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Ameliorating Effect of *Nigella sativa* Oil in Thioacetamide-induced Liver Cirrhosis in Albino Rats

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ABSTRACT

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The present study was conducted to study the ameliorating effect of *Nigella sativa* seed oil (5ml/ kg body weight, 10 ml/kg body weight) on the liver damage caused by thioacetamide (20 mg/kg body weight) in albino rats for a period of eight week. A significant ($p<0.05$) improvement in the altered levels of bilirubin, albumin, total protein, alanine transaminase, alkaline phosphatase, γ-glutamyl transferase was observed after treatment with 10 ml/kg body weight of *Nigella sativa* oil. Antioxidant enzymes like catalase, superoxide dismutase glutathione peroxidase, thiobarbituric acid reactive substances and reduced glutathione also showed significant improvement in their altered levels in *Nigella sativa* (10 ml/kg body weight) treated rats. The results confirm the ameliorating effect of *Nigella sativa* oil on liver injury caused by thioacetamide and suggest the ability of *Nigella sativa* oil in scavenging the free radicals and protecting the liver cell against oxidative damage. The histopathological examination of liver section also confirm the ability of *Nigella sativa* oil in decreasing the severity of histopathological injury caused by thioacetamide.

Keywords: Hepatic cirrhosis, Thioacetamide, *Nigella sativa*, Liver enzymes, Antioxidants.

INTRODUCTION

Thioacetamide (TAA), originally used as fungicide, is a well known experimental hepatotoxin[1] which is a thio-sulfur containing compound endowed with liver cirrhosis and hepatocarcinogenic activity[2,3]. It has been established that TAA metabolize into an obligatory intermediate, thioacetamide-s-oxide by the mixed function oxidase system[4].The reactive oxygen species from TAA binds with liver macromolecules and induces liver cirrhosis that resembles the human disease. Recent evidences suggest that cytochrome P450 2E1(CYP2E1), a member of mixed function- oxidase system plays a key role in the mediation of TAA hepatotoxicity[5,6,7]. Liver cirrhosis is one of the most important world-wide health problem. In spite of tremendous advances in modern medicine, there is no effective drug available to protect liver from damage or help to regenerate hepatic cells[8]. It is therefore necessary to search for alternative drugs for treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

Nigella sativa L (*N. sativa*) commonly known as 'kalongi'[9], a member of ranunculaceae family contains more than 30% of fixed oil and 0.4-0.45%wt/wt volatile oil.[10] The volatile oil contain thymoquinone (TQ) nigellone and monoterpene such p-cyme and α piene as the major constituents[11]. Recently clinical and animal studies have shown that extract of *N. sativa* have many therapeutic effects such as bronchiodialative[12], anti-inflammatory[13], antibacterial[14], hypotensive[15] and antidiabetic[16]. Houghton *et al*[17] also reported that *N. sativa* and its derivative TQ inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation. However hepatoprotective effect of this plant against thioacetamide induced liver cirrhosis remain unclear.

Therefore, the present study was undertaken to investigate the hepatoprotective effect of *N. sativa* oil on TAA induced liver cirrhosis in male albino rat through biochemical histopathlogical analysis.

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MATERIALS AND METHODS

Animals: Male albino rat (100-150g) were procured from Lucknow and were acclimatized in laboratory condition for fifteen days. They were fed with rodent chow and water *ad libitum*. All the animals received humane care during the study under a protocol that was in accordance with institutional guidelines. Liver cirrhosis was induced in rats by intraperitoneal injection of Thioacetamide (Merck Co.) at 20 mg/kg body weight twice a week for eight weeks[18]. The animals were divided into four groups each containing six animals.

Group 1: Normal control receiving the equivalent amount of vehicle (distilled water) for the same period of time *i.e* eight weeks.

Group 2: Thioacetamide control receiving 20 mg/kg body weight.

Group 3: Thioacetamide induced rat treated with *Nigella sativa* (5 ml/kg body weight) throughout the experimental period.

Group 4: Thioacetamide induced rat treated with *Nigella sativa* (10 ml/kg body weight) for the same period of time.

After eight weeks of experiment, blood sample was collected through retero orbital vein and the serum obtained was stored in eppendrof tube at -20˚ for further analysis. The live tissue was excised, rinsed in ice cold water, cut into small pieces and homogenized with Potter-Elvehgan glass-teflon homogenizer in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10min. Supernatant was used for various analysis. The remaining portion of liver was fixed in alcoholic buoin solution and processed for histopathological evaluation using haematoxyline (H) and eosin (E).

Biochemical investigation:

Blood sera were used for determination of various biochemical parameters like Albumin level[19], Total protein level[20,] Bilirubin level[21], Alanine tansaminase (ALT)[22], Alkaline phosphatase activity[23],Gamma-glutamyl transferase activity[24]. The liver homogenate was used to analyse thiobarbituric acid reactive substances (TBARS)[25],reduced glutathione (GSH)[26], superoxide dismutase (SOD)[27], glutathione peroxidase (GPx)[28], catalase (CAT)[29]. All spectrophotometric analysis were carried out in Shimadzu U.V.1601spectrophotometer and the data were statistically evaluated by one way analysis of variance (ANOVA) followed by least significant difference test. p values < 0.05 were considered statistically significant.

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

Rats were treated with thioacetamide alone and combination of both thioacetamide and *N. sativa.* No significant changes were observed in any of the rats regardless of the treatment.

The levels of total protein and albumin in the blood plasma significantly decreased ($p< 0.05$) in TAA injected cirrotic rats as compared to normal ones. The oral treatment of TAA induced rats with *N. sativa* oil exerted a significant increase ($p<0.05$) in the total protein and albumin level as compared to TAA induced rats. The TAA induced rats exhibeted a significant increase ($p<0.05$) in the value of serum bilirubin, γ glutamyl transferase, alkaline phosphatase and ALT as compared to normal rats. In contrast, the oral administration of *N. sativa* significantly decreased ($p<0.05$) the value of bilirubin, γ glutamyl transferase, alkaline phosphatase and ALT as compared to TAA induced rats (Table 1).

Administration of TAA significantly increased ($p<0.05$) the level of TBARS in rat hepatic tissue. In contrast, treatment with *N. sativa* oil decreased the TBARS level as compared to TAA induced rats (Table2). The activity of CAT, SOD, GPX and GSH significantly decreased ($p<0.05$) in TAA induced rats. It clearly indicate that TAA is affecting the liver negatively by increasing the level of peroxidation and meanwhile decreasing the level of antioxidant enzymes. The oral administration of *N. sativa* oil produced marked improvement in the activity of hepatic CAT, SOD, GPX and GSH. (Table 2). However changes are not significant at a low dose of *N. sativa oil* (5 ml/kg body weight). The results clearly indicate the hepatoprotective effect of *N. sativa* oil but at a high dose of 10 ml/kg body weight.

In histopathological analysis, it is observed that the liver section of the control group (Fig A) shows normal architecture of liver cells and bile ducts. The liver section of TAA treated group (Fig.B,C) shows marked dysplasia of liver cells with cirrhotic nodules and bile duct proliferation. *N. sativa* oil treated group (Fig.D) shows reduced cirrhosis, dysplasia and bile duct proliferation which is very much near to normal architecture of liver.

Table 1: Effect of *Nigella sativa* oil on thioacetamide induced changes in different serum parameters in male albino rat.

Parameters	Control	Thioacetamide Induced	Thioacetamide + *N.sativa* oil (low dose)	Thioacetamid + *N.sativa* oil (high dose)
Total protein U/l	10.65±.54	8.4 ± 4.5*	8.56 ± 3.4	9.67 ± 0.905#
Bilirubin U/l	0.26±0.08	0.53 ± 0.095*	0.55 ± 0.086	0.44 ± 0.109#
Albumin U/l	3.31±.202	2.13 ± 0.216*	2.13 ± 0.202	2.68 ± 0.25#
γ –glutamyl transferase U/l	49.79±5.8	74.11 ± 5.39*	74.0 ± 5.56	65.075 ± 8.4#
Alanine transaminase U/l	9.51±2.91	10.73 ± 4.1*	9.98 ± 4.2	10.10 ± 2.4#
Alkaline phosphatase U/l	14.8±1.61	34.4 ± 3.062*	34.0 ± 2.56	30.5 ± 1.54#

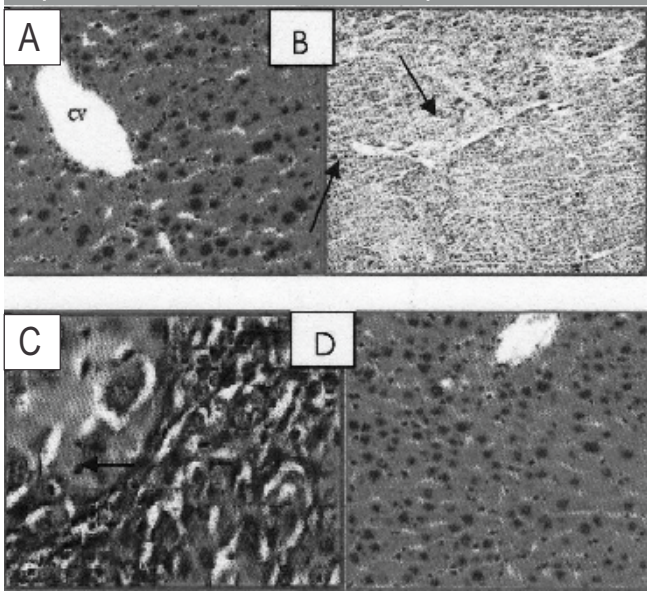
Low dose = 5 ml/kg body weight; High dose= 10 ml/kg body weight., Results are expressed as ± SD from six animals in each group.
*significant difference from control group., # significant difference from acetamide group., $p<0.05$.

Table 2: Effect of *Nigella sativa* oil on thioacetamide induced changes in hepatic antioxidative enzymes in male albino rat.

Parameters	Control	Thioacetamide Induced	Thioacetamide + *N.sativa* oil (low dose)	Thioacetamid + *N.sativa* oil (high dose)
CATU/mg protein	0.225 ± 0.021	0.139 ± 0.023*	0.14 ± 0.021	0.19 ± 0.018#
SODU/mg protein	22.15 ± 1.6	16.24 ± 1.35*	17.0 ± 1.55	19.85 ± 2.1 #
GP_xU/mg protein	0.178 ± 0.30	0.129 ± 0.016*	0.128 ± 0.14	0.168 ± 0.037#
TBARS mM/100 g of wet tissue	0.83 ± 0.50	1.63 ± 0.21*	1.65 ± 0.32	1.1 ± 0.31#
GSH mg/100 g of wet tissue	54 ± 2.0	30.2 ± 2.2*	30.5 ± 3.5	51.0 ± 2.30#

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 thioacetamide group, p<0.05.

[A] Photomicrographs of liver sections of normal control rats showing normal hepatic architecture (X40) [B] TAA treated (X10) showing severe damage of liver structure including the formation of pseudolobules with fibrotic septae (arrows) and necrotic areas (head arrows). [C] TAA treated rats (X40) showing severe damage of liver structure including the formation of pseudolobules with fibrotic septae (arrows) and necrotic areas (head arrows). [D] TAA plus *Nigella sativa* oil treated rats (X40) showing disarrangement of hepatic strands and an absence of fibrotic septae.



DISCUSSION

In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage. The ROS from TAA induces rat liver cirrhosis that resemble human disease and it can serve as a suitable model for studying human liver cirrhosis[30]. The result of the present study indicate that administration of *N. sativa* oil protect the liver against TAA toxicity in male albino rat. TAA causes injury to liver cells due to the production of a metabolite, thioacetamide s- oxide, which is a direct hepatotoxin responsible for change in cell permeability, inhibition in mitochondrial activity followed by cell death[31].

Leakage of cellular enzymes into plasma is an obvious sign of hepatic injury[32]. Their estimation in the serum is an important marker of hepatocellular damage. The activities of ALT, Alkaline phosphatase, γ glutamyl transferase are the most sensitive test employed in the diagnosis of hepatic disease[33]. In the present study the level of these enzymes increased in TAA induced rats which may be due to the leakage from damaged tissues[34]. This indicate necrosis of hepatocytes that results in the elevation of serum alkaline phosphatase, γ glutamyl transferase and ALT[35]. Treatment of TAA induced rats with *N.sativa* oil decreased the level of these enzymes reflecting ameliorating effect of *N.sativa* oil. The present result is in agreement with Daba and Abdel Rahman (1998)[36] who showed, thymoquinone's (the major component of *N. sativa*) protection against tertiary –butyl hydroperoxide induced hepatotoxicity in rat's isolated hepatocytes. This indicate the property of *N. sativa* oil in maintaining the integrity of cell membrane of the liver cells and thus protecting liver from the adverse effect of TAA. The present results also reveal decrease in total protein and albumin level in TAA induced rats which is an indication of liver cirrhosis. Administration of *N. sativa* oil significantly increased the total protein and albumin level in TAA induced rats. The data of the present result are compatible with the previous result demonstrating that *N. sativa* treatment for five days protected mice against hepatotoxicity induced by CCl_4[37]. Nagi *et al* (1999)[38] showed that oral administration of thymoquinone immediately before CCl_4 administration offers some protection to the hepatic cells from toxicity induced by CCl_4 in mice Decrease in serum bilirubin after treatment with *N. sativa* oil indicate the effectiveness of *N. sativa* oil in normal functional status of the liver. This is in agreement with the report by Rajkapoor[39]

The present study clearly demonstrate the ability of TAA to causes severe oxidative stress in rat liver, as evidenced by significant rise in lipid peroxidation product (TBARS) and significant decline in endogenous antioxidants, GSH, GP_X, CAT and SOD. These findings are in agreement with Akbay *et*

al(1999)[1] and Balkan *et al* (2001)[40]. Natural or synthetic compounds with antioxidant property may help to alleviate the liver damage. The result of present study show that treatment of TAA induced rat with *N. sativa* oil significantly decreased the hepatic content of TBARS content while significantly increased the concentration of antioxidant enzyme, GSH, $GP_{X,}$ CAT and SOD. The observed restoration of GSH, $GP_{X,}$ CAT and SOD activities on treatment with *N. sativa* oil may be due to direct stimulatory effect of thymoquinone, one of the major constituent of *N. sativa* oil on these antioxidants which could protect the hepatic cell and ameliorate most of the biochemical adverse effects induced by TAA. These findings are in good agreement with the previous finding that the hepatoprotective effect of thymoquinone against CCl_4 induced toxicity is mediated through decreased hepatic liver peroxidation[37,41].

The histopathological study of TAA induced (Fig B,C) liver section exhibit marked increase in extracellular matrix, dysplasia of liver cells, cirrhotic nodules and bile duct proliferation which are characteristic of cirrhosis[42,43,44]. *N sativa* oil treated rats (Fig D) showed correction of most of the harmful alteration. The results are in harmony with the findings of Ali and Blunden (2003)[45], El-Tahir *et al* (1993)[12].This action may strongly relate to the chemical composition and activity of *N. sativa*. Thymoquinone is the main active component of *N. sativa* and is able to inhibit lipid peroxidation[25]. Moreover, its ability to preserve the membrane integrity can be proven by the restoration.

The results of the present study conclude that *N sativa* oil is effective in the treatment and prevention of thioacetamide induced hepatic cytotocicity. This study along with other research targets *N. sativa* oil as a potentially safe and effective plant product that has important medicinal values and benefits. However, further detailed studies are required to establish its clinical application.

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