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Hepatoprotective Activity of Allicin Against Carbon Tetrachloride Induced Hepatic Injury in Rats

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Abstract: Oral treatment of rats with carbon tetrachloride (CCl₄) have induced severe histopathological changes in the rat liver tissues characterized with proliferation of both inter and intralobular fibrous cells and around the branches of central vein (central cirrhosis), accompanied by fatty changes and ballooning degeneration or foamy cytoplasm of the hepatocytes, with focal necrosis and hypertrophy in some nuclei. Moreover, bile duct adenocarcinoma and tumor giant cells were prominent findings after four weeks of CCl₄ treatment, where tumor cells showing pleomorphism and mitosis. In addition, telangectasis was seen in some cases especially in the subcapsular area of the liver. In this study, allicin, an organosulfur compound derived from garlic, was orally administered to a group of animals treated with CCl₄ to inhibit the development of deleterious pathological changes in liver tissues of such intoxicated animals. Such group of animals, the liver specimens revealed focal areas of ballooned cells instead of diffused cells in group received CCl₄ only. Also the central cirrhosis became dispersed instead of compact fibrous tissue. The perturbations changes were completely absent at the 4th week of administration and hepatocytes became more organized in hepatic cords. Bile duct adenocarcinoma and tumor cells that found in liver tissues of rats administered CCl₄ alone for four weeks were not noticed when CCl₄ given together with allicin.

Key words: Hepatoprotection, allicin, CCl₄ intoxication, liver tissues, rats

INTRODUCTION

Carbon tetrachloride is a clear, sweet-smelling solvent that has been used extensively in the past either as a non-flammable degreasing agent, a dry-cleaning fluid, a fabric-spotting fluid, a fire-extinguisher fluid, a grain fumigant or as a reaction medium (DeShon, 1979). Oral exposure to such hazardous chemical has been observed to result in a wide spectrum of adverse structural effects on the liver tissues such as cloudy swelling, extreme hydropic changes, necrotic lesions, binucleated cells, balloon cells, enlarged pyknotic nuclei, centrilobular necrosis, lipidosis and/or neutrophil infiltration and eventually fibrosis and cirrhosis (Allis *et al.*, 1990; Wolfgang *et al.*, 1990; Blair *et al.*, 1991; Fischer-Nielsen *et al.*, 1991; Waterfield *et al.*, 1991; Weber *et al.*, 1992; Guo *et al.*, 2000; Song and Yen, 2003). Furthermore, Blair *et al.* (1991), Hsu (1998) and Boll *et al.* (2001) stated that carbon tetrachloride has also an adverse ultra structural effects on the liver, the most prominent of which are destruction of the smooth and rough endoplasmic reticulum and its associated enzyme activities (Reynolds and Yee, 1968), reduction of Golgi complexes and mitochondrial figures in

the cytoplasm (Juñnila *et al.*, 2000) inhibition of protein synthesis (Lutz and Shires, 1978; Weber *et al.*, 2003), impaired secretion of triglycerides with resultant fat accumulation (Recknagel and Glende, 1973; Fischer-Nielsen *et al.*, 1991; Waterfield *et al.*, 1991). In general, carbon tetrachloride induces liver tumors which were either hepatomas or hepatocellular carcinomas (both neoplasms and carcinomas) (Della Porta *et al.*, 1961; Costa *et al.*, 1963; Reuber and Glover, 1970).

Garlic acquired a reputation in the folklore of many cultures over centuries as a formidable prophylactic and therapeutic medicinal agent. Garlic has attracted particular attention of modern medicine because of its widespread health use around the world and the cherished belief that it helps in maintaining good health warding off illnesses and providing more vigor.

The characteristic compounds of the garlic bulb include a complex series of sulfur substances. It contains high levels of phosphorus, potassium, sulfur and zinc; moderate levels of selenium, vitamin A and C and low levels of calcium, magnesium, sodium, iron, manganese and B-complex vitamins. In the whole bulb the principal sulfur compound is alliin (85%) and to a lesser degree,

isoalliin (5%) and methyl alliin (10%). These are completely odorless. Opening the bulb frees an enzyme alliinase which rapidly transforms the three compounds into their respective sulfenic acids. These acids are responsible for the characteristic odor of garlic. A series of spontaneous chemical reactions is then triggered leading to the formation of tens of diverse thiosulfates (THS), such as allicin (70%), allyl methane (THS) (12%), trans-1-propenyl-2-propene THS (6%), methyl-2-propene THS (6%), methyl methane THS (2%), allyl trans-1-propene THS (2%) and smaller percentage of other compounds. From allicin, a series of vinylthiols and diallyl disulfurs and trisulfurs, such as diallyl sulfur (DAS), diallyl disulfur (DADS), diallyl trisulfur, allyltrimethylsulfur and ajoene are endowed with special biological activities (Woodward, 1996).

Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl sulfides. However, it was not until 1944 that Cavallito and his colleagues (Cavallito and Bailey, 1944) isolated and identified the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur molecule, which they termed allicin, from the Latin name of the garlic plant, *Allium sativum*. Allicin, a diallylsulfinothiolate which imparts much of garlic's pungent characteristics (Stoll and Seebach, 1951), is considered to be the precursor compound from which other thioallyl compounds are derived (Block, 1985).

Experimental investigations performed by Dorant *et al.* (1993) and Milner (1996) have implicated specific thioallyl constituents and their derivatives regarding the anti-cancer actions of garlic.

Reicks and Crankshaw (1996) showed that garlic organosulfur compounds exert chemo-preventive effects at several organ sites in rodents after administration of chemical carcinogens, possibly by inhibiting carcinogen activation via cytochrome P-450-mediated oxidative metabolism. Allicin was shown to scavenge hydroxyl radicals and prevent lipid peroxidation in liver homogenates (Parsad *et al.*, 1995). The garlic components DAS, DADS and DAT were shown to play a differential modulatory role on the GSH related antioxidant system in rat liver and red blood cells and increased hepatic GST activity (Wu *et al.*, 2001). Protective effect of garlic extracts on hepatic lipid peroxidation during N-methyl-N-nitroso-N-nitrosoguanidine (MNNG) induced gastric carcinogenesis in Wistar rats was considered to be due to enhanced levels of glutathione and glutathione dependent enzymes noted in garlic treated group (Arivazhagan *et al.*, 2000).

Garlic treatment inhibited the development of murine transitional cell carcinomas significantly (Riggs *et al.*, 1997; Radaeva *et al.*, 2004). Moreover Samaranayake *et al.* (2000) reported that garlic inhibited diethylnitrosamine (DEN) induced hepato-carcinogenesis in Wistar rats. Also, Shobana and Naidu (2000), Chang and Chen (2005) and Chiang *et al.* (2005) reported that several spices including garlic possess antioxidant activity, thereby in addition to imparting flavor to food; they provide health benefits by inhibiting lipid peroxidation. An investigation on the scavenging property of garlic preparations against oxygen radicals in human granulocytes, activated with Phorbol Myristyl Acetate (PMA), suggested alliin metabolite (allicin) to be responsible for the oxygen radical scavenging property of garlic (Siegers *et al.*, 1999; Borek, 2001).

Administration of the therapeutic dose of garlic induced slight cytoplasmic granulation in some hepatic cells. However, administration of double the therapeutic dose caused swelling, necrosis and damage of the gastric glandular epithelia together with signs of erosion, exfoliation and necrosis of the surface mucosal cells. It also induced swelling and coalescence of the hepatic cells, loss of the normal arrangement of the hepatic cords and hypertrophy of Kupffer cells (Rahmy and Hemmaid, 2001).

However, no publications were encountered regarding the effect of Allicin ingredients on the histopathological changes in liver of carbon tetrachloride intoxicated animals, thus this research was designed to assess the effect of allicin as a natural products on the deleterious histological changes in the liver of CCl₄-intoxicated rats.

MATERIALS AND METHODS

Seventy-two white adult males albino rats (*Rattus norvegicus*), weighing about 130-190 g, were used as experimental animals in this investigation. Animals were kept under observation for about 10 days before the onset of the experiment to exclude any incipient infections. The animals were housed in stainless steel cages in the departmental animal house at normal temperature and given enough food and water. Animals were divided into four groups designated as follows:

Group I (Normal control group): Rats of this group were given corn oil six days per week by gastric gavage, at a dose level of 2.5 mL kg⁻¹. body weight (b.wt.)

Group II (CCl₄ control group): Rats of this group were treated orally three days per week with 0.25 mL kg⁻¹.

b.wt./day (MacDonald *et al.*, 1986; Koporec *et al.*, 1995; Tanaka, 2001) carbon tetrachloride, obtained from ADWIC company and produced by EL-Nasr Pharmaceutical Chemical Company in Egypt, suspended in corn oil (2.5 mL).

Group III (CCl₄ group treated with allyl disulfide): Rats included in this group were treated orally six days per week with allicin (2-propene-1-sulfinothioic acid S-2-propenyl ester), obtained from Aldrich Company, at a dose level of 100 mg kg⁻¹. b.wt/day (Augusti and Mathew, 1974) dissolved in corn oil and carbon tetrachloride at dose level 0.25 mL kg⁻¹ b.wt., three days per week.

Group IV (normal group treated with allicin): Rats included in this group were treated orally six days per week with allicin at a dose level of 100 mg kg⁻¹. b.wt. dissolved in the same volume of corn oil as control.

At the end of the specific time intervals (2nd, 3rd and 4th weeks of treatments), six animals were sacrificed after administration of the tested compounds (normal control group, CCl₄ control group, CCl₄ group treated with allicin and normal group treated with allicin rats) under diethyl ether anesthesia and small pieces of liver tissues were fixed in neutral buffered formalin and processed for sectioning and stained with haematoxylin and eosin for histopathological studies.

RESULTS

Normal control group: Figure 1a and b shows the normal architecture of rat liver which appeared consisting of numerous hepatic lobules (classical lobules) and connective tissue septa in between. However, these septa are not well conspicuous so as to the liver sinusoids appear continuous from one lobule to another. In the center of each of these hepatic lobules, there is a branch of central vein (cv). Radiating from the central vein toward the periphery of the lobules are plates of hepatic cells or trabeculae (t), locating in between the hepatic sinusoids (s) and Kupffer cells (kc) (Fig. 1b and c).

The portal areas containing the interlobular branches of the portal veins (pv), hepatic arteries (a) and bile ducts (bd) are clearly observed around the peripheries of different lobules (Fig. 1c).

The CCl₄ group: The distinctive microscopic feature in the liver sections of rat after 2 weeks administration of CCl₄ showed proliferation of both inter and intralobular fibrous connective tissue cells together with massive

diffused hepatocellular ballooning (Fig. 2a). Also, the cytoplasm was either vacuolated and or foamy degenerated, while the nuclei were either pyknotic or completely absent (Fig. 2b).

The examination of liver sections post 3 weeks CCl₄ administration showed massive vacuolation in hepatocytes with fibrous proliferation around the branches of central vein (central cirrhosis) (Fig. 3a). The proliferating, fibrous cells seemed compact and dense and vary in shape from stellate or oval to round (Fig. 3b). Some nuclei of the hepatocytes were markedly enlarged (Fig. 3c). Liver section also showed macrophages laden with hemosiderin pigments in some cases (Fig. 3d).

Ballooning and/or vacuolation together with focal necrosis and hypertrophy in some nuclei were the prominent feature in the liver sections taken from rat received CCl₄ for 4 weeks (Fig. 4a). The lesions appeared much severe near to the portal areas with focal mononuclear leucocytes inflammatory cells infiltrating near to the portal triad in addition to hemorrhage (Fig. 4b). In the same group, the livers of some rats showed central cirrhosis beside the previous mentioned lesions (Fig. 4c). Giant hepatocytes, which contain one or more enlarged nuclei, were the obvious feature in other cases (Fig. 4d). Moreover, bile duct adenocarcinoma was a prominent finding in this group. The adenocarcinoma characterized by infiltration of the tumor cells between hepatocytes and between the hepatic lobules. Also, tumor giant cells could be observed in the same liver (Fig. 4e). The tumor cells showed pleomorphism and mitosis (Fig. 4f). In addition, telangectasis was seen in some cases especially in the sub-capsular area of the liver (Fig. 4g and h).

The group-received allicin only: The liver sections did not show obvious alterations 2 or 3 weeks allicin post administration (Fig. 5a). At the 4th week, the liver tissue did not show marked changes other than Kupffer cell (kc) proliferation and activation (Fig. 5b and c).

The group received allicin and CCl₄: The examined liver sections after 2 weeks post administration revealed focal areas of ballooned cells especially around the branches of central vein, instead of diffused cells in group received CCl₄ only (Fig. 6a). Also the central cirrhosis became dispersed instead of compact fibrous tissue in the group received CCl₄ only (Fig. 6b). The vacuolation or ballooning of the hepatocytes and central cirrhosis were minimal by the 3rd week of administration (Fig. 7), while such changes were completely absent by the 4th week and the hepatocytes became more organized in hepatic cords (Fig. 8).

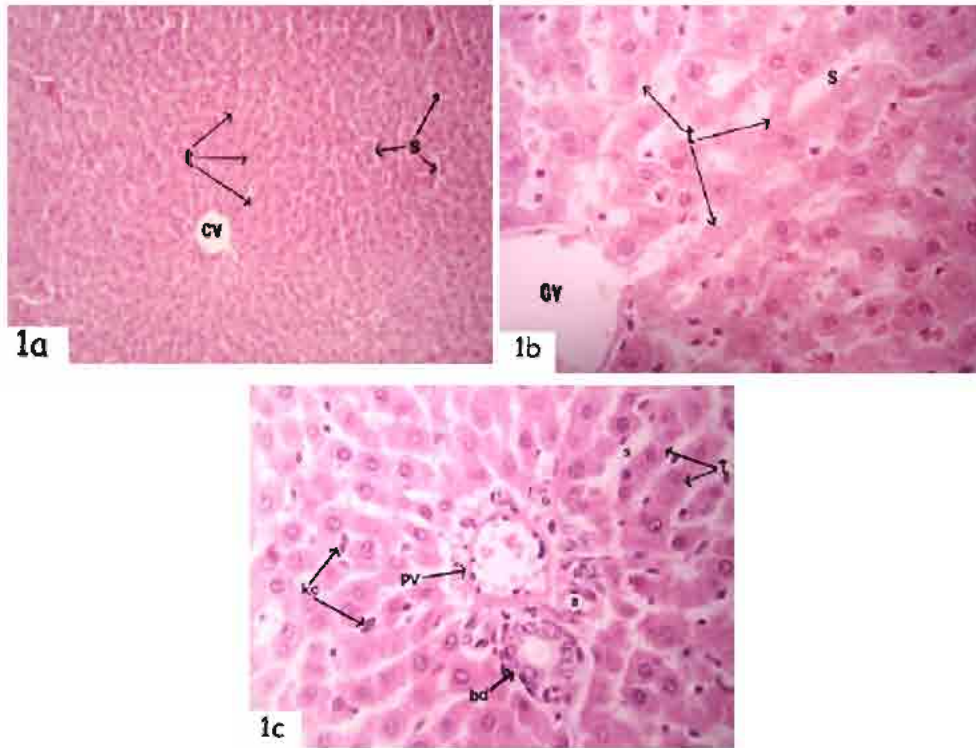


Fig. 1a: Normal control rat liver section showing the normal architecture by low magnification. (CV): central vein branch (t) trabeculae or hepatic strands (s) sinusoids. H. & E. X 100
b: High magnification of the normal control rat liver section showing central vein (CV) & hepatocytes arranged in hepatic strands or trabeculae (t) radiating from the central vein with sinusoids (s) in between. H. & E. X 400
c: A section of a liver of normal control rat showing the portal triad forming of branches of portal vein (pv), hepatic arteries (a) and bile duct (bd). Kupffer cells (Kc), trabeculae (t) and sinusoids (s) are also seen. H. & E. X 400

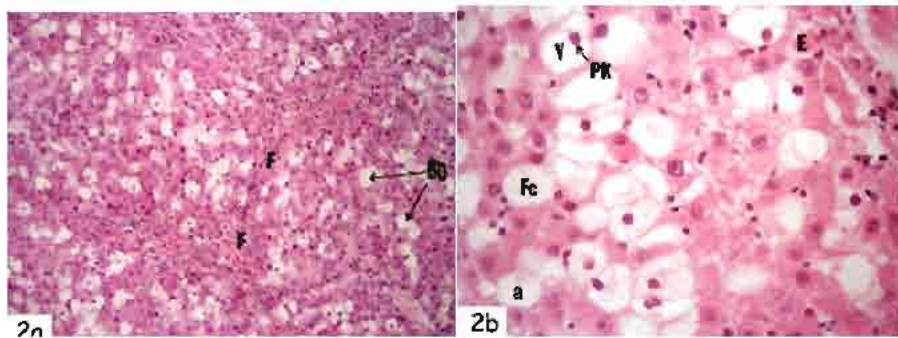


Fig. 2a: A section of a liver of rat received CCl_4 for 2 weeks showing massive hepatocellular ballooning (BO) and proliferation of inter and intralobular fibrous connective tissue cell (F). H. & E. X 100
b: Higher magnification of the last figure, showing either vacuolated cytoplasm (V) or foamy cytoplasm (Fc) where the nuclei may be pyknotic (PK) or completely absent (a). Also, eosinophilic bodies could be seen (E). H. & E. X 400

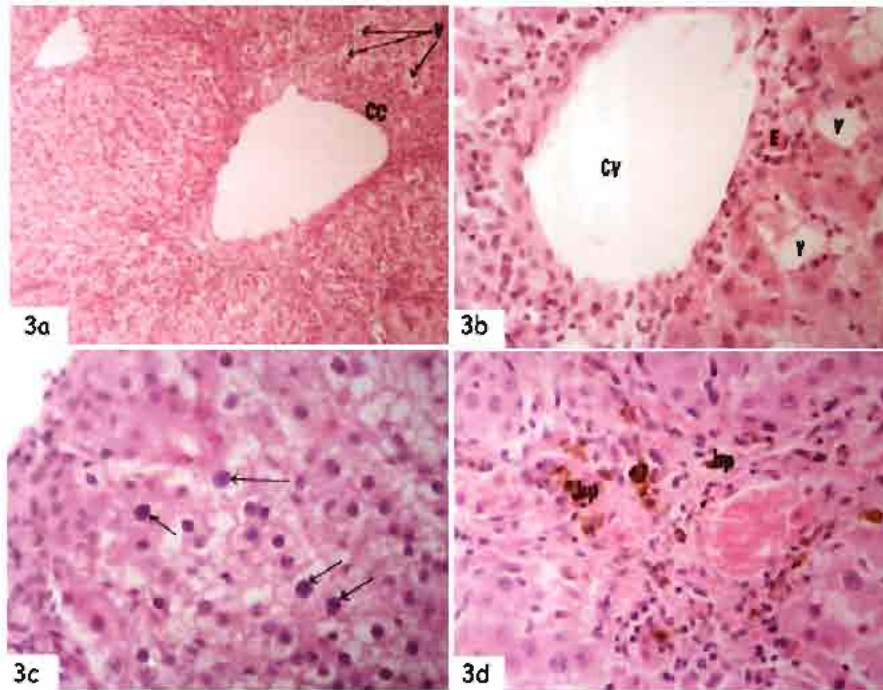


Fig. 3a: A liver section of rat received CCl_4 for 3 weeks showing massive vacuolation (V) and central cirrhosis (CC). H. & E. X 100
b: Higher magnification of fig. 3a showing vacuolation (V), eosinophilic bodies (E), oval, stellate and round fibrous cells around the central vein (CV). H. & E. X 400
c: A liver section of rat received CCl_4 for 3 weeks showing massive vacuolation (V) and enlarged nuclei (arrow). H. & E. X 400
d: A photomicrograph of a liver section of rat received CCl_4 for 3 weeks showing numerous macrophages laded by hemosidrin pigments (hp). H. & E. X 400

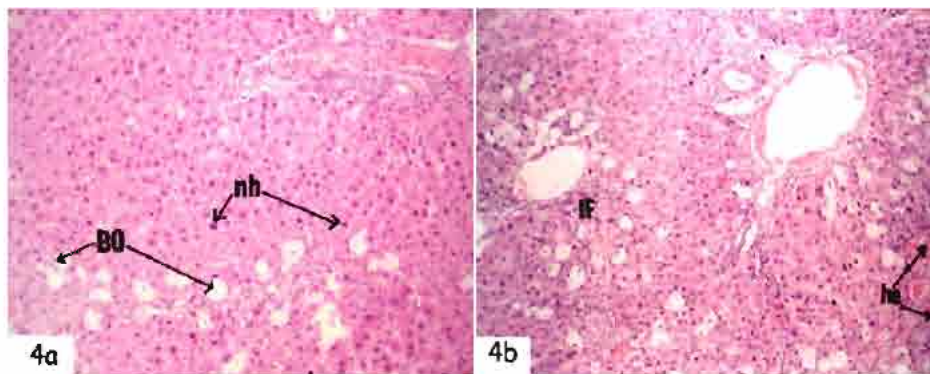


Fig. 4a: Liver section from rat received CCl_4 for 4 weeks showing focal necrosis (n), nuclear hypertrophy (nh) and hepatocellular ballooning (BO). H. & E. X 100
b: A Liver section from rat received CCl_4 for 4 weeks showing much severe alterations with focal mononuclear leucocytes inflammatory cells infiltrating (IF) near to the portal triad in addition to hemorrhage (he). H. & E. X 100

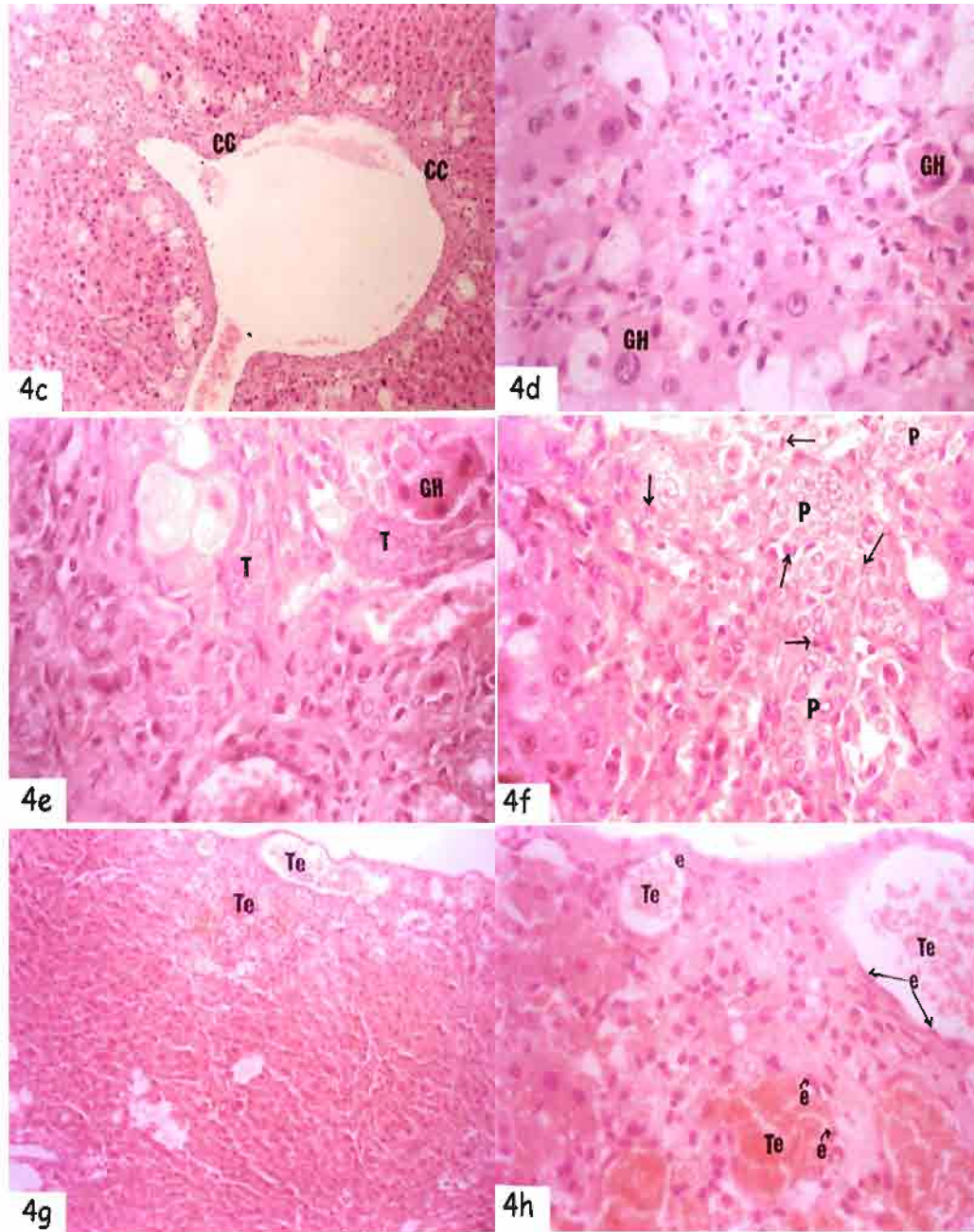


Fig. 4c: A Liver section from rat received CCl_4 for 4 weeks showing condensed central cirrhosis (CC). H. & E. X 100
d: A Liver section from rat received CCl_4 for 4 weeks showing giant hepatocytes (GH). H. & E. X 400
e: A Liver section from rat received CCl_4 for 4 weeks showing infiltrated tumor cells between hepatocytes and hepatic lobules (T). Also, the figure shows tumor giant hepatocytic cells (GH). H. & E. X 400
f: A Liver section from rat received CCl_4 for 4 weeks showing pleomorphism (P) and mitosis (arrow). H. & E. X 400
g: A Liver section from rat received CCl_4 for 4 weeks showing telangectasis especially in the sub-capsular area (Te). H. & E. X 100
h: Higher magnification of Fig. 4g showing telangectasis (Te) lined by endothelial cells (e). H. & E. X 400

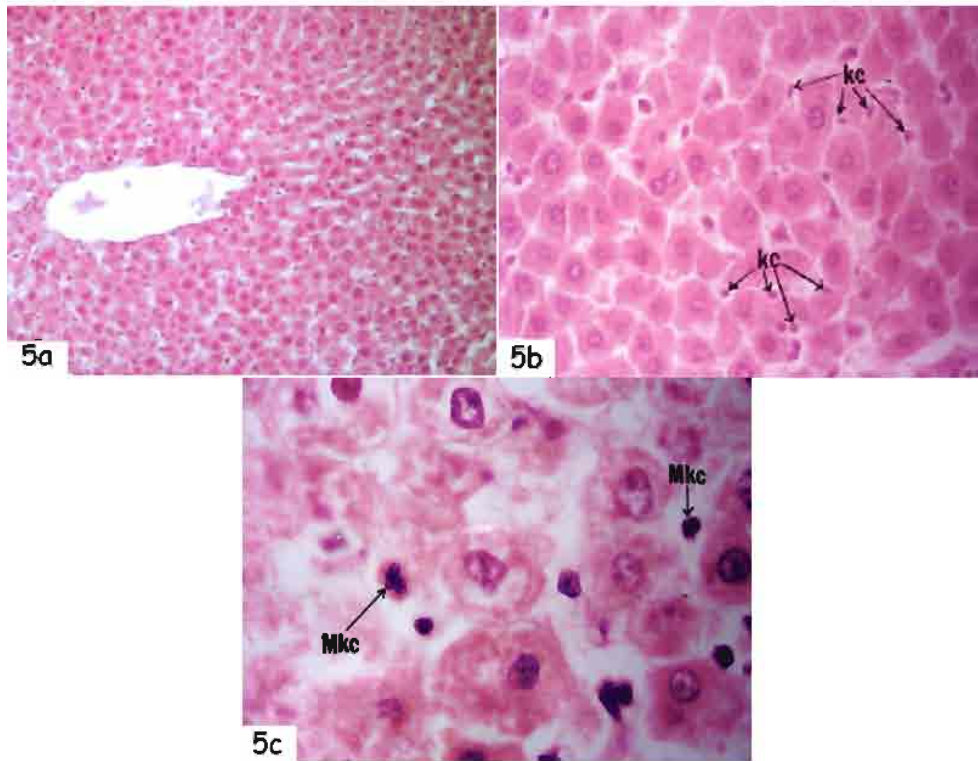


Fig. 5a: A liver section of rat received allicin only for 2 or 3 weeks, showing nearly normal hepatic arrangement of the hepatic strands without any obvious alterations. H. & E. X 200
b: A liver section of rat received allicin only for 2 or 3 weeks, showing Kupffer cells (Kc) proliferation and activation. H. & E. X 400
c: Higher magnification of Fig. 5b showing mitotic activity in Kupffer cells (MKc). H. & E. X 1000

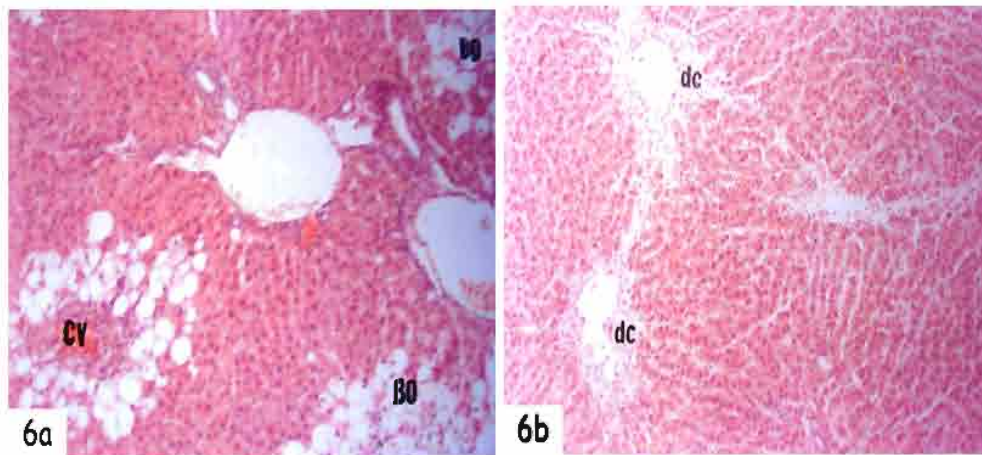


Fig. 6a: A liver section of rat received CCl_4 and allicin for 2 weeks showing focal areas of ballooned cells (BO) especially around the branches of central veins (CV). H. & E. X 100
b: A liver section of rat received CCl_4 and allicin for 2 weeks showing dispersed central cirrhosis (dc). H. & E. X 100

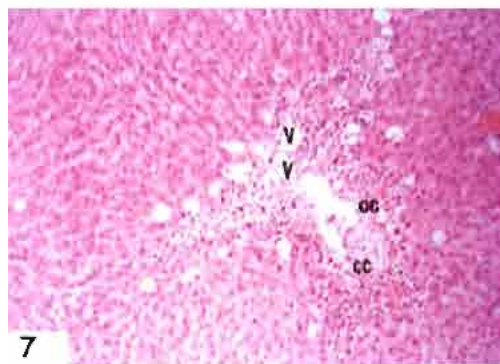


Fig. 7: A liver section of rat received CCl_4 and allcin for 3 weeks showing minimal vacuolation (V) and central cirrhosis (CC). H. & E. X 100

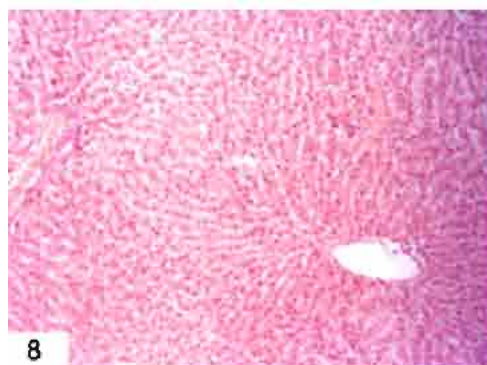


Fig. 8: A liver section of rat received CCl_4 and allcin for 4 weeks showing normal organized hepatic cord. H. & E. X 100

DISCUSSION

Several reports have been published on the effect of garlic upon the deleterious alterations produced by various hepatotoxic substances.

In the present study, we have evaluated the effect of allcin, an organosulfur compound derived from garlic, on rats treated with carbon tetrachloride as a hepatotoxic agent.

The present results indicated that oral treatment of rats with carbon tetrachloride (CCl_4) induced severe histopathological changes in the liver tissues characterized with proliferation of both inter and intralobular fibrous cells especially around the branches of central vein (central cirrhosis), ballooning and/or vacuolation together with focal necrosis and hypertrophy in some nuclei. Moreover, bile duct adenocarcinoma and tumor giant cells were prominent findings in such intoxicated liver sections, where tumor cells showing pleomorphism and mitosis. In addition, telangiectasis was seen in some cases especially in the sub-capsular area of the liver.

Song and Yen (2003) revealed that oral gavage of CCl_4 to albino rats induced a large area of necrosis and fibrosis in liver tissue. Ferezza *et al.* (1993) depicted that the treatment of rats with CCl_4 induced liver cirrhosis and hepatocellular carcinoma. Ashok Shenoy *et al.* (2001) showed that the sections of liver of CCl_4 treated rats exhibited hydropic changes in centrilobular hepatocytes with single cell necrosis, congestion in branches of central vein and sinusoids associated with chronic inflammatory cells infiltration. Hewawasam *et al.* (2004) reported that there is centrilobular necrosis accompanied by fatty changes and ballooning degeneration in the remaining hepatocytes in the mice liver specimens treated with carbon tetrachloride. Altug and Agaoglu (2000) noticed necrosis in the centrilobular and midzonal regions in the liver of acute CCl_4 -administered dogs and fibrosis in the portal region and around the central vein in the chronic treated animals. Suja *et al.* (2004) demonstrated that liver of CCl_4 -intoxicated rats showed fatty changes, gross necrosis, broad infiltration of lymphocytes and Kupffer cells around the central vein and loss of cellular boundaries. Youssef (2000) reported that in the early

weeks of CCl₄ treatment, liver tissues showed typical centrilobular coagulative necrosis and fatty changes, but during the last 3 weeks of his experiment, CCl₄-induced necrosis was more extensive and associated with fibrotic response. These observations are in accordance with the present ones of this study.

The present study are also concomitant with those of Itoh (1988) and Cinar *et al.* (1999) who revealed that necrosis observed in the hepatocytes of centrilobular area of the liver of acutely CCl₄-intoxicated rabbit and increases of connective tissue masses around central vein leading to fibrosis and cirrhosis in the chronically intoxicated animals. These latter observations were evidenced in this study after the 4th week of CCl₄ administration.

Various mechanisms have proposed for CCl₄-inducing liver injury. Most of them suggesting that the hepatic injury produced by CCl₄ is mediated by its reactive metabolites (trichloromethyl free radical or trichloromethyl peroxy free radical) catalyzed by cytochrome P-450 dependent monooxygenase (Recknagel *et al.*, 1989; Azri *et al.*, 1991; Hughes *et al.*, 1991; Wong *et al.*, 1998). The deleterious histological effect of CCl₄ on liver is thought to be due to the binding of these free radicals to centrilobular hepatocytes, which in turn initiates lipid peroxidation and protein oxidation in liver resulting in widespread membrane damage and necrosis (Sheweita *et al.*, 2001). In response to the parenchymal cell damage, perisinusoidal cells or stellate cells may be stimulated to release extracellular proteins that contribute to hepatic fibrogenesis which at least may be mediated by hepatic macrophages (Kupffer cells) production of peptide growth factors (Belyaev *et al.*, 1992; Ishiki *et al.*, 1992; Johnson *et al.*, 1992). Another factor that may be of importance in CCl₄-histological perturbations is the disturbance of normal cellular calcium homeostasis following exposure. Judah *et al.* (1966), Long and Moore (1987) and Kodavanti *et al.* (1990) suggested that early increase in cytoplasmic calcium after CCl₄ administration results in altered membrane function (perhaps, especially at plasma membrane) which ultimately results in irreversible changes that produce cellular necrosis.

In view of the carcinogenicity of CCl₄, it was reported that the reactive metabolites of CCl₄ appear capable of binding to targets of putative relevance to cancer induction (chromosomal DNA and nucleosome proteins) and may even be generated within the nucleus itself (ATSDR Toxicological Profiles, 1999). Malondialdehyde (MDA), a product of lipid peroxidation (a process which

can be induced by CCl₄), may also be involved in CCl₄-induced carcinogenesis. MDA-initiated tumors have been also reported in Swiss mice by Shamberger *et al.* (1974).

Regarding the allicin effect in this study, the liver did not show obvious alterations in the second and third week of its administration. At the fourth week, the liver did not show marked changes other than Kupffer cell proliferation and activation.

As a result of treatment with allicin of CCl₄-intoxicated rats in this study, the liver revealed focal areas of ballooned cells instead of diffused cells in group received CCl₄ only. Also the central cirrhosis became dispersed instead of compact fibrous tissue in CCl₄ group. Such changes were completely absent at the 4th week and hepatocytes became more organized in hepatic cords. Bile duct adenocarcinoma and tumor cells that found in liver tissues of rats administered CCl₄ alone at the 4th week were not noticed when CCl₄ given together with allicin. These findings are in agreement with Turkdogan *et al.* (2003) who found that *Nigella sativa*, known to have antioxidant activity like allicin, prevents coagulative necrosis and hydropic degeneration in the periacinar region and in the portal tracts that occurred as a result of CCl₄ treatment. The present results are also in agreement with the findings of others who reported that allicin and organosulfur compounds found in garlic have an anti-tumor and anti-cytotoxic action *in vitro* or in animals' models (Hayes *et al.*, 1987; Wargovich *et al.*, 1988; You, 1988; Dausch and Nixon, 1990).

Several mechanisms have been proposed to explain the cancer-preventative effects of *Allium* vegetables and related organosulfur compounds including allicin. These include inhibition of mutagenesis, modulation of enzyme activities, inhibition of DNA adduct formation, free radical scavenging capability and effects on cell proliferation and tumor growth (Bianchini and Vainio, 2001). Organosulfur compounds have been shown to modulate the activity of glutathione S-transferase (GST), a family of enzymes important in detoxification of carcinogens and cytochrome P-450 (CYP), a family of enzymes that activate many chemicals carcinogens in experimental animals (Sparmins *et al.*, 1988; Brady *et al.*, 1991; Bianchini and Vainio, 2001). Inhibition of tumor cell proliferation by organosulfur compounds has been also reported in several studies using cell culture (Welch *et al.*, 1992; Milner, 1996; Sakamoto *et al.*, 1997).

In conclusion, it was found that allicin have a potent protective effect against hepatotoxicity produced by carbon tetrachloride (CCl₄) administration to the albino rats, where it produced marked amelioration of the liver

histological perturbations in CCl₄-intoxicated animals. The alleviations of these deleterious effects by allicin may be mediated via improvements in the antioxidant defense system.

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