RESEARCH ARTICLE

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REPAIR OF CARBON TETRACHLORIDE-INDUCED RAT LIVER INJURY BY GINGER EXTRACT (ZINGIBER OFFICINALE)

ABSTRACT:
Carbon tetrachloride is the organic compound that is widely used in fire extinguishers, as a precursor to refrigerants, and as a cleaning agent. The medicinal properties of Ginger (Zingiber officinale) are attributed to its anti-arthritic, anti-migraine, anti-thrombotic, anti-inflammatory, hypolipidaemic, hypocholesterolaemic, and anti-nausea properties. Treating albino rats with Carbon tetrachloride induced various histological changes in the liver. These changes include congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and pyknosis. Administration of carbon tetrachloride decreased the total serum protein and globulin, besides it increased the serum albumin value and significantly increased the albumin/globulin ratio. Treating animals with ginger extract ameliorate the total protein, albumin, globulin, and the ratio of albumin to globulin. Moreover, ginger led to an improvement in the histological liver picture. The results of the present work indicated that the ameliorative effect of ginger against liver damage induced by Carbon tetrachloride is mediated by its potent antioxidant activities.

INTRODUCTION:
Carbon tetrachloride (CCl₄) is a well-known environmental hepatotoxin pollutant, and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals (Ulicná et al., 2003; Manna et al., 2006). CCl₄ metabolism produces a profound oxidative stress through its metabolism to trichloromethylperoxy (CCl₃OO) and to trichloromethyl (CCl₃) that damage cell membrane and causes lipid peroxidation (Pišťiková, 1991).

It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Recknagel, 1967).

Ginger (Zingiber officinale) is a perennial plant with narrow, bright green, grass-like leaves and yellowish green flowers with purple markings. Ginger is cultivated in the tropics for its edible rhizome at approximately 10 months of age, with the root stocks serving a variety of purposes, including culinary and medicinal (Grant, 2000; Ursell, 2000; Portnoi et al. 2003). The efficacy of ginger is reported to be a result of its aromatic, carminative and absorbent properties (Govindarajan, 1982 a&b).

Ginger is known to contain a number of potentially bioactive substances, mainly gingerols and their related dehydration products, the shogaols, as well as volatile oils including sesquiterpenes, such as b-bisabolene and (-)-Zingiberene, and monoterpenes, mainly geranial and neral.

KEY WORDS:
CCl₄, Liver, Rat, Histopathology, Albumin, Globulin, Albumin/Globulin ratio.

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(Ursell, 2000; McKenna et al., 2002). In particular, gingerols have been shown to inhibit both prostaglandin and leukotriene biosynthesis (Kiuchi et al., 1992) and angiogenesis (Kim et al. 2005). In addition, several ginger components exhibit serotonin receptor-blocking activity (Huang et al., 1991; Abdel-Aziz et al., 2005).

Lu et al. (2003) studied the antioxidative activity and protective effect of ginger oil on DNA damage. They found that ginger oil has dominantive protective effect on DNA damage induced by H2O2. Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant. The hepatoprotective activity of aqueous extract of Zingiber officinale was evaluated by Ajith et al. (2007) against single dose of acetaminophen-induced acute hepatotoxicity in rat. They concluded that the hepatoprotective effect of ginger is mediated either by preventing the decline of hepatic antioxidant status or due to its direct radical scavenging capacity. Verma and Asnani (2007) investigated the effect of paraben (p-hydroxybenzoic acid) on acidic, basic, and neutral proteins content, as well as carbohydrate and cholesterol contents in liver and kidney of mice. They reported a significant reduction in protein types, carbohydrate contents and an increase in cholesterol content in liver and kidney. The authors suggested that the oral administration of aqueous extract of Z. officinale along with paraben caused significant amelioration in all the protein types, carbohydrate and cholesterol of liver and kidney.

Kadnur and Goyal (2005) found that fructose supplementation produced a significant elevation in body weight, while treatment with methanolic extract of dried rhizomes of Zingiber officinale led to reduction in fructose and elevation in body weight. Al-Amin et al. (2006) studied the hypoglycaemic potentials of ginger in rats and found that, streptozotocin-injected rats exhibited hyperglycaemia accompanied with weight loss; ginger- treated diabetic rats sustained their initial weights during the treatment period.

Goyal and Kadnur (2006) suggested that goldthioglucose induces a significant increase in body weight of mice, while treatment with methanol and ethyl acetats of ginger extracts produced significant reduction in body weight. York et al. (2007) studied the effectiveness of Chinese herbal extract number ten (NT) in dietary formulation prepared from rhubarb, ginger, astragalus, red sage and turmeric and demonstrated that NT reducing weight gain in rodents. Also, Roberts et al. (2007) demonstrated that NT combined with garlic acid was ineffective in causing weight loss or in suppressing food intake.

Ajith et al. (2007) reported that ginger ameliorated cisplatin-induced nephrotoxicity and this protection is mediated either by preventing the cisplatin-induced decline of renal antioxidant defence system or by their direct free radical scavenging activity.

So, the present study aims to evaluate the ameliorative effect of aqueous ginger extract on the reduction of pathological injuries induced by CCl4 administration.

MATERIAL AND METHODS:

Extract preparation:

Aqueous ginger extract was prepared from locally available ginger roots. The ginger roots were peeled on crushed ice, and 50 g ginger were cut into small pieces and homogenized in 75 ml cold, sterile 0.9 % NaCl in the presence of some crushed ice. The homogenisation was carried out in a blender at high speed using 2 min bursts for a total of 12 min. The homogenised mixture was filtered three times through cheesecloth. The filtrate was centrifuged at 2000 Xg for 10 min and the clear supernatant fraction was made up to 100 ml with normal saline. The concentration of this ginger preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50 g/100 ml). The aqueous extract of ginger root was stored in small samples at 22°C until used.

Animals and diet:

Sixty male adult albino rats (Rattus norvegicus) (weighing 180-230 g), purchased from the animal house of Theodor Bilharz Animal house were involved in the present study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in quite temperature room (26 ± 4°C). Rats had free access water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

Experimental design:

Animals were allocated into four experimental groups (n=15):

Negative control (C1): Sham-treated rats.

Positive control (C2): Rats orally administered 1 ml/day of the final ginger extract by stomach tube for four consequent weeks.

CCl4 treated group (G1): Rats orally administered the same dose of ginger extract given to animals of C2 for three consequent weeks followed by daily oral doses of 0.1 ml/100 g BW of CCl4 for one week.

CCl4 + Ginger treated group (G2): Rats orally administered the same dose of ginger extract given to animals of C2 for three consequent weeks followed by CCl4 at a dose of 0.1 ml/100 g BW of CCl4 combined with 1 ml/day of the final ginger extract throughout the fourth week.
After the experimentation periods, the animals were sacrificed by cervical dislocation; quickly dissected and small pieces of liver were fixed in Bouin's fluid, dehydrated, and embedded in wax. Five µm thick sections were stained with haematoxylin and counterstained with eosin and evaluated by light microscopy.

Biochemical assays:
The total serum proteins, albumin, and globulins were measured according to Henery (1964).

RESULTS AND DISCUSSION:
Table 1 and figures 1 & 2 show that aqueous extract of ginger Zingiber officinale affected the total serum protein, albumin, globulin, and altered the albumin to globulin ratio. The results recorded a significant decrease in total protein in G1 (treated with CCl4) compared with the shame-treated group (C1) and ginger treated one (C2), and a significant decrease in globulins in G1 and G2 groups (treated with CCl4 and CCl4+ginger) compared with control ones (C1 and C2). Ginger caused significant increase in albumin to globulin ratio in G1 and G2 compared with C1 and C2.

Table 1. Total serum protein and globulin values, and A/G ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
<th>Globulin (g/l)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>- ve control</td>
<td>106.98 ± 8.92</td>
<td>60.2 ± 4.36</td>
<td>45.05 ± 1.36</td>
<td>0.75</td>
</tr>
<tr>
<td>+ ve control</td>
<td>105.86 ± 6.17</td>
<td>60.57 ± 2.34</td>
<td>45.2 ± 1.98</td>
<td>0.75</td>
</tr>
<tr>
<td>CCl4 treated</td>
<td>77.98 ± 4.12</td>
<td>29.09 ± 1.72</td>
<td>20.9 ± 1.17</td>
<td>1.94</td>
</tr>
<tr>
<td>Ginger + CCl4</td>
<td>94.57 ± 4.36</td>
<td>42.83 ± 2.12</td>
<td>49.96 ± 3.68</td>
<td>1.66</td>
</tr>
</tbody>
</table>

The present results showed that treatment with CCl4 reduced the liver weight, body weight, and liver/body weight ratio. These parameters were significantly decreased in G1 compared with those of controls. The liver weight of G2 showed a non-significant decrease compared with controls. However, the body weight and the liver/body weight ratio of G2 showed a significant decrease compared with controls (Table 2 and Figs 3-5). In contrast, Shih et al. (2005) studied the effects of Anoectochilus formosanus (AFE) on liver fibrogenesis in carbon tetrachloride-induced cirrhosis and suggested that CCl4-induced liver atrophy, while AFE increased the liver weight.
These results are in agreement with those of Mandal et al. (1992) who studied the effect of Mikania cordata root extract on the rate of hepatic protein synthesis in CCl4-induced liver damage. They found that pretreatment of the root extract lead to tissue repair and hence to a functional improvement of the hepatocytes that were disorganized with CCl4 intoxication. Mandal et al. (1998) studied the effect of the Trianthema portulacastrum L. on the CCl4-induced chronic hepatocellular damage. The authors suggested that CCl4 administration alone caused alterations of plasma albumin and globulins. The administration of plant extract restored parameters to the normal level.

Gole and Dasgupta (2002) suggested that Aphananxmis polyschachya has a beneficial effect on toxic liver injury. The antihepatotoxic activity was evaluated on CCl4-induced liver injury. The leaf extract ameliorated the depressed value of serum albumin caused by CCl4.

Bhandarkar and Khan (2003) found that administration of Lawsonia alba afforded good hepatoprotection against CCl4-induced reduction in total serum protein. They suggested the hepatoprotective and antioxidant activity of L. alba bark extract. Moreover, Manjunatha et al. (2005) reported that leaf extracts of Leucas hirta have hepatoprotective activity against CCl4-induced liver damage and reported a significant decrease in total protein level caused by CCl4. The authors mentioned that leaf extracts showed a significant increase in total protein.

Shih et al. (2005) studied the effects of Anoectochilus formosanus (AFE) on liver fibrogenesis in carbon tetrachloride-induced cirrhosis in rats. They reported that CCl4 led to the drop of serum albumin concentration, while the treatment with AFE increased the albumin concentration. The CCl4-induced decreased the protein content in rat's liver and AFE significantly increased the contents of protein in the liver.

Lin and Lin (2006) investigated the effects of Reishi mushroom and Ganoderma lucidum extracts (GLE), on liver fibrosis induced by carbon tetrachloride and mentioned that CCl4 caused decrease in plasma albumin, albumin to globulin ratio (A/G ratio) and hepatic protein levels. They found that administration of GLE significantly reduces CCl4-induced hepatic fibrosis in rats, probably by exerting a protective effect by its free-radical scavenging ability.

The present results showed no changes in total protein, albumin, globulin, and albumin to globulin ratio in C1 (Sham-treated) compared with C2 (ginger treated). Al-Naqeeb et al. (2003) in their studies on the biochemical and histopathological toxicity of an aqueous extract of ginger in female rats found that the serum proteins were unaffected by ginger treatment, while liver protein content was decreased. Moreover, the present results indicated non-significant changes in body weight in C2 compared with C1. These results confirm that of Al-Naqeeb et al. (2003) who mentioned that ginger treatment did not affect the weight gain of the animals.

Amin and Hamza (2006) demonstrated that Z. officinalis increased the activities of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalase and reduced level of malondialdehyde. Ansari et al. (2006) showed that the ethanolic Z. officinalis extract pretreatment for 20 days in isoproterenol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibits cardioprotective property. Ajith et al. (2007) reported that ginger ameliorated cisplatim induced nephrotoxicity and this protection is mediated either by preventing the cisplatin – induced decline of renal antioxidant defense system or by their direct free radical scavenging activity. Siddaruju and Dharmesh (2007) reported that ginger–free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properities.

Mandal et al. (1992) studied the effect of Mikania cordata root extract on the rate of hepatic protein synthesis in vivo in CCl4-induced liver damage. They reported that pretreatment with the root extract (100 mg/kg, once daily for successive 5 days) showed a marked enhancement in the levels of hepatic DNA, RNA and protein content that were adversely affected with CCl4 treatment in the experimental mice. Increase in the total protein mass, fractional rate of protein synthesis (% of protein synthesized/day), total rate of protein synthesis (fractional rate x protein mass), ribosomal capacity (RNA/protein), ribosomal efficiency (rate/ribosome) and high turnover rate of protein (protein/DNA) in response to the pretreatment of the root extract in hepatic tissue indicated the tissue repair leading to a functional improvement of the hepatocytes that were disorganized with CCl4 intoxication.

Thomson et al. (2002) investigated the effect of an aqueous extract of ginger (Zingiber officinale) on serum cholesterol and triglyceride levels, as well as platelet thromboxane-B2 and prostaglandin-E2 production. These results suggest that ginger could be used as a cholesterol-lowering, antithrombotic and anti-inflammatory agent.

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Histological observations:

The control animals which given water or Ginger (C and C1) showed normal liver structure (Figs 6&7). Examination of liver sections obtained from rats of G1 showed obvious histopathological alterations of the liver. The normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was almost lost. The central and portal veins were congested (Fig. 8 A&B). Some liver sections demonstrated a considerable number of damaged hepatic cells that lost their characteristic appearance while others showed marked cytoplasmic vacuolizations which were so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells – frequently forming a narrow peripheral rim was left. The nuclei of these cells were pyknotic. In addition, congestion of the intrahepatic blood vessels and inflammatory leucocytic infiltrations were observed. Some liver cells were degenerated and suffered from fatty infiltrations (Figs 8 &9).

Fig. 6. Section in the liver of a C1 control rat showing the normal structure where the hepatocytes (H) are arranged in plates radiating from central vein (CV). S: sinusoids, arrowhead: pointing to Kupffer cells. X 250

Fig. 7. Liver section of the rat treated with ginger supplemented C2 demonstrated normal arrangement of hepatocytes, although no significant difference was observed between control and ginger group X 400.

In animals treated with CCl4 combined with ginger (G2), the majority of these histopathological changes were diminished but some of the intrahepatic vessels were congested and some hepatocytes appeared with vacuolized cytoplasm and Kupffer cells were activated (Fig. 10).

Fig. 10. Section in the liver of G2 showing almost normal liver histology, where the hepatocytes are arranged in characteristic strands pattern radiating from the central vein (CV) and sinusoids (S) became nearly normal. Kupffer cells: arrowhead. X 800

The obtained results showed that treating rats with CCl4 and ginger improved the histopathological changes induced in the liver by CCl4. This indicated the effectiveness of ginger in prevention of CCl4 hepatotoxicity. These results are in agreement with Yemitan and Izegbu (2006) who studied the effect of the ethanol extract

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of the rhizome of *Zingiber officinale* against carbon tetrachloride and acetaminophen-induced liver toxicities in rats. The protective effect of the extract on CC\(_4\)-induced damage was confirmed by histopathological examination of the liver. These results indicated that the oil from the rhizome of *Zingiber officinale* could be useful in preventing chemically induced acute liver injury. Again, the hepatoprotective effects of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and vitamin E pre-treatment against carbon tetrachloride (CC\(_4\))-induced liver damage in male Wistar albino rats were investigated by Patrick-Iwuanyanwu et al. (2007). They found a severe infiltration of inflammatory cells in liver of rats treated with CC\(_4\) alone. However, the observed alteration in the normal architecture of the hepatic cells decreased remarkably in pre-treated rats.

It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Recknagel, 1967).

From this study it could be concluded that ginger extract can be used as antioxidant, free radical scavenger and protective against carbon tetrachloride-induced oxidative damage in liver.

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