ORIGINAL ARTICLE

The Hepato-protective effect of *Nigella sativa* (Black Seeds) on Alcohol induced Hepatotoxicity in rodents

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ABSTRACT

Aim: To determine the effect of aqueous extract of *Nigella sativa* seeds in experimentally induced hepatotoxicity by ethanol in rats.
Study Design: Cohort study.
Place of study: Animal research lab. Bakhtawar Amin Medical & Dental College, Multan
Duration of study: From February 2018 To March 2018.
Methods: The rats were separated in a control group (A), hepatitis induced group (B) while the left over two groups(C and D) worked as investigational groups. They received "*Nigella sativa"* extract as hepatoprotective agent. Group C rats (experimental group I received 3g/kg body weight of ethanol as the intra-peritoneal injection and 250mg/kg body weight of "*Nigella sativa"* seeds extract orally daily . On the other hand group D rats (experimental group II) received 3g/kg body wt of ethanol by intraperitoneal injections and aqueous seeds extract of "*Nigella sativa"* 500mg/kg body wt orally daily.
Results: Blood samples were taken at the end of 6th week and liver was taken out for microscopical exam. The rats of experimental groups showed increase in ALT, AST, and ALP levels. 250mg/kg body weight of "*Nigella sativa"* seeds extract treatment diminished the level of these factors in rats. On the other hand 500mg/kg body weight of "*Nigella sativa"* dose considerably reduced levels of these biochemical factors. The histopathological investigation of experimental group I rats exhibited slight recovery whereas the rats in group II revealed a substantial recovery.
Conclusion, "*Nigella sative"* aqueous seeds extract showed hepato-protection against alcohol induced hepatotoxicity in rats in dose dependant manner.
Keywords; Hepato-protection, *Nigella sativa*, Hepatotoxicity

INTRODUCTION

Hepatitis is one of the important microangiopathic complications of alcohol ingestion[1]. About 50 years ago it was believed that alcohol in itself was not toxic, rather that the nutritional deficiencies often accompanying it, were the actual causes of liver damage. However, it was shown by Lieber and De Carli that in rats, ethanol induced liver damage developed despite adequate nutrition[2].. Later on it was shown to be related to its metabolism by alcohol dehydrogenases. Ethanol ingestion, generates more reactive oxygen metabolites[3] which may prompt enactment of resistant cells to express expert fibrotic and inflamatory cytokines. Macrophages synthesize TNFα in different conditions that produce oxidative pressure[4]., in addition IL-1 and IL-6[5].. Additionally, oxidative pressure prompts the age of lipid peroxidation items and protein adducts, which in the long run invigorate a break in self-resilience[6,7] and an insusceptible response related with hepatitis[8]. Furthermore, it has been shown that the increase in cellular oxidative stress by which alcoholism worsens progression of chronic hepatitis C, as antibodies towards albumin adducted with lipid peroxidation products were greater in consumers[9]. After heavy alcohol consumption, the MEOS (Microsomal Ethanol Oxidizing System) pathway of alcohol metabolism becomes more important. This pathway consists of several enzymes located in the liver microsomes (small spherical structure found in all cells)[10]. The primary component of MEOS is the molecule cytochrome P450, which exists in several variants[11]. The variant most important for the alcohol consumption is cytochrome P450 2E1 (CYP2E1)[12]. Alcohol treatment results in increment in production of endotoxins from the gut microbes and penetrability of gut to the endotoxin or both. Females are progressively touchy to these changes. Raised dimensions of endotoxins enact kupfer cells to discharge substances, for example, eicosanoids, TNF alpha and free redicals[13,14]. Prostaglandins increment the oxygen take-up and are in all likelihood in charge of the hypermetabolic state in liver. The expansion in oxygen request prompts the hypoxia in liver and on re-perfusion, alpha hydroxyl-ethyl free redicals are framed which lead to tissue harm in oxygen poor pericentral locale of the liver lobule.

Alcohol affects almost all organs of body. Chronic alcohol consumption lead to several metabolic disorders including hepatic and extrahepatic diseases[15].

Pakistan being a Muslim country bans free sale of alcohol drinks. Hence statistics are not available for the incidence of liver damage caused by alcohol use. A study was carried out in Aga Khan University, concluded that "the reason for using alcohol was peer pressure (96%), academic stress (90%), and curiosity (88%)[16].

The seeds of "*Nigella sativa"* an annual Ranun-culaceae herbaceous plant, commonly known as the "BLACK SEEDS" is a medicinal plant that grows throughout greater part of Middle East, Northern Africa, Far East and Asia. It has traditionally been used for the treatment of skin diseases, inflammations, arthritis[17] and now a days being used for the treatment of asthma (18). *"Nigella sativa"* is known to posses immunomodulator, anti-inflamatory and anti tumour activities. It holds more than 30% of fixed oils[19] and 0.40-0.45 w/w of volatile oil.The volatile oil has been revealed to have 18.4-24% thymoquinone[20] and 46% many monoterpenes such as p-cymene and alpha pinene[21]. "Nigella sativa" contain thymoquinone (TQ) and dithymoquinone (DTQ), are presumed to be the active ingredients[22,23]. The liver has a vital role in the breakdown of nutrients and drugs[24,25] which may produce intermediates toxins to the liver itself. *Nigella sativa* was used to treat liver diseases and its oil is reported to have a choleretic effect[26]. It is also found that administration of thymoquinone resulted in significant protection against drug induced hepatotoxic effects[19,27,28]. It contains unsaturated fats including omega 3 and omega 6[29].. These are important for the

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development of prostaglandin E1 which adjusts and fortifies the insusceptible framework, enabling it to keep from contaminations, hypersensitivities and control unending ailments. Solid cells are shielded from infections, so shielding from tumors.

The purpose of the present study was to observe the effect of *Nigella sativa* on alcohol induced hepatotoxicity in albino rats."

MATERIALS AND METHODS

Animals: Fifty six (56) adult male albino rats weighing 200-300g were procured for this study. They were kept in the experimental research laboratory under day and night conditions. They were divided in to 4 groups, each having 14 animals. Prior to the initiation of the trial, all rats were kept for one week under a similar research center conditions, at a temperature of 25 ± 2 ^{0}C, relative dampness of 70 ± 4% and 12 hour light/day cycle. They got healthfully standard eating regimen and faucet water. The consideration and treatment of rodents were as per the universally acknowledged standard rules for utilization of animals.

Plant materials and preparation of the extract: Seeds of "*Nigella sativa"* were collected from local market of Multan and were authenticated from a Botanist. *Nigella sativa* seeds were coarsely chopped in a grinder. 10g of chopped seeds was boiled with 100 ml distilled water for 15 minutes and allowed to cool at room temperature. Then the extract was filtered with clean cotton, and the volume of extract was adjusted to 100 ml by evaporation. Therefore the final concentration of extract was 10g/dl. This filtered extract was further allowed to evaporate until its final volume was reduced to 10 ml and it was concentrated to 100g/dl. The extract was stored in a deep freezer (-20 degree Celsius) until used. At the time of use it was defrosted and administered to animals for six weeks.

Experimental Procedure: After acclimatization, the rats were divided into four groups. Group A (Control group) received daily intra-peritoneal injection of 0.9% w/v NaCl only, while Group B, C and D were given ethanol (3g/kg) dissolved in 0.9% w/v NaCl, intra-peritoneally daily, for six weeks. In addition Group C and D received 250mg/kg and 500mg/kg *Nigella sativa* aqueous seeds extract orally daily for a period of six weeks.

Sample collection: Twenty four hour after organization of the last portion of concentrate the creatures were gauged and anesthetized under ether vapors. An sample of 2ml blood was drawn from all rats, via venesection of heart. Blood was drained to the clean vacuotainers with gel and permitted to cluster at room temperature for 60 minutes. It was then centrifuged for ten minutes at a speed of 3000 rpm. Serum was isolated and put away in sterile eppendorf tubes at - 20°C for examination of biochemical parameters.

Biochemical Analysis: Plasma ALT, AST and ALP was determined by using their respective kits, manufactured by Dia Sys Diagnostic Systems, Germany, by IFCC method. Controls were analyzed followed by test samples, 0.4 ml of non - hemolyzed blood was contained into the sample cup, and procedure was done. The alteration in the absorbance was gauged at 340 nm by using kinetic mode with a factor -2300. Control materials utilized were, Assayed bovine sera level 2 (Normal) Lot No: 355SN and Assayed bovine sera level 3 (Abnormal) Lot No: 280SE/1 of Randox (UK). CV of the method was 0.64- 0.74 percent.

Histopathological procedure: Slice of liver removed at the time of termination of experiment were dehydrated using ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were made using paraffin wax and sections of 4-5 microns were cut for staining with H&E. Finally the slides were mounted, using DPX.

Statistical Analysis: The data was entered and analysed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant."

RESULTS

Effect of *Nigella sativa* on the serum levels of liver enzymes (ALT, AST, ALP)_ showed that the injection of ethanol caused a significantly (p<0.01) increased serum ALT, AST, ALP parameters in the animals of group B, C and D as compared to control group. On the other hand, simultaneous administration of aqueous extract of *Nigella sativa* resulted in a significant (p<0.01) decrease in the serum ALT, AST, ALP levels of the rats in groups C and D when compared with that of group B in dose dependant manner.

Table 1: Mean± SEM values of different parameters in all groups

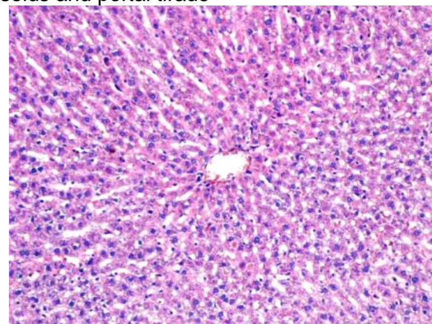
	Group A	Group B	Group C	Group D
Serum AST u/l	183.36±39.63	288.94±42.96	189.98±14.19**	144.97±19.63**
Serum ALT u/l	0.63±0.03	1.80±0.054*	1.31±0.10**	0.90±0.04**
Serum ALP u/l	127.16±20.73	234.95±.40.46*	180.10±13.15	215.57±39.82**

*p<0.05 when compared with group A **p<0.05 when compared with group B

Histopathological examination: In histopathological studies of livers of male albino rats, the control group showed normal lobular pattern and normal architecture. The hepatocytes and portal system are normal (Fig 1) and those given alcohol for six weeks showed liver damage characterized by, clear cytoplasm, vascular congestion and apoptosis (Fig.2)

These changes were found to be much reduced in livers of rats treated with *N.S* extract in a dose of 250mg/kg (Fig 3). The liver of rats treated with 500mg/kg of *N.S* extract showed that liver is normal and there is no obvious change of architecture (Fig 4). The apoptotic bodies as the marker of toxicity were counted per HPF were counted from each group resulted numerous apoptotic bodies in each slide from Group "B", and 32-50 and 3-8 apoptotic cells were counted per HPF in slides of Group "C" and "D" respectively.

Group-A (control) Fig.1 (H&E, Objective magnification, 20 x Normal rat liver showing normal liver architecture, normal central vein, sinusoids and portal tirade



Group B **(Ethanol treated rats liver)**

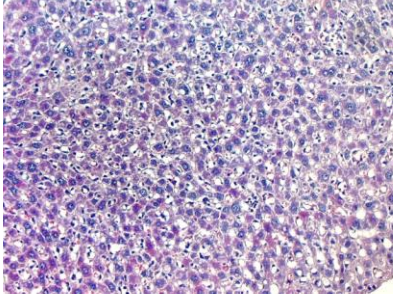


Fig.2: (H&E, objective magnification 100x) Photomicrograph of ethanol treated rats liver showing scattered apoptotic bodies.

Group C

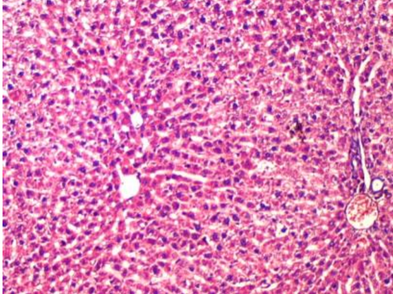


Fig.3 (PAS, objective magnification 60x Photomicrograph of **(**Ethanol+*N.s* extract 250mg/kg) treated rat liver showing mild fatty change along with rare apoptotic bodies. .

Group D

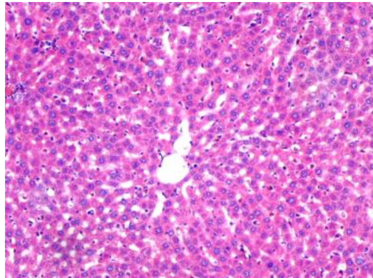


Fig.4. Photomicrograph of **(**Ethanol+*N.s* extract 500mg/kg) treated rat liver showing normal hepatic architecture with only occasional apoptotic bodies. (H&E ×60)

DISCUSSION

Frequent use of alcohol induces acute liver injury. Typically, hepatotoxicity is assessed by the measurement of serum levels of total billirubin and the activity of aminotransferases, which are commonly used methods in clinical practice. In general, the increased values of all these three parameters are associated with and reflect the status of liver injury.

Ethanol induced hepatic damage covers a major portion of the clinical and pathological expressions of hepatic dysfunctions. There was significant elevation observed in the levels of liver enzymes in the serum of alcohol administered rats as compared to normal rats. Higher levels of these enzymes in serum are apparent indicators of drug produced lesions in the hepatocytes. Higher predisposition of hepatocytes cell membrane to alcohol tempted peroxidative destruction might be resulted in the bigger release of these enzymes into the systemic circulation.

Drug-induced hepatotoxicity is one of the most common causes of drug withdrawal at the later stages in drug development (30) (31). As patients are receiving the drug, and a lot of resources have already been put into compound development, the need for better prediction of hepatotoxicity at an earlier stage cannot be overemphasized. The chemokines have a role in attracting neutrophils to sites of injury. CXCL2 expression is regulated by NF-κB, which in turn is activated by oxidative stress for instance after toxic insult[32,33].

It has also been noted that IFNγ levels are critical for determining the ethanol affects on IL-12[34]. Additionally, the secretion of IL-12 is regulated by cAMP and IL-10. As TGF-β can be considered as the indicator of fibrinogenic response, because it induces the synthesis of collagen in stellate cells[35,36]. As fibrosis is a slow process, so a longer duration of ethanol treatment is required for the development of fibrosis. Apart from this our data are generally more in line with acute innate immune response to ethanol, as proposed by Wheeler and colleagues[37].

Products of lipid peroxidation have been involved in the establishment of acute liver disease. It has been shown in alcoholics that the products of lipid peroxidation increase the synthesis of collagen in stellate cells. The products of lipid peroxidation can form the aggregates of protein that interfere the cell function, contribute to Mallory bodies formation (38) (39), and signaling pathways. Products of lipid peroxidation have an effect on inflammation. It has been shown in human alcoholics that antibodies directed towards aggregates that are formed in response of lipid peroxidation are readily detected and T cells of alcoholics respond to these. Cytokine 8 over expression has previously been shown to induce Mallory body formation in mice[40]. Cytokeratins 8 and 18 have also been shown to be up regulated in response to oxidative stress by CYP2E1 *in vitro*[41]. Cytokeratins normally have a high rate of turnover, but accumulation of these proteins lead to their aggregation into Mallory bodies. Mallory bodies then compromise the function of the microtubule network as well as the centrosomes which will eventually lead to cell death.

In the present study, co-administration of *N.S* extract maintained the levels of these diagnostic marker enzymes in the serum of Group C and Group D animals towards normalcy as compared to Group B rats, indicating the cytoprotective and antioxidant effect of black seeds aqueous extract. It probably did so by preventing the alcohol induced damage by its membrane stabilizing action.

The comparison of mean values of ALT, AST and ALP results in all groups indicates alcohol induced rise in liver enzymes and then protection of black seeds extract in a dose dependent manner. Several plant derived compounds have been planned as antioxidants and antifibrotics in the treatment of chronic liver disease. The antioxidant systems guard the cells against lipid peroxidation, which is the base of many pathologic processes[42].

In present study, we found that *N.s* treatment for 6 weeks diminished the higher levels of liver enzymes in ethanol treated rats, which shows that NS has a defending effect against oxidative injury in quarantined rat hepatocytes. It has been detected that *N.s* has a substantial hepatoprotective effect in CCl_4-administrated rabbits. The hepatocellular degenerative and necrotic changes were slight in *N.s* treated group. However, *N.s* can avoid liver fibrosis and cirrhosis, suggesting that *N.s* shields liver against fibrosis possibly through immunomodulator and antioxidant deeds[43]. Most of the hepatoprotective drugs belong to the group of free radical scavengers, and involves membrane stabilization, neutralization of free radicals and immuno-modulation. The inhibitory effects of the crude fixed oil and pure thymoquinone on membrane lipid peroxidation have been demonstrated.

In this study it is proven that the co-treatment of *N.s* extract with alcohol, prevents alcohol induced hepatotoxicity in rats that is probably caused by its antioxidant and free oxygen radicals scavenging ability.

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