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Effect of *Ginkgo biloba* Leaves Aqueous Extract on Carbon Tetrachloride Induced Acute hepatotoxicity in rats

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Abstract

Bachground: Oxidative stress plays a pivotal role in the pathogenesis and progression of various liver diseases. *Ginkgo biloba* leaves extract (GbE) have been proved to be an effective antioxidant, thereby can contribute to the prevention and treatment of diseases associated with oxidative stress. The present study aimed to investigate the hepatoprotective effect of GbE on acute liver injury induced using carbon tetrachloride (CCl_4) in rats.

Material and Methods: Hepatotoxicity was induced in male rats by intraperitoneal (i.p) injection of CCl_4 1mL/ kg body weight (b.w.) for every 72 h for 14 days, GbE was administered orally at a dose of 150 mg/kg b.w., daily started two weeks prior to CCl_4 injection and continued until the end of the experiment**.**

Results: CCl_4 caused acute liver damage in rats, as evidenced by significant increase serum enzymes activities of aspartate and alanine aminotransferase (ALT & AST) and alkaline phosphatase (ALP), and hepatic malondialdehyde (MDA), as well as significant decrease in weight gain percent, serum total protein (TP), high-density lipoprotein cholesterol (HDL-C), and hepatic reduced glutathione (GSH). Pretreatment with GbE prior to CCl_4 injection elicited hepatoprotetcive activity by significant decreased the activities of liver enzymes and hepatic MDA, and significant increased the levels of TP, and hepatic GSH, as well as induced significant ameliorated in weight gain percent and lipid profile parameters as compared with CCl_4 group. Histopathological examination of the liver tissues of CCl_4 group represented the presence of hepatic necrosis associated with cells infiltration and vacuolar degeneration of hepatocytes, while the pretreatment with GbE overcome these changes, the majority of the cells tend to be normal.

Conclusion: The present findings indicated that the hepatoprotective effect of GbE against CCl_4-induced oxidative damage may be due to its potent antioxidant activity. Therefore, GbE could be of potential help as a medicament or food supplement for alleviation of liver toxicity.

Key words: *Ginkgo biloba* – aqueous extract, male rats, carbon tetrachloride, liver enzymes, lipid parameters, malondialdehyde, reduced glutathione, hepatoprotective.

Introduction:

The liver, due to its metabolic enzymes, plays a vital role in maintaining the homeostasis of the body via the metabolism of endogenous and exogenous molecules and it eases their detoxification and elimination. Liver function can be impaired and hepatocytes damaged upon exposure to drugs, alcohol, infections, or malnutrition (Mroueh *et al*., 2004). It has been demonstrated that oxygen-derived free radicals and lipid peroxidation play a critical role in the pathogenesis of various liver diseases (Loguercio and Federico, 2003 and Das *et al*., 2005). Thereby, it has become the key to prevent and cure hepatic damage by eliminating free radicals and preventing lipid peroxidation (Han *et al*., 2004 and Gedik *et al*., 2005), and are applied in clinical medicine (He *et al*., 2004).

Ginkgo biloba L. (Family: Ginkgoaceae*)*, is an important herb medicine, achieving unprecedented popularity over the past decade, and the recognition of the important therapeutic effects shown by this plant (Guo *et al*., 2011). Chemically, the active constituents of *Ginkgo biloba* leaf are mainly (kaempferol, quercitin and isorhamnetin), diterpene lactones namely Ginkgolides A, B, C, M and J and bilobalide, biflavones (ginkgetin, isoginkgetin, bilobetin) and organic acids such as 4-hydroxybenzoic acid, that

have presented various pharmacological activities (Ahlemeyer and Krieglstein, 2003 and Boonkaew and Camper, 2004). The extract of *G .biloba* leaves have been proved to be an effective antioxidant and found to possess cardioprotective, antiasthmatic, antidiabetic, and potent central nervous system activities, including enhancement of memory, concentration, mental alertness and decrease in mental fatigue (Naik *et al*., 2006 and Naik and Panda, 2007). Also, it is used in the management of cerebral insufficiency that occurs during normal aging and treatment of neurological diseases like Alzheimer's, dementia, and other cognitive dysfunctions (Kwon *et al*., 2004).

This extract has been shown several *in vivo* effects, including augmentation of blood flow and inhibition of platelet activating factor, it protects the cell membrane against damage induced by free radicals and presents protective effects against myocardial and brain ischemia/reperfusion injury (Zhang *et al*., 2000 and Ahlemeyer and Krieglstein, 2003). The herb in addition posses other important pharmacological actions. *Ginkgo biloba* extract decreased gastric injury caused by ethanol (Wang *et al*., 2000), protected against chemically induced oxidative injury and fibrosis (Ding *et al*., 2005). Therefore, the purpose of this study was designed to investigate the protective effect of GbE on CCl_4-induced acute liver injury in rats.

Material and Methods:

Drugs and chemicals:

Carbon tetrachloride and liquid paraffin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carboxymethl cellulose, Thiobarbituric acid, 1,1,3,3-tetramethoxy-propane, trichloroacetic acid, and diethyl ether were obtained from Sigma-Aldrich (USA). Chemical Kits were obtained from Biodiagnostic Co. Egypt. All chemicals used were analytical grade of the highest laboratory purity. Casein was obtained from Misr Scientific Co. Dokki, Giza, Egypt. Cellulose and L-cystine were purchased from Morgan Co. Cairo, Egypt. Starch and corn oil were obtained from local market. Vitamins and minerals constituent and sucrose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt.

Plant material:

Ginkgo biloba L. family (*Ginkgoaceae*), leaves were obtained from International Garden, Abbas El-Akkad St., Nasr City, Cairo, Egypt. It was identified and collected by **Agri. Eng. Mohammed Abdul Latif Jadallah,** Director General of the International Garden. The plant leaf of *Ginkgo biloba* was confirmed by **Prof. Dr. Al-Nowaihi, A. S. M**., Prof. of Taxonomy, Botany Department, Faculty of Science, Ain Shams University.

Preparation of aqueous extract of *G. biloba*:

Ginkgo biloba leaves (100 gm dried powder) were soaked in 1 liter boiling distilled water. After 2 h it homogenized in the same distilled water, stirred by using magnetic stirrer at 40° C for 1 h, then filtered through a two-layer of cheese cloth. The residue was re-extracted with fresh boiling distilled water by the same way. The later aqueous extract was added to the first one. This combined aqueous extract was condensed in rotary evaporator under vacuum then lyophilized and stored at 4 ºC until further use according to Guo *et al*. (2011). Lyophilization was conducted at Mycotoxins Central Lab & Food Safety, National Research Center, Dokki, Cairo by using Freeze-Dryer Lyophilizer Heidolph (Dura-Top-Digital Programmer Bulk Tray Dryer FTS-Systems, Dura-Dry MP, Egyptian Canadian Co. Laborota, 4000 efficient, 90 rpm). 100 gm of *G. biloba* L. leaves yielded 17.561 gm extract.

Pretreatment with GbE:

Ginkgo biloba extract was dissolved in carboxymethyl cellulose (CMC), and a dose of 150 mg/kg b.w. was administered by gavage in (1 mL of 1%, w/v, CMC) according to Yapar *et al*. (2010).

Induction of hepatotoxicity by CCl_4:

Animals were injected intraperitoneally (i.p) with CCl_4 (1 mL/kg b.w., 1:1 v/v mixture of CCl_4 and liquid paraffin) every 72 h for 14 days according to Karthikeyan and Deepa (2010).

Experimental animals:

Forty-two adult male albino rats, *Sprague Dawley* strain, weighing (170 ± 10) g were purchased from the animal house of the National Research Center, Dokki, Egypt. Animals were housed in plastic cages, fed on standard casein diet according to Reeves *et al*. (1993) and given

tap water *ad libitum*. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Experimental design:

After the period of adaptation (one week), animals were divided into four groups (each of 6 rats) as following: **Control group:** Rats were orally administered a single daily dose of (1 mL of 1% , w/v, CMC), after two weeks injected i.p. with liquid paraffin at a dose of 1 mL/kg b. w. every 72 h for 14 days. **CCl_4 group:** Rats were injected i.p. with CCl_4 in liquid paraffin (1:1) at a dose of 1 mL/kg b.w. every 72 h for 14 days. **GbE group:** Rats were administered orally by gavage GbE at a dose of 150 mg/kg b.w. dissolved in (1 mL of 1%, w/v, CMC) for 28 days. **Pretreated GbE group:** Rats were administered orally with GbE at the same dose in GbE group, started two weeks prior to CCl_4 injection and continued until the end of the experiment. During the experimental period, food intake was recorded daily, and all animals were weighed at the beginning and biweekly intervals to monitor changes and to adjust the dose of GbE and CCl_4 accordingly.

Blood collection and serum separation:

Blood samples were withdrawn from the retro orbital plexu of each animal, 48 h after the last dose of the drug under anesthesia with diethyl ether according to the method of Cocchetto and Bjornsson (1983). Blood was allowed to clot, and then centrifuged at 3000 rpm for 15 min to separate serum, which kept at -20 ºC till biochemical analysis. Immediately after blood sampling, animals were sacrificed and the liver of each animal was dissected out, a part of liver was fixed in 10% formalin for histopathological studies and the other part was washed with ice-cold saline to remove as much blood as possible and stored at -20 ºC until assayed.

Determination of liver enzymes activities and total protein:

Separated serum samples were used for determination of alanine and aspartate aminotransferase activities (ALT&AST) (Reitman and Frankel, 1957) and alkaline phosphatase (ALP) (Belfied and Goldberg,1971). Furthermore, serum samples were used for determination of total protein (TP) (Henry, 1964).

Determination of lipid profile parameters:

Serum samples were used for determination of triacylglycerol (TG) (Fossati and Prencipe, 1982), total cholesterol (TC) (Allain *et al*., 1974) and high-density lipoprotein cholesterol (HDL-C) (Demacker *et al*., 1980). While, low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to the equation of (Friedewald *et al*., 1972).

Determination of hepatic malondialdehyde and reduced glutathione:

Liver homogenized (10%) was prepared in ice cold saline (0.9%), and the homogenized tissues were centrifuged at 3000 rpm at 4 ºC for 30 min. The obtained supernatants were used for determination of malondialdehyde (MDA) as a measure of lipid peroxidation (Yoshioka *et al*., 1979), and reduced glutathione (GSH) (Beutler *et al*., 1963).

Histopathological examination:

Specimens from liver were fixed immediately in 10% neutral buffered formalin, dehydrated in different grades of alcohol, cleared in xylol, embedded in paraffin wax, sectioned at 4-6 u thick and stained with Haematoxylin and Eosin (Bancroft *et al*., 1996) and examined microscopically.

Statistical analysis:

Results were expressed as a (mean ± SE). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to Snedecor and Cochron (1989). An IBM computer with a software system SPSS version 20 was used for these calculations.

Results:

Biological evaluation:

The result in Table (1) shows the effect of GbE on biological evaluation (weight gain percent, food intake and food efficiency ratio) in CCl_4 intoxicated rats. It is observed that there was significant decrease in weight gain percent ($p< 0.001$), food intake ($p< 0.001$) and FER ($p < 0.01$) in CCl_4 intoxicated group as compared to control group. Concerning the effect of GbE on rats, the ingestion showed slightly increase in weight gain percent, food intake and FER, there were no significant difference

as compared with control group. Pretreatment of rats with GbE showed a significant increase in weight gain percent ($p< 0.001$), food intake ($p< 0.001$) and FER ($p<0.01$) as compared with CCl_4 intoxicated group indicating the sign of amelioration.

Biochemical results:

The effect of GbE on serum liver enzyme activities (AST, ALT & ALP), and TP levels in CCl_4 intoxicated rats is illustrated in Table (2). CCl_4 intoxication caused a sharp significant increase in serum AST, ALT and ALP activities ($p<0.001$), and significant reduction in serum TP level compared to control group ($p<0.001$). Administration of GbE to rats showed non-significant changes in serum liver enzyme activities, and TP level as compared to control group. Pretreatment of rats with GbE caused a marked protection evidenced by significant reduction ($p<0.001$) in serum AST, ALT and ALP enzyme activities, and significant increase ($p<0.001$) in TP level compared to CCl_4 group.

Table (3) shows the effect of GbE on lipid profile parameters in CCl_4 intoxicated rats. Serum TC, TG, LDL-C and VLDL-C levels were significantly increased ($p<0.001$) along with a significant decrease in serum HDL-C levels ($p<0.001$) in CCl_4 intoxicated rats, as compared to control rats. Administration of GbE to rats revealed non-significant changes in all tested lipid profile parameters compared to control group. GbE pretreatment showed a significant improvement in the levels of lipid parameters, there was significant decrease in serum TC, TG, LDL-C and VLDL-C levels ($p<0.001$) along with a significant increase in serum HDL-C levels ($p<0.001$) in rats group pretreated with GbE as compared to CCl_4 group.

Effect of GbE on hepatic malondialdehyde (MDA) and reduced glutathione (GSH) in CCl_4 intoxicated rats is presented in Table (4). Results showed that, the level of MDA in the rats' liver tissue, significantly elevated ($p<0.001$) in CCl_4 intoxicated group compared to control group. On the other hand pretreatment of rats with GbE revealed amelioration in hepatic MDA content, since the value of MDA showed significantly reduced ($p<0.001$) as compared to CCl_4 group, while the results of rats receiving GbE tended to match control value. Regarding, hepatic GSH, the results revealed significant reduction ($p<0.001$) in rats intoxicated with CCl_4 as compared to control group. Pretreatment of rats with GbE markedly preserved hepatic GSH, the value of GSH near to normal levels comparing with CCl_4 group, at the same time there was significant difference ($p< 0.001$) as compared with CCl_4 group.

Histopathological results:

Microscopically, liver from control rat group showed the normal histological structure of hepatic lobule and portal vein without alterations Fig. (1). Liver tissues in CCl_4 intoxicated rats showed focal area of hepatic necrosis associated with mononuclear cells infiltration Fig. (2) and ballooned hepatocytes and pyknosis of their nuclei Fig. (3). Moreover, vacuolar degeneration of hepatocytes (fatty change) Fig. (4). Liver tissues of rats group received GbE showed no histopathological changes Fig. (5). Pretreatment of rats with GbE showed apparent normal histological structure Fig. (6).

Discussion:

Liver diseases reduce people's quality of life and frequently lead them to death. The occupational exposure to chemical compounds like aliphatic hydrocarbons alters the liver structure and functions (Jaeschke, 2008). Carbon tetrachloride (CCl_4) is one of the most studied hepatotoxic compounds and is frequently used as a model of experimental liver damage (Rinco´n *et al*., 1999). Administration of CCl_4 causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane (De Andrade Belo *et al*., 2012).The hepatotoxic effect of CCl_4 is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Pandit *et al*., 2004). *G. biloba* is known as an antioxidant, it shows potential in treating cerebrovascular dysfunctions and peripheral vascular disorders, in part owing to its potent antioxidant properties (Diamond *et al*., 2000 and McKenna *et al*., 2001). It can also enhance antioxidant defenses in *vitro* in cancer cells (Gohil and Packer, 2002). In the present investigation, GbE was evaluated for the

hepatoprotective activity using CCl_4 induced acute hepatotoxicity in rats.

In this Study, results showed that rats group intoxicated with CCl_4 revealed significant decrease in weight gain percent, food intake and FER as compared to control group. These results were in agreement with (Venukumar and Latha, 2002, Chang *et al*., 2007 and Balamurugan and Muthusamy, 2008). Pretreatment of rats with GbE showed a significant increase in weight gain percent, food intake and FER as compared to CCl_4 intoxicated group. These findings suggested that the extract administration has significantly neutralized the toxic effects of CCl_4 and helped regeneration of hepatocytes. These observations were in perfect conformity of Farooq *et al*. (1997). Guo *et al*. (2011) reported that pretreatment with *Ginkgo* leaf extract significantly suppressed the effect of CCl_4. These results indicate that GbE is a potent hepatoprotective agent against CCl_4-induced liver injury. On the other hand, oral administration of GbE induced slightly increase in weight gain percent, food intake and FER as compared to control group, this confirmed its safe use and agree with Dias *et al*. (2008) who found no significant alterations was observed in body weight gain or food consumption associated with *G. biloba* extract ingestion.

In the present study, following injection of CCl_4 serum ALT, AST and ALP activities have dramatically significant elevation when compared to control group. These results were consistent with those studies where ALT, AST and ALP activities were significantly increased following CCl_4 injection (Tirkey *et al*., 2005 and Anand *et al*., 2011). Elevated levels of serum liver marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Drotman and Lawhorn, 1978), since SGPT is thought to be one of the indices of the degree of cell membrane damage and SGOT is an indicator for mitochondrial damage; mitochondria contain 80% of this enzyme (Dabba and Abdel-Rahman, 1998). This effect of CCl_4 may be attributed to hepatocellular necrosis or membrane damage leads to very high levels of serum transaminases (ALT and AST) released from liver to circulation (Achliya *et al*., 2003). Serum ALP level on the other hand, is related to the function of hepatic cell, the increase in ALP serum level is due to increase its synthesis, in the presence of increased biliary pressure (Muriel *et al*., 1992).

There were significant restorations of these enzymes level by pretreatment with GbE. The reversal of increased serum ALP enzymes in CCl_4-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). Hepatoprotective activity may be due to presence of compounds in this extract with high antioxidant capacity. Studies have shown that flavonoid (ginkgo-flavone glycosides) and terpenoid (ginkgolides and bilobalides) are the most important active substances in the *G. biloba* extract which have antioxidant effect (Itil and Martorano, 1995). This agrees with Ding *et al*. (2005) and Cha´vez-Morales *et al*. (2010).

The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin and fibrinogen and also the other important components like α and β-globulin. The metabolic biotransformation of amino acid in liver by synthesis, transamination, etc., may be impaired due to the escape of both non-proteins and protein nitrogenous substances from injured cells as mediated by a raise in the serum enzyme activities of AST, ALT and ALP. The reduction in the TP is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Suresh Kumar *et al*., 2007). Pretreatment with GbE enhanced the synthesis of TP which accelerates the regeneration process and the protection of liver cells that is clearly demonstrated in the present study. Therefore, the increased level of TP in serum indicates the hepatoprotective activity. Moreover, Zhang *et al*. (2004) reported that GbE has a protective effect on hepatic endothelial cells and hepatic microcirculation in rats with chronic liver injury induced by CCl_4, the mechanisms may

involve its inhibition on platelet-activating factor and lipid peroxidation.

The present results revealed significant elevation in serum TC, TG, LDL-C and VLDL-C levels along with significant reduction in serum HDL-C levels in CCl_4 intoxicated rats, as compared to control rats. These results were in agreement with the previous results of El-Habibi *et al*. (2009) and Al-Dosari (2010). CCl_4 intoxication increases the synthesis of fatty acids and triglycerides from acetate, this could be attributed to CCl_4 positively affects the transport of acetate into the liver cell, resulting in increased acetate availability, CCl_4 intoxication also results in inhibition of synthesis of the bile acids from cholesterol which is synthesized in liver or derived from plasma lipids leading to increase cholesterol level (Boll *et al*., 2001). On the other hand, CCl_4 lowers β- oxidation of fatty acids and hydrolysis of triglycerides, this increases the availability of fatty acids to esterification (Lieber, 2000). The current study showed that pretreatment of rats with GbE resulted in significant improvement in the tested lipid profile parameters, it could be attributed to the active components of GbE, mainly flavonoid fraction, which have many beneficial effects, and antioxidant properties (Oteiza *et al*., 2005 and Bhendrich, 2006).

Lipid peroxidation is one of the principal causes of CCl_4-induced liver injury and is mediated by the free-radical derivatives of CCl_4 (Manibusan *et al*., 2007). The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation hepatic MDA of rats intoxicated with CCl_4 was observed. The increase in hepatic MDA levels leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (Amresh *et al*., 2007). Pretreatment of rats with GbE significantly reduced the elevated levels of MDA through scavenging oxygen radicals as the extract possesses a potent antioxidant activity. *Ginkgo*'s antioxidant activity is attributed to its ability to increase levels of free radical-scavenging enzymes and to neutralize ferryl ion-induced peroxidation ((Bridi *et al*., 2001 and Naik and Panda, 2007).

Glutathione is one of the most abundant tripeptide non-enzymatic biological antioxidant, its functions include removal of free radicals such as H_2O_2 and superoxide anions, maintenance of membrane protein thiols and acting as a substrate for glutathione peroxidase and glutathione reductase (Meister, 1984). In the present study, significant decrease in hepatic GSH level was observed in CCl_4 intoxicated group as compared to control group. The depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to CCl_4 (Hewawasam *et al*., 2003). The increase in hepatic GSH level in the rats pretreated with GbE may be due GSH regeneration. This effect of GbE may be due to an initial reduction in hepatic peroxidative activities, thereby leading to restoration of the GSH content (Naik and Panda, 2007). These effects would also contribute to partially explain the hepatoprotective effect of GbE by inhibiting the biotransformation of CCl_4 and the consequent production of free radicals (Cha´vez-Morales *et al*., 2010).

Histological results were in agreement with the measured activities of serum liver enzymes and provided supportive evidence for the biochemical analysis, in the current study, histopathological examination of the liver tissues showed congestion in portal vein with infiltration of mononuclear inflammatory cells, necrosis, vacuolar degeneration and kupffer cells activation in CCl_4 group. Accordance to these findings, Padhy *et al*. (2007) observed leukocytic infiltration, centrilobular necrosis and vacuolation in CCl_4 treated rats. Gupta *et al*. (2011) observed also fatty change, congestion in portal vein, necrosis, ballooning degeneration and loss of cellular boundaries. These finding relates with high activities of serum liver enzyme activities found in the CCl_4 group in the present results. Liver sections of rats pretreated with GbE showed regeneration of hepatocytes near normal liver architecture. This may be explained by the constituents of GbE are scavengers of free radicals, and inhibit lipid peroxidation, thus help to maintain the integrity and permeability of cell membranes and protects cells and tissues against oxidative stress induced by free radicals (Naik and Panda, 2007). Protective effect of GbE against CCl_4 induced hepatotoxicity in rats appears to be

related to inhibition of MDA and enhancement of antioxidant enzymes in addition to free radicals scavenging activity. Therefore, the hepatoprotective action, combined with antioxidant activity, has a synergistic effect in preventing the process of initiation and progress of hepatocellular diseases.

In conclusion, the present results demonstrated that GbE was effective in the prevention of CCl_4 induced acute toxic effects in rat liver, which were proven by biological evaluation, biochemical analysis, and further supported by the histological examinations in the liver tissues. This hepatoprotective activity is both preventive and curative. As a possible mechanism an aqueous extract of *G. biloba* leaf consists of many chemical constituents which could scavenge oxidative free radicals, inhibit lipid peroxidation, possess antioxidant activity and then alleviate acute liver toxicity. This absence of toxicity of GbE should be taken into account if safety measures for public health are to be implemented in response to increased ingestion of this herbal by human populations, given that GbE has been clinically prescribed for the treatment of various diseases. Further studies are required to isolate the active constituents in aqueous extract responsible for hepatoprotective activity and developing new drugs to treat drug/chemical-induced liver toxicity.

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Table (1): Effect of GbE on weight gain percent, food intake and food efficiency ratio (FER) in CCl_4-induced hepatoxicity in rats.

Experimental groups	Weight gain percent	Food Intake (g/ rat/day)	FER
Control	27.71 ± 0.72	19.16 ± 0.74	0.09 ± 0.002
CCl_4	a*** 12.28 ± 0.88	a*** 11.62 ± 0.55	a** 0.068± 0.006
GbE	26.54 ± 1.14	19.47 ± 0.58	0.086± 0.006
Pretreated with GbE + CCl_4	a * b *** 24.16 ± 0.83	a * b *** 17.31 ± 0.57	b ** 0.087± 0.001

- GbE *: Ginkgo biloba L. leaf* extract - CCl_4 :Carbon tetrachloride.
- Each value represents the mean of 6 rats ± SE.
- [a] Significant difference from control group at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$*** from control group.
- [b] Significant difference between CCl_4 group and CCl_4 group pretreated with GbE at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$***.

Table (2): Effect of GbE on serum aminotransferase (ALT, AST) and alkaline phophatase (ALP) enzyme activities, as well as total protein (TP) levels in CCl_4-induced hepatoxicity in rats.

Experimental groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T P (g/dl)
Control	39.1 ± 1.73	99.99 ± 3.99	70.68 ± 1.44	6.498± 0. 14
CCl_4	a*** 106.17 ± 1.54	a*** 215.86 ± 3.12	a*** 143.59 ± 1.93	a*** 4.54± 0.15
GbE	38.92 ± 1.16	97.98 ± 2.02	69.14 ± 1.24	6.71± 0.12
Pretreated with GbE + CCl_4	a * b *** 46.01 ± 2.28	a * b *** 111.6 ± 1.88	a * b *** 77.66 ± 2.23	a * b *** 5.92± 0.16

- GbE *: Ginkgo biloba L. leaf* extract - CCl_4 :Carbon tetrachloride.
- Each value represents the mean of 6 rats ± SE.
- [a] Significant difference from control group at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$*** from control group.
- [b] Significant difference between CCl_4 group and CCl_4 group pretreated with GbE at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$***.

Table (3): Effect of GbE on serum lipid profile parameters in CCl_4-induced hepatoxicity in rats.

Experimental groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	92.08 ± 2.57	86.29 ±2.47	44.99± 1.27	29.83± 3.33	17.26 ± 0.49
CCl_4	a*** 151.25± 2.24	a*** 121.16±2.11	a*** 33.37 ± 1.32	a*** 93.65 ± 2.35	a*** 24.23 ± 0.42
GbE	90.77 ± 2.69	85.13 ±2.62	45.36 ± 1.61	28.38 ± 2.87	17.03 ± 0.53
Pretreated with GbE + CCl_4	a* b*** 102.02 ± 2.41	a* b*** 94.45 ± 2.36	a* b*** 40.62 ± 1.01	a* b*** 42.51 ± 3.89	a* b*** 18.89 ± 0.47

- GbE *: Ginkgo biloba L. leaf* extract - CCl_4 :Carbon tetrachloride.
- Each value represents the mean of 6 rats ± SE.
- [a] Significant difference from control group at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$*** from control group.
- [b] Significant difference between CCl_4 group and CCl_4 group pretreated with GbE at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$***.

Table (4): Effect of GbE on hepatic lipid peroxide as (MDA) and reduced glutathione (GSH) in CCl_4-induced hepatoxicity in rats.

Experimental groups	MDA (nmol/g tissue)	GSH (mg/g tissue)
Control	185.6 ± 3.01	20.78 ± 0.34
CCl_4	a*** 259.17 ± 4.08	a*** 14.38 ± 0.50
GbE	182.00 ± 2.57	22.84 ± 0.95
Pretreated with GbE + CCl_4	a* b*** 198.34 ± 2.93	a* b*** 18.73± 0.64

- GbE *: Ginkgo biloba L. leaf* extract - CCl_4 :Carbon tetrachloride.
- Each value represents the mean of 6 rats ± SE.
- [a] Significant difference from control group at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$*** from control group.
- [b] Significant difference between CCl_4 group and CCl_4 group pretreated with GbE at $p < 0.05$*, highly significant difference at $p < 0.01$** and very highly significant difference at $p < 0.001$***.

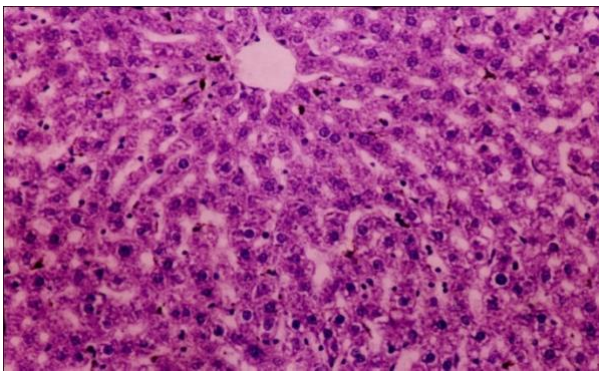

Fig. (1): Liver section of control rats showed normal histological structure of hepatic lobule and portal vein without alterations. (H&E stain x200)

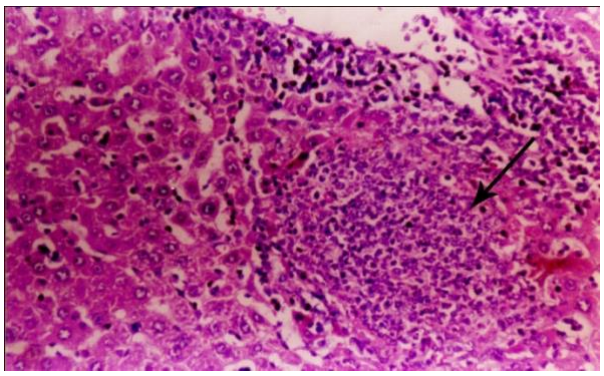

Fig. (2): Liver section of CCl_4 intoxicated rats showed focal area of hepatic necrosis associated with mononuclear cells infiltration (arrow). (H&E stain x200)

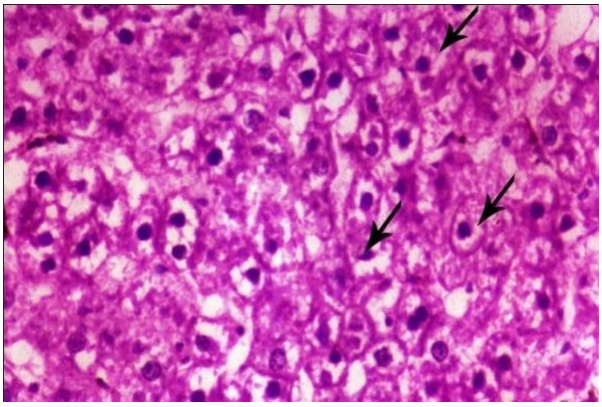

Fig. (3): Liver section of CCl_4 intoxicated rats showed ballooned hepatocytes and pyknosis of their nuclei (arrow). (H&E stain x200)

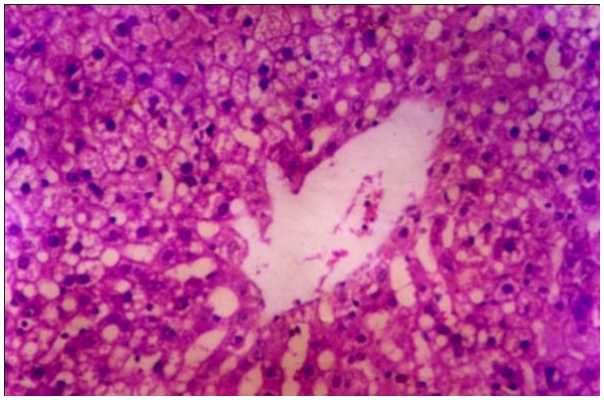

Fig. (4): Liver sections of CCl_4 intoxicated rats showed vacuolar degeneration of hepatocytes (fatty change). (H&E stain x200)

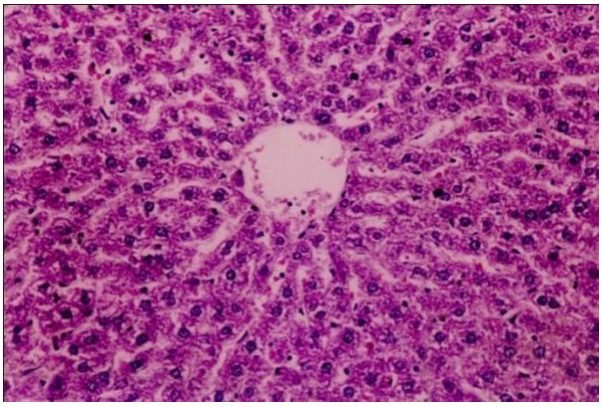

Fig. (5): Liver section of rats received GbE showed no histopathological changes. (H&E stain x200)

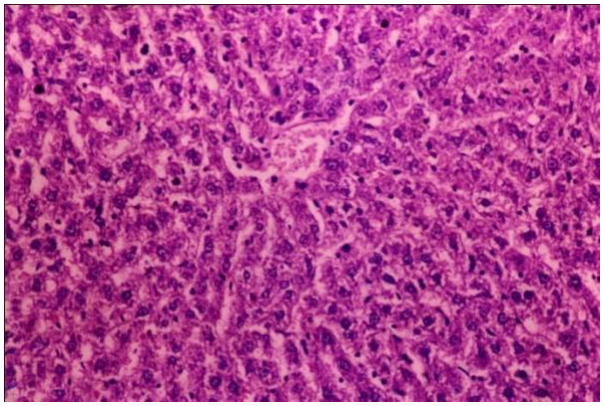

Fig. (6): Liver section of rats group pretreated with GbE showed apparent normal histological structure. (H&E stain x200)

تأثير المستخلص المائي لأوراق نبات الجنكوبيلوبا علي التسمم الكبدي الحاد المُحدث برابع كلوريد الكربون في الفئران

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الملخص العربي

الضغط التأكسدي له دور بالغ الاهمية في احداث وتقدم مختلف امراض الكبد. وقد أثبت المستخلص المائي لأوراق نبات الجنكوبيلوبا فعاليتة كمادة مضادة للاكسدة، بذلك فإن له دور في الوقاية والعلاج للامراض المرتبطة بالضغط التأكسدي. تهدف هذه الدراسة الي تقييم التأثير الواقي للكبد للمستخلص المائي لأوراق نبات الجنكو بيلوبا علي التسمم الكبدي الحاد المُحدث برابع كلوريد الكربون في الفئران. تم إحداث التسمم الكبدي في ذكور الفئران وذلك بالحقن داخل الغشاء البريتوني برابع كلوريد الكربون بجرعة مقدارها 1 مل/كجم من وزن الجسم كل 72 ساعة لمدة 14 يوماً ، بينما تم إعطاء المستخلص المائي لأوراق نبات الجنكو يومياً عن طريق الفم بجرعة مقدارها 150 مجم/كجم من وزن الجسم لمدة أسبوعين قبل الحقن برابع كلوريد الكربون واستمرحتى نهاية التجربة.

أظهرت النتائج أن الحقن برابع كلوريد الكربون قد أحدث تلف حاد في الكبد, حيث وجد إرتفاع ذو دلالة إحصائية في مستويات إنزيم ناقلة أمين الأسبارتات، إنزيم ناقلة أمين الآلانين، وإنزيم الفوسفاتيز القلوي في مصل الدم ، ومحتوي الكبد من المالونداي ألدهيد، وكذلك وجد انخفاضا ذو دلالة إحصائية في النسبة المئوية للوزن المكتسب، البروتين الكلي، الكوليستيرول المرتبط باللبيوبروتينات ذات الكثافة العالية في مصل الدم ومحتوي الكبد من الجلوتاثيون المختزل. وقد أظهرت المعالجة المسبقة بالمستخلص المائي لأوراق نبات الجنكو قبل الحقن برابع كلوريد الكربون فعالية في حماية الكبد، حيث وجد إنخفاض ذو دلالة إحصائية في نشاط انزيمات الكبد ومحتواه من المالونداي ألدهيد، متزامنا مع حدوث إرتفارع ذو دلالة إحصائية في البروتين الكلي، ومحتوي الكبد من الجلوتاثيون المختزل، أيضا أظهرت النتائج حُدوث تحسنا معنويا في النسبة المئوية للوزن المكتسب وكذلك مقاييس الدهون عند المقارنة بالمجموعة التي تم حقنها برابع كلوريد الكربون . وقد اظهر الفحص الهستوباثولوجي لانسجة الكبد أن الحقن برابع كلوريد الكربون قد تسبب في حُدوث تغييرات نسيجية وموت موضعي، مع وجود ارتشاح الخلايا الالتهابية والتنكس الفجوي في الخلايا الكبدية، بينما أدت المعالجة المسبقة بالمستخلص المائي لأوراق نبات الجنكو الي التغلب علي هذة التغييرات المُحدثة، حيث أن أغلبية الخلايا الكبدية أصبحت أقرب الي الحالة الطبيعية.

تشير نتائج هذه الدراسة إلى أن التأثير الواقي للكبد للمستخلص المائي لاوارق نبات الجنكو ضد التلف التأكسدي المَحدث برابع كلوريد الكربون قد يرجع إلى فعاليتة كمادة مضاد للأكسدة ودورة في تثبيط الضغط التأكسدي. ولذلك قد يمكن أستعمال هذا المستخلص المائي لاوراق نبات الجنكو في المستحضرات الدوائية ومكملات الاغذية لفائدتة في التقليل من السمية الكبدية.

الكلمات المفتاحية: نبات الجنكو بيلوبا، المستخلص المائي، ذكور الفئران، رابع كلوريد الكربون، أنزيمات الكبد، مقاييس الدهون، المالونداي ألدهيد، الجلوتاثيون المختزل، التأثير الواقي.