ORIGINAL ARTICLE

Hepatoprotective Effect of Black Garlic Extract against Carbon Tetrachloride Induced Liver Injury in Rats

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ABSTRACT

Objective: To investigate the protective effect of Black Garlic extract (BGE) in carbon tetrachloride (CCl4) induced liver injury in adult male Wistar rat model.

Study design: Experimental / Analytical study

Place and Duration: Animal House, Isra University Hyderabad from May to September 2013.

Material & Methods: Sixty adult male Wistar rats were divided into three groups; Group I controls received 0.9% isotonic saline, Group II received CCl4 orally (1.9mg/kg) mixed in olive oil, and Group III. received the BGE (100 mg/kg) + CCl4. After 4 weeks blood samples of animals were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 10% formaldehyde, was embedded in paraffin. Tissue sections of 5μ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on Statistix 8.1 (USA) using one-way ANOVA, Bonferroni and Chi-square tests. P-value of=0.05 was taken statistically significant.

Results: The biochemical and histological findings of liver showed statistically significant differences between the controls, CCl4 and BGE+CCl4 groups (p=0.001). Liver enzymes and histology was deranged significantly in CCl4 group compared to controls and BGE+CCl4 group (p=0.001). The BGE+CCl4 group showed less rise of liver enzymes and derangement of liver histology when compared to CCl4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis were prominently found in CCl4 group.

Conclusion: The Black Garlic extract (BGE) protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model.

Key words: Carbon tetrachloride, Liver injury, Black Garlic extract.

INTRODUCTION:

Garlic is a traditional food item used worldwide, and recent studies have reported many of its therapeutic uses[1-5].

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The studies have reported an inverse association between garlic consumption and a reduced risk of gastric and colon cancer[2], antithrombotic activity[3], and its cardioprotective effect by reducing blood lipids[4]. Black garlic extract (BGE) is an odorless product obtained from extraction of fresh garlic at room temperature. The BGE exhibits high biological activity in vitro. The BGE contains water-soluble allyl amino acid derivatives, which account for most of its organo-sulfur content, stable lipid-soluble allyl sulfides, flavonoids, saponins, and essential macro- and micronutrients[5]. The BGE is a new form of garlic preparation produced by aging garlic at high temperature and humidity. The BGE, like other aged garlic products, is rich in bioactive organic sulfur compounds such as S-allylcysteine, S-allylmercaptocysteine, and

tetrahydro-β-carboline[6]. It is rich in superoxide dismutase–like activity, hydrogen peroxide (H_2O_2) scavenging activity, and polyphenols than normal garlic[6]. Therefore BGE has stronger in vitro and in vivo antioxidant activity than raw garlic and may have greater efficacy for preventing metabolic diseases and alcoholic hepatotoxicity[7,8].

Carbon tetrachloride (CCl4) is a hepatotoxic compound. The CCl4 has been used extensively in laboratory animals for induction of liver injury, elucidate the underlying mechanism of liver injury and hepatoprotective effects of various therapeutic agents[9]. One of the postulated mechanism of CCl4 induced liver injury is the formation of Reactive oxygen species (ROS). The ROS disrupts the hepatocyte at cell membrane level through the lipid peroxidation[9,10], causing anatomical disruption of liver architecture and physiological disturbances[11]. The hepatocyte injury causes leakage of cytoplasmic and mitochondrial enzymes in the blood streams[12]. The cytoplasmic and mitochondrial enzymes are clinically used as markers of liver injury, and for monitoring and treating the liver diseases also. The liver enzymes which appear in the blood as a result of liver injury include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) and are often employed in assessing liver injury[9,13].

The present study aimed to investigate the possible protective effects of black garlic extract (BGE) against Carbon tetrachloride (CCl4)-induced liver damage in experimental rat model.

MATERIAL & METHODS:

An experimental study was conducted at the animal house of Isra University on rat model over a period of six months, from May to September 2013. Sixty adult male Wistar rats of 250-300 grams were included in the study. Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chow diet and clean water were provided ad-libitum.

Animals and experimental design

The garlic was fermented in a thermo-hygrostat (model TH- 500 [patent pending], Hanyoung nux Co., Incheon, Korea) for 9 days maintaining proper temperature and humidity. The BGE was rendered black by fermentation. The BGE group was orally administered 100 mg/5mL of distilled water/kg of body weight. Carbon tetrachloride was purchased from scientific drug store at Hyderabad City. The CCl4 dissolved in olive oil as vehicle (1:1 Ratio) at a dose level of 1.9 ml/kg orally on alternate day for four weeks and sacrificed at the end of their respective period of time[13].

The rats were divided into three groups;
Group I. Control Group (n=20) Rats received 0.9% isotonic saline orally on alternate day for four weeks and served as control group.
Group II. Experimental Group (CCl4) (n=20) Rats were given CCl4 orally mixed in olive oil on alternate day for four weeks.
Group III. Experimental Group (CCl4+BGE) (n=20) Rats received BGE and CCl4 on alternate days for four weeks
After 4 weeks of administration of the test compound, 12-hour-fasted rats were anesthetized during the post absorptive period. Blood was drawn from the vena cava into heparin tubes centrifuged and stored at 4°C; the plasma was stored in a freezer for later assays.
Blood sample: The blood samples were collected from vena cava into EDTA tubes centrifuged and stored at 4°c; the plasma was stored in a freezer for later assays. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes and plasma lipids.
Liver enzyme: Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehydrogenase (LDH) using commercially available diagnostic kits.

Plasma lipid profile: Plasma triglyceride (TG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) were enzymatically determined using immunoassay kits (Asia Pharmaceuticals, Seoul) and an enzyme-linked immunosorbent assay reader (Pharmacia

Biotech, Cambridge, United Kingdom) according to the manufacturer's protocol. Plasma low-density lipoprotien-cholesterol (LDL-C) was calculated using the Friedewald equation.

Sacrifice of animals: The animals were sacrificed using standard method as described[11]. In order to examine the liver tissue, the liver of the sacrificed animals was removed promptly and preserved in formaldehyde.

Histopathology and Grading of tissue damage: After fixation in 10% formaldehyde, liver tissue slices were embedded by the paraffin slice technique, sections of 5μ thickness were subjected to haematoxylin and eosin. Hepatic morphology was assessed by light microscopy and photographed; later the microscopic slides of the liver sections were photographed.

In H & E staining, damaged hepatocytes graded as 0= normal, += mild damage (swollen and pale cytoplasm), ++= moderate damage (vacuolated cytoplasm), +++= severe damage and ++++= very severe damage (pyknotic nucleus and eosinophil cytoplasm)[12].

The data was analyzed on Statistix 8.1 (USA). The continuous variables were presented as mean±SD using one-way ANOVA and Bonferroni test. Chi-square test was used for categorical variables. A p-value of = 0.5 was taken statistically significant.

RESULT:

The present study observes major differences in liver injury between and among groups as indicated by blood enzyme levels in different animal groups. The ALT, AST, LDH, and ALP in serum of rats treated with carbon tetrachloride were found elevated compared with control group after four weeks, with a highly significant p-value for multiple comparisons (p=0.001). The CCl4+BGE group shows a significant reduction in the liver enzymes compared with the CCl4 group (p=0.001) and control group (p=0.001). The animals CCl4+BGE group shows significant reduction in the liver enzyme levels compared to CCl4 group alone (p=0.001). The finding shows significant hepatoprotection by the BGE in CCl4 induced injury. The liver enzyme assays among different groups are shown in table-1. Different parameters of histological score of liver injury are shown in Table-2. The Liver sections of the control group animals show intact central venules and hepatocytes arranged in compact cords. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm are seen in control group (Figure.1). On the contrary, the CCl4 group shows derangement of hepatocytes cords, hydropic changes with congestion of central venules and sinusoids, and abundant inflammatory cell infiltration (Figure.2). The centrilobular hepatocytes show hydropic changes and necrosis, while mid zonal and peripheral hepatocytes show vacuolar degeneration and fatty changes in CCl4 group (Figure.2). In CCl4+BGE animals, liver tissue sections reveal less significant derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl4 group (Figure.3).

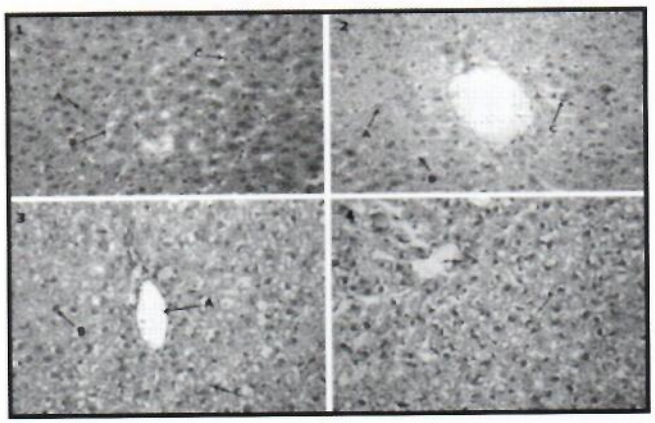

Figure-1. Liver slide of control group shows normal looking hepatocytes arranged in cords. Central vein (CV) is shown (A) separated by sinusoids

DISCUSSION:

The present study is an original research work, which investigates the effect of black garlic extract (BGE) on carbon tetrachloride (CCl4) induced liver injury in adult male Wistar rats. The Null hypothesis is rejected because the study observes hepatoprotective effects of BGE as evidenced by biochemical and histological marker of liver injury. The present study shows liver damage caused by the carbon tetrachloride as

Table. 1. Liver enzyme levels in controls, *CCl_4 and CCl_4+ Black garlic extract groups

Groups	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	ALP (IU/L)
Group. I (Controls)	48.7±3.29	92.1±15.91	713.5±47.8	95.5±7.98
Group. II (*CCl_4)	185.7±10.97	493.7 ±19.9	2768.9±137.6	171.1±7.02
Group. III (*CCl_4 + BGE†)	88.9±16.98	169.9±20.3	2148.6±141.3	135.8±17.15

*Carbon tetrachloride † Black garlic extract

Table. 2. Histology of liver injury of controls, *CCl_4 and CCl_4+ Black garlic extract groups

Groups	Congestion	Vacuolar degeneration	Inflammatory cell infiltrate	Necrosis
Group. I (Controls)	0	0	0	0
Group. II (*CCl_4)	++++	+++	++++	++++
Group. III (*CCl_4 + BGE†)	++	++	+++	++

*Carbon tetrachloride † Black garlic extract

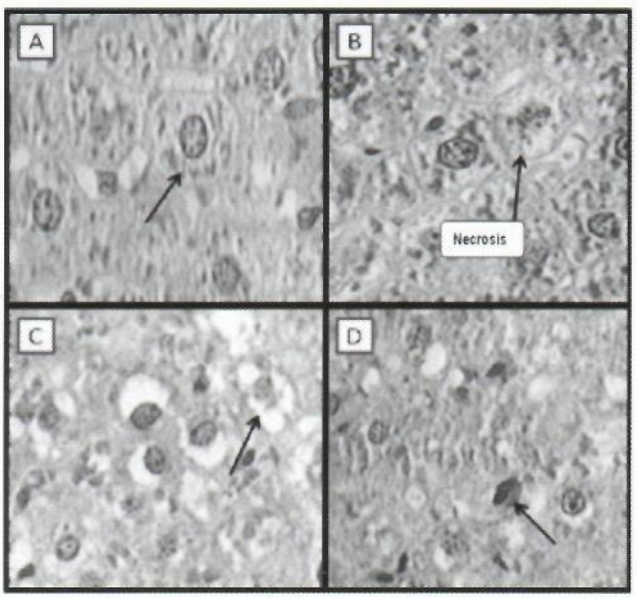

Figure-2. CCl4 group showing hydropic degeneration (arrow), inflammatory cell infiltrate and necrosis

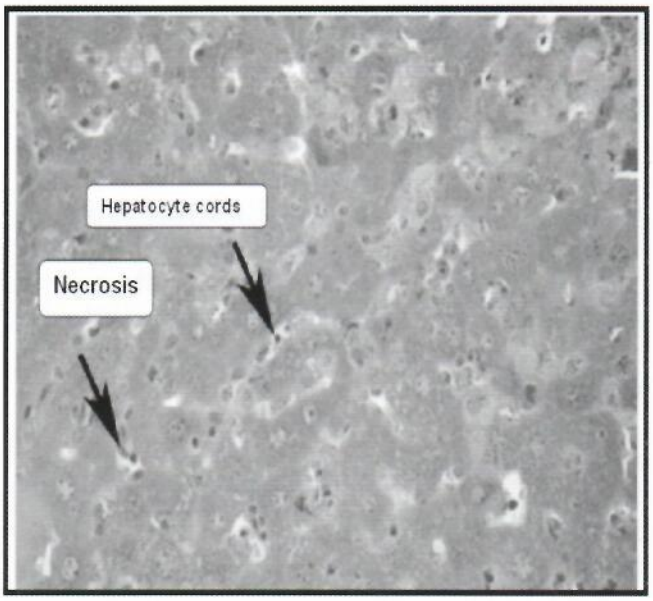

Figure-3. CCl4 + Black garlic extract group showing normal hepatocyte arranged in cords with congested sinusoids, and few lymphocytic infiltrations.

indicated by serum levels of liver enzymes compared to control group in rat model. The carbon tetrachloride induced liver injury with release of liver enzymes is comparable finding to reported previously by Hurkkeri et al[15]. The Hurkkeri[15] reported elevated hepatocyte enzyme of liver as a consequence of CCl4 induced liver injury in animal model. The release of large quantities of cytoplasmic and mitochondrial enzymes of liver is a clinical indicator of hepatocyte cell membrane damage and rupture that to produce change in enzyme levels in blood. The histological examination of present research study correlates in parallel to disturbance in biochemical markers of liver injury. The histology of liver tissue shows disruption of liver architecture, hepatocytes, hepatic lobules and derangement of hepatocytes in cords. The hepatocytes show findings of cellular injury with marked cytoplasmic vacuolization. The injured hepatocytes show pyknotic nuclei with lymphocyte infiltrations. The pyknotic nuclei are a sign of severe cellular injury caused by carbon tetrachloride. The Kim et al[14] conducted study on rat model and reported that the aged black garlic exerts strong antioxidative properties and may be a promising agent for protecting against chronic alcohol induced liver damage. The results of present study are highly consistent with previous report of Kim et al[14].

Antioxidative compounds in BGE might inhibit the oxidative attack of endogenously produced ROS after ethanol exposure. This result is comparable to that of an earlier study[16] in which an antioxidant-rich vegetable and fruit diet containing vitamin C, vitamin E, and ß-carotene significantly reduced the DNA tail moment and induced a significant decrease in oxidative DNA damage, finally leading to the resistance of lymphocyte DNA to oxidative damage.

The histological and biochemical findings of present study are comparable to those mentioned previously[16-20]. The carbon tetrachloride is metabolized to free radical during its metabolism and detoxification in smooth endoplasmic reticulum by the cytochrome P450[17-20].

CONCLUSION:

The black garlic extract protects against oxidative damages of liver caused by carbon tetrachloride in rat model. The black garlic extract may be used as an effective protector against chemical induced liver damages; however, further studies on humen being are needed.

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