±.20	4.75±.23#

Low dose = 5 ml/kg body weight; High dose= 10 ml/kg body weight.
Results are expressed as ± SD from six animals in each group.
*Significant difference from control group.
#Significant difference from alcohol group.
$p<0.05$.

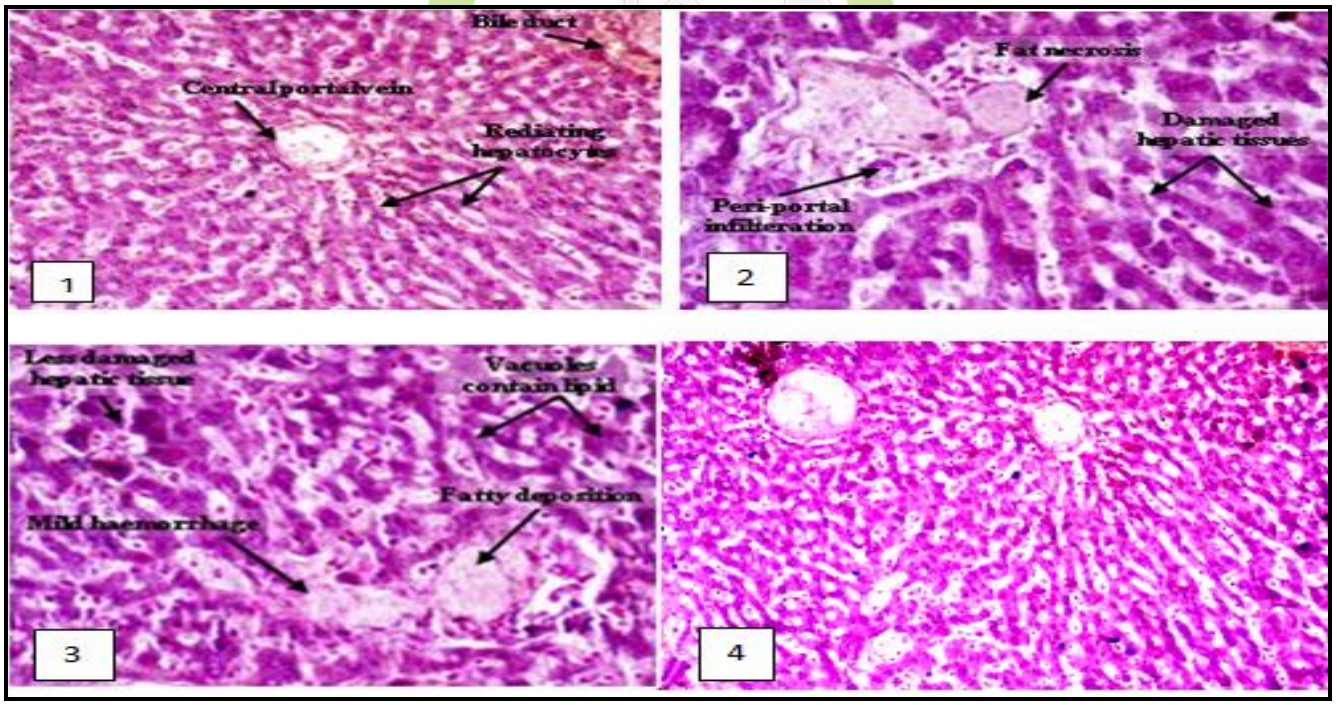

Figure : [1] Photomicrograph of liver section of normal rat (gr.1) showing normal cellular structure with central portal vein, bile duct and radiating hepatocytes(x40). [2] Alcohol treated rat (gr.2) showing degenerated cellular structure with fat necrosis, peri-portal fatty infilteration andvacuoles containing lipid (x40). [3] *N.satva*,5ml/kgb.w treated rat (gr3) showing restoration of normal cellular structure with less damage (x40). [4] *N.sativa*,10ml/kgb.w.(gr.4) showing restoration of normal cellular population of hepatocytes woth central portal vein and bile duct (x40)

The hepatoprotective effect of *Nigella sativa* was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of normal group showed normal hepatic cells with well-preserved cytoplasm, prominent nucleolus and central vein (Fig 1). In alcohol treated animals, the sections showed several degree of liver damage, showing congestion, periportal infilteration, fat necrosis (Fig 2). In *Nigella sativa* (5ml/Kg bw.) treated animals, (Fig 3) restoration of normal hepatocyte population with less damage was observed. In *Nigella sativa* (10 ml/Kg bw) treated animals, (Fig 4) liver section showed restoration of normal cellular architecture of liver with central portal vein and bile duct.

DISCUSSION

The present study was undertaken to demonstrate the haepatoprotectiver effect of *N.sativa* seed oil on alcohol – induced liver injury in albino rat. The results of the current study showed significant increase in the serum enzyme such as AST and ALT in alcohol intoxicated rat. The elevated level of these enzymes was primarily due to leakage of cellular enzymes in the blood stream leading to their increase in serum.[24] This indicates liver injury. Increased level of ALP was also recorded in the alcohol –induced group which was similar to the results of Soliman *et al* (2006).[25] The increase in ALP was attributed to the damage in lysososomal membrane.[26] This was in agreement with Salah *et al* (1976)[27] who noticed that lysosomal enzymes were activated in conditions characterized by increased tissue catabolism. The increase of cytosolic calcium could contribute to more breakdown and damage of lysosomal and other cellular membrane structure. In our study, treatment of alcohol- intoxicated rat with *N. Sativa* oil, alleviated the increased level of ALT, AST, ALP to near normal which could be manifested to reduction in cell membrane disturbances. Our result is in agreement with Danladi *et al* (2013)[28] who also showed the restoration of ALT, AST and ALP to near normal level in CCl_4 induced animals when treated with *N.sativa*. Serum GGT is a sensitive marker enzyme widely used as a laboratory test for the hepatobillary disease especially alcoholic liver disease and alcohol induced damage.[29] In our present study we observed that GGT has invariably decreased after *N. Sativa* treatment reflecting the reduction in liver damage and hence the ameliorating effect of *N. sativa* seed oil.

Albumin is one of key component of serum protein. As albumin is synthesized in the liver, it can be used as biomarker to monitor liver function.[30] In the present study albumin level was reduced in alcohol-induced rat which may be due to disturbance in protein synthesis in the liver.[31] A significant increase in serum albumin was observed in *N.sativa* treated rats. This is a clear indication of *N.sativa* being related to an improvement in the functional status of the liver cells. Mahmood *el al* (2008)[32] observed similar result in malathion induced wistar albino rat after treating with *N.sativa* oil.

Oxidative stress plays an important role in the pathogenesis of ethanol toxicity.[33] Ethanol is toxic to liver as 80% of ingested alcohol is metabolized in the liver without feedback mechanism. Ethanol is metabolized to acetaldehyde, the excess of which alters the intracellular redox status, induces fat deposits and triggers inflammatory and immune response. Lipid peroxidation mediated by excessive production of reactive oxygen species (ROS) due to ethanol toxicity is considered to play a pivotal role in the liver and other hepatic tissues damage.[34] ROS damages every major cellular component like membrane, lipids, carbohydrates and DNA which ultimately leads to widespread tissue damage.[35] Oxidative stress is a constant threat to all living organisms and an endogenous antioxidant defence system is employed to fight with it.[36] In the present study significant increase in MDA level in alcohol intoxicated rat was observed which means enhanced lipid peroxidation leading to tissue injury and failure of antioxidant defence mechanism.[37] Decrease in the level of MDA, following the treatment with *N. Sativa* oil indicate the antioxidative property of *N. Sativa*

seed oil. Similar results have been reported by many workers like Houghton *et al* (1995)[15] Mansour *et al* (2002)[38], Kanter *et al* (2005)[39] Mahmood *et al* (2007)[32] and Danladi *et al* (2013)[28]. The decrease in MDA may be due to inhibition of lipid peroxidation that causes damage to cell wall, cell lysis and necrosis.[40]

The present study showed significant decrease in antioxidant defence activity of SOD, CAT, GPx in alcohol induced rat. This decrease could be attributed to the feedback inhibition or oxidative inactivation of enzymes because of excess ROS generation. The generation of α-hydroxyl radical may lead to inactivation of these enzymes.[41] Decreased activity of GPx may be due to reduction in the reduced glutathione and increase in peroxides. The depletion of glutathione causes proportional decrease in H_2O_2 detoxification by glutathione peroxidase.[42] Thus, the balance of this enzyme system is important in removing superoxide anion and peroxides generated in liver. The relation between reduced glutathione peroxidase level and development of necrosis have been reported by Kim and Mahan (2003).[43] In the present study, significant increase in the activity of these enzymes was observed after *N. Sativa* administration. It is reported that *N.sativa* suppresses the formation of superoxide anion and hydrogen peroxide by increasing the activity of SOD, CAT, GPx.[44] Turkdogan *et al* (2001)[45] suggested that *N.sati*va protects liver possibly through immunomodulator and antioxidant activities. Studies conducted by Daba and Abdel-Rahman(1998)[46] have also shown that *N.sativa* has protective role against oxidative damage in isolated rat hepatocytes Therefore multiple action may take place during hepatoprotective activity. Modulation of lipid peroxiation and antioxidant status may be one of the important mechanism by which *N.sativa* oil exert toxic inhibitory effect.

Histopathological analysis of the liver sections is in good agreement with biochemical changes. Besides, results from histological images showed accumulations of fatty droplets in the hepatocytes which also provided clear cut evidence that the preinduction with alcohol induce liver damage, including loss of cell membrane integrity, accumulation of fatty acids, and necrotic cell death in rats[37] (Figure-2). Treatment with *N.sativa* oil was able to reduce the accumulation of fats in the mice and bring back the normal architecture of liver cells and bile ducts similar to that of the normal group. (Figure-4).

Thus, this study suggest that *N.sativa* has ameliorating effect on alcohol induced injury and mechanism may involve the prevention of cell membrane disturbances and reduction of oxidative stress by radical scavenging and antioxidant activity, this in turn prevents kupffer cell activation and pro-inflammatory mediators and normalization of altered redox state in addition to hastened elimination of ethanol and acetaldehyde from the blood. Further studies are needed to unravel the precise mechanism of action.

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