Study of the Protective Effects of Ginger Extracts Against Induced by Carbon Tetrachloride in Rats.

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Summary

The present study was designed to evaluate the possible hepatoprotective and Antioxidant activity of ethanol and aqueous extracts of *Zingiber officinale* using carbon tetra chloride (Ccl4) - induced hepatotoxicity in rats. The study was conducted of 42 male rats; the animals were allocated into 6 groups (7 rats in each group) and treated as follow: group I treated with oral doses of normal saline and saved as a control; group II treated with 0.5 ml of volume dose oral doses of Ccl4 suspended in olive oil doses of Ccl4 suspended in olive oil (1:1, v/v) 0.5ml at 3 days in week; group III treated with 0.5 ml of volume dose from Ccl4 plus ethanolic extract at concentration 300 mg/kg at the same time; group IV treated 0.5 ml of volume dose from with Ccl4 plus ethanolic extract at concentration 150 mg/kg at the same time; group V treated with 0.5 ml of volume dose from Ccl4 plus aqueous extract at concentration 300 mg/kg at same time; group VI treated with 0.5 ml of volume dose from Ccl4 plus aqueous extract at concentration 150 mg/kg at the same time; at the end of treatment period (8 weeks), rats were scarified, blood sample obtained for assessment of oxidative stress parameters and antioxidant parameters MDA, GSH and vitamin C. Additionally, serum levels of ALT and AST were evaluated after and before treatment, the result indicated that ethanol extract at concentration 300mg/kg has the ability to a significant decrease (p<0.05) in activities of serum liver enzyme AST, ALT, vitamin C, and MDA level, a significant increase (p<0.05)in GSH level. The results of this study indicate that the two concentrations(150, 300)mg/kg of the ethanolic *Zingiber officinale* rhizomes extracts gave a good results, even better than aqueous extract.

Introduction:

Herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy (Venukumar and Latha, 2002). Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases (Dash *et al*., 2007).

Ginger (*Zingiber officinale Roscoe*; Zingiberaceae) has used as spice for over thousand years (Bartley and Jacobs, 2000). Its roots contain polyphenol compounds (6-gingerol and shogaols), which have a high antioxidant activity (Stoilova *et al*., 2007).

The antioxidant property of ginger has been proposed as one of the major possible mechanisms for the protective effects of the plant against toxicity and lethality of radiation (Jagetia *et al*., 2003; Haksar *et al*., 2006) and a number of toxic agents such as carbon tetrachloride, arsenic and cisplatin (Amin and Hamza, 2006; Yemitan and Izegbu, 2006; Morakinyo *et al*., 2010).
Liver is a major detoxifying organ in vertebrate body, it plays a central role in carbohydrate, protein and fat metabolism and allows the detoxification of various xenobiotics. Additionally, it regulates the synthesis and secretion of bile (Casarett and Doull's, 2008).

Many xenobiotics such as acetaminophen, Ccl4 and yellow phosphorus produce liver damage, in a predictable and dose-dependent manner; the most frequent mechanism of hepatocellular injury involves production of injurious metabolites by the cytochrome p450 system (Sunita et al., 2008). Toxic injury occurs in the liver more often than other organs, because all ingested substances that are absorbed are first presented to the liver and that the liver is responsible for the metabolism and elimination of many substances (Casarett and Doull's, 2008).

Materials and methods:

Collection & Classification of plant:

This study was conducted at the Clinical researches laboratory/ Pharmacy College and Animal physiology laboratory/ Sciences College/ university of Kufa during the period from 10/9/2012 to 02/1/2013. The dried rhizomes of zingiber officinale were pushed from a local market and botanical authentication was provided by Assit. Proff. Dr. Ahmed abais in Department of Biology, faculty of science, Kufa university, Iraq. The dried rhizomes were cut into small pieces and crushed by blender to obtain powder, and the powder kept in refrigerator until used.

Animals:

42 Rattus Norvegicus of male sex weighing 210-290 g were obtained from the animals house in college of medicine, university of Karbalaa are used in this study.

The animals were housed in the animal house of faculty of science, university of Kufa, under standard environment condition (temperature 25-28 C° and 12 hr light - dark cycle) and allowed access to standard laboratory feed and water.

Methods:

preperation of Crude Plant Extracts:

Ethanol Extract

20 g of the powder kept in thimble was extracted with 200 ml 90% ethanol in a soxhlet extractor for 24 hour. The extract was concentrated in a vacuum at 60 C° using rotary evaporator, to evaporate the remaining solvent. The extract was kept in a freeze dryer for 24 hour yielding semisolid residues of EGE. (Laad et al., 1978 and Hassan, 2011).

Aqueous Extract

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Aqueous extract of ginger was prepared by maceration method according to (Parekh et al., 2005). With slight modification, a total 10 g of the ginger powder was steeped in 100 ml of sterilized distilled water for one day, and then filtered through eight layered of cotton. It was further filtered using filter paper (Whatman No.1) and centrifuged at 3000x for 10 minutes, then the filtrated was kept in a freez dryer for 48 hour yielding solid residues of AGE.

**Study Design:**

The rats were allowed to acclimatize to the laboratory condition for a week before they were used for the experiment, 6 groups were formed 7 rats for each group and treated for 8 weeks.

**group I:** control animals gives of volume dose 0.5 ml/kg of normal saline only. **group II:** give 0.5 ml/kg of Ccl4 was suspended in olive oil (1:1 v/v) and administrated orally by oral needle 0.5 ml of volume dose for three time per week for 8 weeks to induce liver damage. **group III:** was administrated with 0.5 ml of volume dose from Ccl4 plus ethanolic extract at concentration (300 mg/kg) at same time for 8 weeks . **group IV :** was administrated with 0.5 ml of volume dose from Ccl4 plus ethanolic extract at concentration (150 mg/kg) at same time for 8 weeks, **group V:** was administrated with 0.5 ml of volume dose from Ccl4 plus aqueous extract at concentration (300 mg/kg) at same time for 8 weeks, **group VI:** was administrated with 0.5 ml of volume dose from Ccl4 plus aqueous extract at concentration (150 mg/kg) at same time for 8 weeks.

**Blood Collection**

At the end of experimentation (8-weeks) Each animal was anaesthetized with chloroform, and scarified. Heart puncture was done with a 5 ml disposable syringe and 2-5 ml blood was drawn very gently and slowly. Each blood sample was divided in 2 parts. The first part (about 0.5 ml) was placed in a tube containing disodium EDTA (22mg/ml) as anticoagulant and mixed thoroughly, Then used for determination of hematological analysis by an automatic analyzer. The remaining blood was placed in a tube without anticoagulant and left for 30 minute in room temperature and used to obtain serum via centrifugation at 3000 rpm for 15 minutes and then preserved at -20 in a freezer for determination biochemical analysis.

**Biochemical analysis:**

**Determination of Serum Transaminase Activity:**

Coloric determination of ALT&AST activity according to the Reitman and Frankel method (Reitman and Frankel, 1957), by using biomerieux kit.

**Determination of Serum Glutathione Activity:**

The test is intended for quantitative of glutathione (GSH) concentration in serum through the immunosorbannt assay (ELISA) using bioelisa reader EL x800 (biokit, U.S.A.) in virology laboratory of AL-Sadder Medical City in Najaf governorate, the
assay Max Glutathione (GSH) ELISA kit was achieved according to the manufacturing company (CUSABIO, U.S.A.).

**Determination of Lipid Peroxidation Activity (MDA):**

Cell Bio labsTM MDA Adduct ELISA Kit (USA) is an enzyme immunoassay developed for rapid detection and quantitation of MDA-protein adducts. The quantity of MDA adduct in protein samples is determined by comparing its absorbance with that of a known MDA-BSA standard curve.

**Determination of Vitamin C**

Vitamin C kit for quantitative determination of vitamin C in human serum was supplied by ALPCO company, USA. The application of Vitamin C for HPLC allows the determination of Vitamin C, the kit includes all reagents in ready to use for preparation and separation of the samples. The columns are not included. The first step in the determination of Vitamin C is a fast and easy sample preparation. During the precipitation higher molecular substances are removed. After centrifugation the supernatant is injected into the HPLC system. The separation via HPLC follows an isocratic method at 25°C using a reversed phase column; one run lasts 12 minutes. Vitamin C is detected by an UV-monitor. The quantification is performed with the delivered calibrator; the concentration is calculated via integration of the peak areas by the external standard method (Falch, 1998).

**Biostatistical Analysis:**

The results were expressed as (mean ± standard deviation). Pooled t-test was used for the comparison between control and other groups in the measured parameters. The level of significance among the various treatments was determined by least squares difference (LSD) analyses at 0.05% levels probability. All statistical analysis were performed using Excell program (2007) from micro soft Co. USA. The difference will be significant when P <0.05.

**Results**

The results in figure 1 showed that serum activity of aspartate aminotransferase (AST) was significantly elevated in Ccl4 - intoxicated animals compared to control group (p<0.05).

Treatment of the rats with EGE at concentration 150, 300mg/kg of body weight along with Ccl4 for 8 weeks showed a marked decline in the serum AST activity compared to Ccl4 -treated animals (p<0.05). Treatment of the rats with AGE at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks showed a marked decline in serum AST activity compared to Ccl4 -treated animals (p<0.05) this show in fig.2.
The results in figure 3 showed that serum activity of alanine aminotransferase (ALT) was significantly elevated in Ccl4 - intoxicated animals compared to control group (p<0.05). Treatment of the rats with EGE at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks showed a marked decline in the serum AST activity compared to Ccl4 -treated animals (p<0.05). Treatment of the rats with AGE at concentration 150, 300 mg/kg of body weight) along with Ccl4 for 8 weeks showed a marked decline in serum AST activity compared to Ccl4 -treated animals (p<0.05) this show in fig. 3.
Figure (3): effect of ethanolic ginger extracts on ALT levels in rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

Figure (4): effect of aqueous ginger extract on ALT levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

The results in figure 5 showed a significant decrease in serum level glutathione reductase (GSH) in animals treated with Ccl4 for 8 weeks compared with control group. Treatment of the animals with EGE at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks showed a significant increase in level of GSH compared with Ccl4 group. Treatment of the animals with AGE at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks (p<0.05), showed a significant increase in GSH levels compared with Ccl4 group (p<0.05) this show in fig. 6.

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Figure (5): effect of ethanolic ginger extract on GSH levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

Figure (6): effect of aqueous ginger extract on GSH levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

The results in figure 7 showed a significant increased in lipid peroxidation products (MDA) with in group treated with Ccl4 for 8 weeks compared to control (p<0.05).

Treated of the animals with EGE at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks showed a significant decrease in level of MDA compared with Ccl4 group.
Treatment of the animals with AGE at concentration 150, 300 mg/kg of body weight) a long with Ccl4 for 8 weeks (p<0.05), showed a significant decrease in MDA levels compared with Ccl4 group (p<0.05), this show in fig. 8.

Figure (7): effect of ethanolic ginger extract on MDA levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

Figure (8): effect of aqueous ginger extract on MDA levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.

The results in figure 9 showed a significant decreased in non- enzymatic antioxidant ascorbic acid (vitamin C) with in group treated with Ccl4 for 8 weeks compared to control (p<0.05). Treated the animals with EGE at concentration 150, 300 mg/kg of body weight) a long with Ccl4 for 8 weeks showed a significant increase in level of vitamin C compared with Ccl4 group. Treatment of the animals with AGE at concentration 150, 300 mg/kg of body weight a long with Ccl4 for 8 weeks (
p<0.05) showed a significant increase in vitamin C levels compared with Ccl4 group (p<0.05), this show fig. 10.

![Figure 9: Effect of ethanolic ginger extract on the vitamin C levels in the rats treated with Ccl4. *p<0.05 significant different to control group, ss= significant different to Ccl4 group. Each value represents mean ±SD.](image)

**Discussion:**

Effect of *zingiber officinale* extracts on hepatic enzyme activity.

ALT and AST are moderately elevated after the administration of hepatotoxic drugs. The Ccl4 is considered as one of the most commonly used hepatoxines in the
experimental study of liver diseases, (Johnston et al., 1998) due to its active metabolite, trichloromethyl radical (Srivastava et al., 1990). Increased activity of liver enzymes are usually regarded as expressions of cellular necrosis, especially in hepatocytes (Halkes et al., 2002). The increase in the levels of transaminase reflects a clear indication of cellular leakage and loss of functional integrity of the cell membrane (Halkes et al., 2002). Assessment of liver function can be made by estimating the activities of serum AST and ALT, which are originally present in higher concentrations in cytoplasm. In hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage (Venkataringanana et al., 1998). These enzymes reflect cellular damage due to Ccl4 administration which according to many authors the enzymes activity become normal before the rats treated with Ccl4, the results in the figure (1), and figure (4) showed the hepatotoxicity of Ccl4 was clear by observed through a significant elevation of serum AST and ALT levels in Ccl4-treated rats compared with control (p<0.05). As it has been previously reported that Ccl4 administration cause deteriorations of liver function tests such as AST, ALT, ALP, TSB and urea revealed hepatic dysfunction, which could be a secondary events following Ccl4-induced liver damage, with the consequent leakage from hepatocyte (Xu et al., 2002).

The figure (1) and figure (3) show a significant increase in AST and ALT levels in the animals treated with Ccl4 (0.5 mg/kg) for 8 weeks compared with control group (p<0.05). This result which agree with the previously reported result (Kew, 2000; Ahmed and Sajida, 2012).

The administration of EGE at concentration 150, 300 mg/kg of body weight along with Ccl4, the results in figure (1) and (3) showed a significant decreased in the levels of ALT and AST compared to Ccl4 group.

Administration of AGE at concentration 150, 300 mg/kg of body weight along with Ccl4, the results in figure (2) and (4) showed a significant decreased in the serum levels of ALT and AST compared to Ccl4 group, these results elucidate the hepatoprotective effect of ginger extracts due to the presence of antioxidant compounds (polyphenoles) in ginger extract, this act as scavenger of free radicals and prevent flow of AST and ALT to the blood stream (Masuda et al., 2004).

**Effect of zingiber officinale on GSH levels**:

Treatment with ginger extracts at concentration 150, 300 mg/kg for each extracts along with Ccl4 for 8 weeks, the result in figure (5 and 6) show normalized the antioxidant levels through their rich of flavonoids that have the ability to scavenge free radicals (Mitra et al., 1998). This results agree with the results that obtained by (Tarek et al., 2011). Treatment of the animals with ginger extracts for 8 week, these results agree with the results that obtained by (Tarek et al., 2011).

GSH is a tripeptide composed of glycine, glutamic acid, and cystein. The important part of the molecules is the SH group of cystein; therefore it exists both in oxidized and reduced formes (Sarkar et al., 2006). Ccl4 is one of an extensively environmental toxicant. The reactive metabolite trichloromethyl radical (Cl3) has been formed the metabolic conversion of Ccl4 by cytochrome p-450. This reactive metabolite initiates the peroxidation of membrane poly-unsaturated fatty acids,
generated PUFA radicals, covalently binds to membrane lipids and proteins and generates ROS (Gowri et al., 2008).

Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to attenuate ROS. However, when a condition of oxidative stress established, the defense capacities against ROS becomes insufficient. Therefore, ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, and decreases in activity of CAT and enhanced lipid peroxidations (Gowri et al., 2008). In agreement with these explanations, the observed decrease in GSH was recorded in Ccl4 treated rats that may be due to inactivation of the antioxidative enzymes. This may cause an increased accumulation of superoxide radicals, which could further stimulated lipid peroxidation. The result in figure (5) show Decrease in GSH activity might be due to decrease availability of GSH resulted during the enhanced lipid peroxidation processes.

**Effect of zingiber officinale on lipid peroxidation levels MDA :**

Free radicals produced from Ccl4 induced peroxidation of membrane lipid which can be very damaging because it leads to alteration, in the biological properties of membrane, such as degree of fluidity, and can lead to inactivation of membrane bound receptors or enzymes, which in true may impair normal cellular function. Lipid peroxidation product, the MDA, commonly used as biomarker of oxidative stress/damage (Dalle- Done et al., 2006).

The results in figure (7) showed a significant increased in the level of lipid peroxidation (MDA) in group treated with Ccl4 for 8-weeks compared to control.

Treatment of the animals with Ethanolic ginger extracts at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8 weeks, the result in figure (7) show a significant increased in the level of MDA compared to control, these results due to Ethanolic ginger extracts express antioxidant activity against free radicals that formed from Ccl4.

Treatment of the animals with aqueous ginger extracts at concentration 150, 300 mg/kg of body weight along with Ccl4 for 8-weeks, the result in figure (8) show a significant decreased in the levels of MDA compared to control, these due to the ginger extract act as scavenge to free radicals that formed from Ccl4, ginger has been reported to increased glutathione reduced lipid peroxidation n vivo and scavenging of various free radicals in vitro (Jagetia, et al.,2003).

**Effect of zingiber officinale extracts on vitamin C levels:**

The results in figure (9) show the increase levels of vitamin C with rats administration with ethanolic ginger extracts at concentration 150, 300 mg/kg of body weight along with Ccl4, and the result in figure (10) show increase in levels of vitamin C with groups administration with aqueous ginger extracts at concentration150, 300 mg/kg of body weight along with Ccl4 this increase due to antioxidant activity of ginger extracts.

In addition to that (Elsayed, 2001) showed that mobilization antioxidants in response to oxidative stress reflects a dynamic process whereby dietary antioxidant...
supplemental factors (Elsayed, 2001). All the above data may explain the increase in serum GSH of group treated with Ccl4 plus ginger extracts.

Antioxidants status during Ccl4 injected, While Ccl4 induced in rats, free radical production, this type of injury is also impairs antioxidant, defense mechanisms injury alter non-enzymatic defense mechanism including decrease in α-tocopherol vitamin E, ascorbic acid vitamin C, reduce in glutathione; in addition to that trace element status is also altered after major liver injury leads to decrease serum levels of glutathione (Farriol et al., 2001). In general, ROS attach can lead to a major depletion of antioxidant status and intracellular redox status (Rossi et al., 2006). In this study GSH serum level decreased in rats treated with Ccl4 compared with control, and other groups.

It is a powerful antioxidant, which works in aqueous environments of the body. As it cannot be synthesized in human, it must be obtained from the diet. In cells, it is maintained in its reduced form by reduction with glutathione (Maiese et al., 2007).

Result obtained by (Bertin–Maghit et al., 2000), they suggested after injury, treated group with Ccl4 exhibit in antioxidant vitamin C. Furthermore, (Nanji et al., 2003), reported that the mean level of ascorbic acid in the group II were lower than control, and other groups.

Consequently, it is reported that ginger extract act as antioxidants give orally after injury by Ccl4 prevent the altered cell energetic. Strongly suggesting a cause- and – effect relationship between increased oxidant release with inflammation, decreased antioxidant activity and altered cell energetic (Lalonde et al., 1988). Vitamin C would serve to scavenge free radicals within the extracellular space, it is a highly water soluble, low molecular weight vitamin; by a stepwise donation of an electron the resulting ascorbate free radical that is formed is more stable than other free radicals and can serve as a free radical scavenger also. On the other hand, it has been found that vitamin C has a strong regulatory influence on neutrophil production of ROS; vitamin C appears to act directly by scavenging ROS in neutrophil (Lalonde et al., 1988). It has been Ccl4 that causing oxidative stress can initiate chain reactions, cause lipid peroxidation and protein oxidation and depletes the antioxidants including natural antioxidant GSH (Horton, 2003).

**Conclusion :**

Antioxidants have proven to be effective in ameliorating Ccl4-induced toxicity in many preclinical and few clinical interventions, this study may explore its nutraceutical role in human diet, and ethanol extract recorded the most potent effect due to its content of flavonoids, sterols, triterpenes, carbohydrate, and alkaloids.

**Reference:**


