Original Article

Nigella Sativa Role in Liver

Role of Nigella Sativa L. Seeds against Carbon Tetrachloride Induced Liver Injury in Rabbit – An Experimental Study

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ABSTRACT

Objective: To investigate the protective effect of Nigella sativa against carbon tetrachloride (CCl_4) induced liver injury in adult male Rabbit model.

Study Design: Experimental/Analytical study

Place and Duration of Study: This study was carried out at the Animal House, Isra University from May, 2012 to October 2012.

Materials and Methods: Thirty male Rabbits were selected according to inclusion and exclusion criteria and studied. Animals were divided into three groups Carbon tetrachloride was used to induce liver injury. N. sativa seeds were administered in experimental group. Blood samples were collected from peripheral veins. Sera were used liver enzyme detection. The animals were sacrificed by over-dose of Ketamine and liver was removed for histological study. The data was analyzed on SPSS version 16.0 for Windows release (Chicago, IL, USA). A p-value of ≤ 0.5 was taken statistically significant.

Results: Liver enzymes found elevated in CCl4 compared with control group after three weeks (p=0.001) The CCl_4+ N.sativa group showed a significant reduction in liver enzymes compared with CCl_4 group (p=0.001) and control group (p=0.001). N.sativa when mixed with CCl_4 showed significant reduction in the liver enzyme. Findings shows significant hepatoprotection by the N.sativa in CCl_4 induced injury. Liver tissue sections showed least derangement of hepatocytes cords, hepatocytes damage and necrosis was minimal compared with CCl_4 group

Conclusion: Nigella sativa protects against carbon tetrachloride induced liver injury in rabbit model.

Key Words: Nigella Sativa, Carbon Tetrachloride, Liver injury.

INTRODUCTION

Liver is the largest gland of human body, which performs many biochemical and metabolic functions.[1] Free oxygen generated radicals known as reactive oxygen species (ROS) are implicated in the pathogenesis of most liver diseases, including ischemia/reperfusion injury, endotoxemia, chronic hepatitis C, alcoholic and non-alcoholic fatty liver disease, and cholestasis.[2] Carbon tetrachloride (CCl_4), a hepatotoxin, has been used extensively for decades to induce liver injury in various experimental models to elucidate the mechanisms behind hepatotoxicity.[3] It has been known for a long time that a part of the liver injury caused by this solvent may have originated through the free radical reactions to the metabolism of CCl_4 in the liver and subsequent initiation of lipid per oxidation.[3,4] In CCl_4 induced liver damage, there is an excessive lipid peroxidation leading to functional and structural disruption.[5] The damage or death of hepatocytes usually results in leakage of enzymes from affected tissue into the blood stream.[6] Serum or plasma enzyme levels have been used as markers for monitoring chemically induced liver damages. The enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) are important enzymes that are often employed in assessing liver injury.[1,3] Rajesh and Latha [7] showed that various herbal extracts like that of Nigella sativa could protect organs against CCl_4 induced oxidative stress by altering the levels of increased lipid peroxidation and enhancing the decreased activities of antioxidant enzymes. [7]

Nigella sativa (NS) is an aromatic plant, native to the Mediterranean region and the Southwest Asia.[8] Botanically, the NS plant belongs to the family of Ranunculaceae. In Pakistan, it is commonly known as "*Kalonji*".[9] The NS plant as well as its seeds has great importance in the old systems of therapeutics such as Unani and Ayurverdic and also in the Allopathic system of medicine. In Southeast Asia, it is publicly known as the Kalonji. In Arabic countries, it is known as the "*habat-ul-sauda*". The English people call the NS seeds as "*black cumin*". The NS plant has been a focus of most of research studies in modern era. As it has been traditionally used for centuries, hence many studies have been conducted to explore its chemical constituents and biological activities by scientific methods. Several studies on animal models have been conducted to identify the biological activities of *N. sativa* on different components of the metabolic syndrome.[10] The most active constituent of NS seeds

and oil is the Thymoquinone (TQ). Its chemical name is the "*2-isopropyl-5-methyl-benzoquinone*" and most of the therapeutic properties are attributed to this constituent. Thymoquinone yields most of the bio-therapeutic properties of NS seeds and oils. Thymoquinone is a promising dietary agent and a chemo- therapeutic and chemo-preventive agent for the treatment of diseases.[11]

However, in spite of large number of antioxidant and hepatoprotective studies carried out worldwide on *N. sativa* oil and TQ, scrutiny of published literature showed that there is a need to investigate whole seeds as they are used for treatment in folk medicine rather than the oil extract or TQ. Therefore, the present study aimed to find out if aqueous extract of whole *N. sativa* seeds possess protective effect against CCl_4-induced hepatotoxicity in experimental animals.

MATERIALS AND METHODS

An experimental study was conducted on rabbit model over a period of six months (May-October 2012). Adult Wistar male rabbits of age 7 months to 1 year, weighing 1.0-1.5 kg were included in the study. Female rabbits, age of <7 months, or >1 years, weight <1.0 Kg or > 1.5 kg and sick rabbits were excluded from the study. Animals were housed in stainless steel cages at room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. Fresh alfalfa and tap water were provided *ad-libitum*. The rabbits were divided into three groups;

Group 1. (n=10) Rabbits received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group 2. (n=10) Rabbits were given CCl_4 orally mixed in olive oil on alternate day for three successive weeks and

Group 3. (n=10) Rabbits received Nigella sativa and CCl_4 on alternate days for three successive weeks

Experimental Details: Carbon tetrachloride was purchased from scientific drug store and Nigella sativa seeds from a local herbal grocery. The N. sativa seeds were dried, cleaned and powdered to mix with isotonic saline solution. Olive oil was used as vehicle to administer CCl_4. Twenty four hours after the end of experimental period, blood samples were collected from peripheral veins. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes. The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006)[12] and liver was removed promptly for histological study.

Liver enzyme assay: Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Histological studies: Each sample of liver obtained was washed in normal saline and tissues were fixed in previously marked containers, containing 10% formaldehyde as preservative. The tissues were embedded in paraffin, cut into 5 um thick sections and stained with Hematoxylin-Eosin (H & E) and Masson`s trichrome staining for histological examination. The histological criteria included vacuolar degeneration, inflammatory cell infiltrate, congestion and necrosis. The histological parameters were graded as follows; 0 = no abnormal findings, + = mild injury, ++ = moderate injury and +++ = severe injury.[13]

Statistical analysis: The data was analyzed on SPSS version 16.0 for Windows release (Chicago, IL, USA). The continous variables were presented as mean±SD and range. The categorical variables were analyzed by Chi-square test. While the continous variables among and between groups were calculated by one-way analysis of variance (one-way ANOVA) and post hoc Tukey`s HSD testing. A p-value of ≤ 0.5 was taken statistically significant.

RESULTS

In present study, we observed major differences in liver enzyme assays among groups. The ALT, AST, ALP and LDH in sera of Rabbits treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant of p-value for all variables (p=0.001) The CCl_4+ N.sativa group showed a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The N.sativa when mixed with CCl_4 showed significant reduction in the liver enzyme elevation in blood sera. The finding shows significant hepatoprotection by the N.sativa in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1

Table No.1: Liver enzyme assays in Controls, CCl_4 and CCl_4+ N.sativa groups.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)
Group 1 (Controls)	47.1± 2.99	89.1± 17.98	91.76± 9.01	712.4± 53.7
Group 2 (CCl_4)	197.7± 12.1	513.7 ± 19.9	176.1± 7.23	2798.8 ±145.8
Group 3 (CCl_4 + N.sativa)	84.79± 19.97	168.3± 21.3	137.9± 19.15	2140.6 ±156.4

Different parameters of histological score of liver injury are shown in Table. II. The Liver sections from control group showed intact central portal venules and compact hepatocytes arrangement. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm were seen in control group. The CCl_4 group showed derangement of hepatocytes cords, hydropic changes with congestion of central venules and

sinusoids, and abundant inflammatory cell infiltration. The centrilobular hepatocytes showed hydropic changes and necrosis, while midzonal and peripheral hepatocytes showed vacuolar degeneration and fatty changes in CCl_4 group. In CCl_4+N.sativa animals, liver tissue sections revealed least derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group.

Table No.2: Histological score of liver injury

Groups	Vacuolar degeneration	Inflammatory cell infiltrate	Congestion	Necrosis
Group 1 (Controls)	0	0	0	0
Group 2 (CCl_4)	+++	+++	+++	+++
Group 3 (CCl_4 + N.sativa)	+	+	++	+

DISCUSSION

Carbon tetrachloride is a commonly used hepatotoxin in experimental study of liver diseases in animal models.[14] The liver toxicity is produced by release of free radicals and lipid peroxidation[15] which causes hepatocyte necrosis, inflammation and fibrogensis.[16] The serum levels of ALT, AST, ALP and LDH reflect the physiological state of liver. The ALT, AST, ALP and LDH are released parallel to the distortion of liver, and cellular injury of the organ caused by toxic metabolites and diseases.[17] The present study indicates that the carbon tetrachloride caused an increase in serum levels of liver enzymes in rabbits as compared to control group; that is carbon tetrachloride induced a detectable damage to liver sufficient to release liver enzymes, as previously reported by Hukkeri et al.[18] The Hukkeri proved elevation in the plasma level of cytoplasmic and mitochondrial enzymes due to liver injury induced by CCl_4 in animal models.[18] Increased blood levels of liver enzymes indicate rupture of the cell membrane and damage of hepatocytes sufficient to release cytoplasmic enzymes into blood circulation.[19] In the present study, damage of liver caused by CCl_4 was evident by the rise in serum marker enzymes beside the histological changes in liver tissue. Administration of CCl_4 significantly increased the serum levels of liver enzymes; AST, ALT, ALP and LDH, which are indices of liver cell damage and leakage of enzymes from cells.[7,20] It is reported that rise in ALT is almost always due to hepatocellular damage; accompanied by rise in AST and ALP.[21] The carbon tetrachloride is found to produce free radicals, which affect cellular permeability of hepatocytes leading to elevated levels of liver enzymes.[22]

The histological examination of current work correlates with disturbance in biochemical markers of hepatocellualr damage. Histological examination of carbon tetrachloride group revealed disruption of normal structural organization of hepatic architecture, hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells. The hepatic cells revealed characteristic appearance of cellular injury and showed marked cytoplasmic vacuolization. The nuclei of these cells were pyknotic. Lymphocytic infiltration and fatty change was also evident. Our findings are supported by previous studies which showed that carbon tetrachloride induces centrilobular hepatocellular vacuolar degeneration and necrosis.[23,24,25] The carbon tetrachloride induced hepatotoxicity has been attributed to the formation of free radicals during its detoxification in hepatocytes smooth endoplasmic reticulum by the cytochrome P450.[26] Balahoroglu et al.[27] reported that carbon tetrachloride induces lipid peroxidation which produces changes in biological membranes resulting in serious hepatocellular injury. Treatment with *N. sativa* seeds significantly reduced effects of carbon tetrachloride induced hepatocellular damage and it was evidenced by the decreased level of liver enzymes and restoration of hepatocellular architecture. Similarly, Al-Razzuqi et al.[28] had reported protective effect of oil extract of *N. sativa* seeds against carbon tetrachloride induced acute liver injury in experimental rabbit models. Also, protective effect of black seed oil against lead acetate-induced hepatic tissue damage in mice was investigated.[29] The findings are consistent with our current work. The present study reveals that the N.sativa seeds possess hepatoprotective potential against oxidative damages caused by carbon tetrachloride. The N.sativa may be used as an effective protector against chemical induced liver damages.

CONCLUSION

The present study concludes that *Nigella sativa* seeds possess hepatoprotective potential against oxidative damages caused by carbon tetrachloride. The *N.sativa* may be used as an effective protector against chemical induced liver damages. Histological findings also indicate liver protection by Nigella sativa against CCl_4 induced liver damages in present study.

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