MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Antioxidant and Hepatoprotective Role of Ginkgo biloba Leaves Extract Against Experimental Hepatotoxicity in Male Rats

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Received:4/8/2019
Accepted: 2/11/2019

Abstract: Background: The rising numbers of patients with liver injury caused by many reasons such as usage of drugs, alcohol and malnutrition which induced an excessive production of free radicals which are responsible for several lived diseases, recent investigations have proved the important role of antioxidants to ameliorate the damage caused by these compounds. In addition, there is a great attention for the usage of herbal medicine in treatment of liver disease.

Objective: The present comparative study was carried out to evaluate the hepato-protective effect of *Ginkgo biloba* leaf extract (GbE) against hepatotoxicity induced experimentally by CCl_4 and comparing these effects with a reference drug (Legalon) that used commonly in Egypt to treat liver disorders.

Methods: Animals were treated orally with GbE at a dose of (100ml/kg) and Legalon drug at a dose (100ml/kg), once daily for one week before the first dose of CCl_4. Thereafter, liver fibrosis was induced by oral administration of CCl_4 at a dose of (2.5 ml/kg) in olive oil day after day for 8 weeks, and the administration of GbE and Legalon was continued at the same doses and duration. At the end of the experiment, biochemical indices changes and histopathological studies were examined to evaluate the protective effect of GbE.

Results: showed that CCl_4 induced a significant ($P>0.5$) increase in ALT, AST, ALP, MDA and lipid profile as compared with control group While, TP, alb, HDL, TAC, GSH, GPx, CAT and SOD showed a significant decrease when compared with control group. On the other hand, treatment with GbE caused alterations to all of these parameters when compared with both CCl_4-intoxicated group and Legalon group. All of these results were confirmed with the histopathological investigations.

Conclusion: pre-treatment of the animals with GbE improved the liver damage that induced experimentally by CCl_4. The biochemical parameters and the histopathological studies showed that GbE plays an important role as a potent antioxidant; it causes suppression of the oxidative stress, which subsequently leading to ameliorate hepatotoxicity and counteracting the progress of liver fibrosis.

keywords: Liver, Hepatotoxicity, Ginkgo, Legalon.

1.Introduction

Liver is the main organ that is involved in metabolic functions. Liver has a very important role in detoxification of hepatotoxicants which results from metabolic reactions, those hepatotoxicants may cause hepatic injury **(1).** Hepatotoxicity is the inability of the liver to perform its functions normally due to its damage by the increased levels of drugs or xenobiotics **(2)**. The substances that cause hepatotoxicity are known as hepatotoxicants or hepatotoxins that may include an overdose of certain medicinal drugs, industrial chemicals, and natural chemicals such as microcystins, herbal remedies and dietary supplements **(3, 4).**

Carbon tetrachloride (CCl_4) is usually used to stimulate liver injury by generation of free radicals that enables the researchers to evaluate hepatotoxicity **(5).** Besides the liver, there are many target organs for CCl_4 such as lungs, heart, testes, kidneys and brain **(6).** It was

reported that induced liver damage by CCl_4 results from activiation of CCl_4 into trichloromethyl CCl_3^* free radical by cytochrome P450 system in liver microsomes leading to lipid peroxidation of liver membranes **(7).**

The early stages of chronic liver injuries without effective treatments, irrespective of cause, results in reversible liver fibrosis that may be converted to irreversible cirrhosis. One of the most common features of liver fibrosis is the accumulation of hepatic extracellular matrix (ECM) proteins **(8).**

Herbal drugs have been used in the treatment of liver diseases since ancient periods; they are developed in Eastern medicine and have a time-honored history. In written records, the study of herbs dates back over 5,000 years – that was illustrated in the ancient Chinese and Egyptian papyrus writings as early as 3,000 BC **(9).** Researchers have reported that people in different countries tended to use the identical or comparable herbs for the purpose of prevention, diagnosis, improvement or treatment of physical and mental illness. It is reported that three-quarters of the world population depends on herbal and traditional medicine as a basis for primary health care **(10).** The activity of any hepatoprotective drug relies on its ability to reverse the toxic effects or restores the normal hepatic physiological functions **(11).** Recently, supplements of the natural source are commonly used in the treatment of several diseases as they have the ability to improve the efficacy of the drug or to reduce the toxic side effects **(12).**

Ginkgo biloba leaves extract GbE is one of the most widely used herbal supplements in traditional Chinese medicine for centuries **(13).** It has the ability to protect the liver from damage by decreasing lipid peroxidation and GSH depletion associated with stimulation gene expression of the antioxidant enzymes **(14).** *Ginkgo biloba* (Ginkgoaceae family) contains various phytoactive compounds like flavonoids, terpen-trilactones, proanthocyanidins, ginkgolic acids, biflavone, and ginkgotoxins **(15).** It also contains Ginkgo toxin that has been reported to be similar in the structure to vitamin B_6 **(16).** Therefore, the present study has been carried out to investigate the possible ameliorative effect of a plant extract (*Ginkgo biloba* leaves extract) and antifibrotic drug (Legalon) against liver toxicity induced experimentally by carbon tetrachloride in male rats. This was achieved in terms of several biomarkers and histopathological investigations.

2. Materials and methods

Chemicals

CCl_4 was purchased from the local distributor of Sigma Chemicals, Egypt, Legalon drug was purchased from a local pharmacy, and *Ginkgo biloba* leaves extract was prepared in the lab of natural products in Chemistry department, Faculty of science, Mansoura University reported by Şener, Ekşioğlu-Demiralp (17). All other reagents used in this study were of high quality and analytical grade.

Animals and experimental protocol

Adult male albino rats, eight weeks old weighing 100-120 g, were kept under a photoperiod of 12h light: 12h darkness schedule with lights-on from 06.00 to 18.00h. They were housed in stainless cages with air conditioned temperature (22-25^{o}C). The animals received standard laboratory diet composed of 40% crushed corn, 30% feed paddy, 20% grinded soybean, 10% barley, molasses and supplied with water *adlibitum* throughout the experimental period. After 2 weeks of acclimation, rats were divided into seven groups each comprising of eight animals.

Animal grouping:

Animals were divided in to seven groups (n=8). Group I: control group in which animals didn't receive any treatment. Group II: GbE control group in which animals have received GbE orally at a dose (100 mg/kg) day after day for 8 weeks (18). Group III: Legalon control(L) group that received Legalon at a dose (100mg/kg) day after day for 8 weeks(19). Group IV: rats were received CCl_4 dissolved in olive oil (V/V) at a dose (2.5 ml/kg body. wt) orally day after day for 8 weeks(20). Group V: animals received GbE and CCl_4 at the same doses mentioned previously. Group VI: rats received Legalon and CCl_4. Group VII: animals were administered GbE in addition to Legalon and CCl_4 at the same doses mentioned previously.

Sampling

At the end of the experimental period (8 weeks), overnight fasted rats were sacrificed and blood samples were collected into clean centrifuge tubes and allowed to clot, then centrifuge at 2000 Xg for 20 min for biochemical analysis. Blood sera were carefully separated and were kept at -20^{o}C for subsequent analysis of future estimations. The rats were sacrificed 24 h after the last treatment and the liver of each rat was excised then cut intotwo parts: one of them was stored in neutral formalin (10%) for the histopathological studies; the other part was weighed and frozen for biochemical analysis.

Preparation of liver homogenate

A tissue sample from a known portion of the liver was carefully homogenized in distilled water to form 10% (w/v) and they were saved at -20^{o}C.

Assessment of biochemical parameters

AST and ALT activities in serum and liver were assayed according to the methods of Reitman and Frankel (21). The activity of ALP and GGT were assayed according to the methods of Belfield and Goldberg (1971) and Persijn and Van der Slik (23) respectively . The total bilirubin was determined according to the method of Walters and Gerarde (24)**.** Serum and liver homogenate were used for the determination the levels of total proteins **(25),** albumin **(26),** globulin **(27),** total cholesterol **(28),** HDL**(29),** LDL **(30),** VLDL **(31)**and triglycerides **(32).** Liver homogenate used for determination of the level of lipid peroxidation (measured as malondialdehyde, MDA) according to the method of **(33),** and assay of antioxidants including GSH **(34),** GPx **(35),** SOD **(36),** and CAT **(37)**.

Histological and ultrastructural methods

Liver tissues were fixed in neutral formalin solution (10%), dehydrated in ascending grades of ethanol, cleared in xylene, embedded in a paraffin wax, sectioned at 5-7 µm and stained with hematoxylin and eosin. The stained sections were examined and photographed under a light microscope to detect histopathological changes **(38)** In addition to routine hematoxylin and eosin stain, Masson's trichrome stains (three-color staining protocol used in histology, connective tissue is stained blue, nuclei are stained dark red/purple, and cytoplasm is stained red/pink) were employed for identification of collagen fibers as a good marker for various diseases such as fibrosis **(39).**

Statistical analysis:

Results were expressed as means±SE.Statistical significance was calculated using one way analysis of variance (ANOVA) followed by post comparsion was carried out with LSD test using GraphPad Prism. Differences were considered significance at $p \leq 0.05$**(40).**

3. Results and Discussion

Evaluation of liver damage

Data represented in table(1) CCl_4-intoxicated group showed a significant increase ($P<0.05$) in the activities of hepatic and serum liver enzyme markers including ALT, AST, ALP and GGT in addition to the increased level of total bilirubin when compared to normal control group. While, a significant decrease ($P<0.05$) in the level of serum and hepatic total protein, albumin and globulin were observed in the CCl_4-intoxicated group as compared with normal control group. On the other hand, oral administration of *Ginkgo biloba* leaf extract against CCl_4-intoxication ameliorated all of these results as compared with both CCl_4-intoxicated group and Legalon administered groups.

Evaluation of lipid profile

Oral administration of CCl_4 resulted in a significant ($P<0.05$) increase in the concentration of total cholesterol, triglycerides, LDL-c and VLDL-c as compared with control group, but a significant decrease ($p<0.05$) in HDL-c level was observed as shown in table (3).On the contrary, rats protected with *Ginkgo biloba* leaf extract against CCl_4-intoxication showed a significant improvement in the lipid profile when compared with CCl_4-intoxicated group as shown in table (3).

Evaluation of lipid peroxidation and the antioxidant activities

Data represented in table (4) illustrated that CCl_4-intoxicated group showed a significant increase in MDA level resulted in tissue damage as well as decreased level of the antioxidants as compared with the control group, While, pretreatment with GbE caused a significant decrease

(P<0.05) in the level of hepatic MDA and a significant (P<0.05) increase in the level of hepatic GSH, GPx, CAT and SOD as compared with CCl_4-intoxicated group that indicated the elevated antioxidant capacity of GbE.

Table (1): Serum and hepatic biochemical parameters in the control and different treated animal groups

Parameters	Animal groups						
	Control	GbE	L	CCl_4	GbE+CCl_4	L+CCl_4	GbE+L+CCl_4
S. ALT (U/L)	51.5±2.58	52.5±1.06	59.8±0.886	199.9^{a}±3.37	88.6^{a,b}±4.19	137.1^{a,b}±3.54	126.6^{a,b}±4.16
S. AST (U/L)	89.3±1.27	85.33±1.273	91.59±1.133	246.5^{a}±1.133	111.9^{b}±9.610	146.2^{a,b}±5.533	150.6^{a,b}±7.97
S. ALP (IU/L)	127.5±1.99	169.0±1.813	175.3±2.635	295.0^{a}±23.39	153.2^{b}±2.745	186.4^{a,b}±3.129	179.5^{a,b}±3.75
S. GGT (U/L)	13.5±0.429	12.64±0.3110	13.38±0.2661	26.75±0.3848	18.73±0.517	21.23±0.5954	20.09±0.537
S. TotalBilirubin(mg/dl)	0.49±0.032	0.523±0.0189	0.578±0.0125	0.725^{a}±0.0144	0.56^{b}±0.024	0.67^{a}±0.01958	0.66^{a}±0.014
S. Totalprotein(g/dl)	5.598±0.42	6.140±0.2410	5.450±0.1963	2.463^{a}±0.196	5.213^{b}±0.106	3.715^{a,b}±0.144	3.695^{a}±0.186
S. TotalAlbumin(g/dl)	4.02±0.038	3.99±0.05935	3.485±0.0718	1.803^{a}±0.1211	3.938^{b}±0.177	3.300^{a,b}±0.136	3.29^{a,b}±0.063
S. TotalGlobulin(g/dl)	2.08±0.022	2.268±0.0427	1.973±0.0206	1.655^{a}±0.0239	2.035^{b}±0.010	1.718^{a}±0.0296	2.045^{b}±0.014
A/G ratio	2.08±0.022	2.268±0.0427	1.973±0.0206	1.655^{a}±0.0239	2.035^{b}±0.010	1.718^{a}±0.0295	2.045^{b}±0.014
H. ALT (U/g)	58.20±1.10	55.83±1.230	71.86±4.287	105.4^{a}±3.215	80.28^{a,b}±1.15	91.02^{a,b}±2.059	87.09^{a,b}±1.66
H. AST (U/g)	57.62±2.655	60.41±1.063	71.07±2.393	122.6^{a}±2.503	84.61^{a,b}±2.05	91.54^{a,b}±±2.68	87.21^{a,b}±1.73
H.Totalprotein(g/100g wet tissue)	5.26±0.113	5.063±0.0551	4.90±0.06745	3.145^{a}±0.0366	4.74^{a,b}±0.168	4.12^{a,b}±0.0591	4.55^{a,b}±0.186

Values are presented as means ±SE (n=8rats per each group).

(P<0.05), a: significance as compared with control, b: significance as compared with CCl_4 group. S:serum, H: hepatic.

Table (2): Lipid profile (serum& liver) in control and different treated animal groups:

Parameters	Animal groups						
	Control	GbE	L	CCl_4	GbE+CCl_4	L+CCl_4	GbE+L+CCl4
S.Total cholesterol(mg/dl)	109.3±0.6989	109.2±0.7596	112.2±0.4966	132.4^{a}±1.223	116.9^{a,b}±1.004	118.8^{a,b}±0.77	117.8^{a,b}±0.64
S.tri glyceride(mg/dl)	87.87±0.7074	86.79±0.8315	89.63±0.7112	109.5^{a}±0.6460	100.6^{a,b}±0.9088	101.5^{a,b}±0.97	99.80^{a,b}±0.64
S.HDL-Cholesterol(mg/dl)	35.39±0.6963	35.18±0.4447	30.75±1.443	23.76^{a}±0.6221	31.63^{b}±0.3856	27.18^{a}±1.693	29.48^{a,b}±1.175
S.LDL-Cholesterol(mg/dl)	55.46±1.300	53.45±0.6763	59.12±1.603	76.05^{a}±1.024	61.81^{a,b}±0.9186	69.40^{a,b}±1.35	67.26^{a,b}±1.61
S.VLDLCholesterol(mg/dl)	17.40±0.2517	17.51±0.2341	17.95±0.3167	21.95^{a}±0.1157	19.07^{a,b}±0.1862	20.35^{a,b}±0.23	19.82^{a,b}±0.13
H.Total cholesterol(mg/g)	96.31±2.248	104.2±1.238	108.9±0.9704	132.5^{a}±1.433	115.2^{a,b}±1.295	119.4^{a,b}±0.49	117.1^{a,b}±1.11
H.tri glyceride(mg/dl)	290.6±5.582	274.4±9.049	282.3±5.195	344.6^{a}±5.496	298.1^{b}±4.251	308.0^{b}±3.660	309.0^{b}±1.988

Values are presented as means ±SE (n=8rats per each group).

(P<0.05), a= significance as compared with control, b= significance as compared with CCl_4 group. S=serum, H= hepatic MDA= Malondialdehyde, SOD= superoxide dismutase, GSH= Glutathione, Gpx= Glutathione peroxidase.

Table (3): Lipid peroxidation product (MDA) levels and antioxidant parameters in control and different animal groups:

Parameters	Animal Groups						
	Control	GbE	L	CCl_4	GbE+CCl_4	L+CCl_4	GbE+L+CCl_4
MDA(nmol/mg)	0.098±0.0026	0.089±0.0045	0.17±0.01486	0.40^{a}±0.00299	0.175^{b}±0.0187	0.31^{a,b}±0.0351	0.23^{a,b}±0.01755
Totalantioxidant capacity(ng/ml)	0.254±0.0045	0.317±0.00868	0.194±0.0132	0.119^{a}±0.0073	0.21^{ab}±0.0036	0.16^{a,b}±0.0072	0.187^{a,b}±0.0056
GSH(ng/ml)	0.24±0.00540	0.2910±0.0086	0.247±0.0098	0.061^{a}±0.0194	0.205^{b}±0.0023	0.167^{a,b}±0.005	0.18^{a,b}±0.00575
GPX(ng/ml)	0.204±0.0020	0.219±0.00168	0.149±0.0108	0.097^{a}±0.0064	0.187^{b}±0.0032	0.109^{a}±0.0087	0.1205^{a}±0.0068
Catalase(ng/ml)	0.288±0.0018	0.357±0.00625	0.307±0.0032	0.145^{a}±0.0036	0.29^{b}±0.00598	0.2^{a,b}±0.00512	0.21^{a,b}±0.00405
SOD(IU/mg)	11.41±0.3534	12.13±0.2241	10.99±0.4251	6.248^{a}±0.2805	9.613^{a,b}±0.369	8.723^{a,b}±0.491	8.885^{a,b}±0.3756

Values are presented ac means ±SE (n=8rats per each group).

(P<0.05), a: significance as compared with control, b: significance as compared with CCl_4 group. S: serum, H: hepatic, MDA: Malondialdehyde, SOD: superoxide dismutase, GSH: Glutathione, GPX: Glutathione peroxidase.

Histological examination of Hematoxylin and Eosin (H&E) stained sections of liver

Histological examination of H&E stained sections of control liver showed normal lobular architecture with normal hepatocytes arranged around the central vein in strands and separated by clear blood sinusoids (Fig. A). The liver section of CCl_4-intoxicated animals showed absence of normal liver architecture with several histopathological changes were observed involving, deforming hepatocytes with atrophied densely stained nuclei, intense

infiltration of inflammatory cells, dialation and congestion of blood vessels, and collapsed blood sinusoids with more dense kupffer cells. Swelling hepatocytes and hepatocytes with cytoplasmic vacuolization and karyolitic nuclei, necrosis and connective tissue hyperplasia were observed (Fig. D). The liver section of CCl_4-intoxicated rats, pre-treated with *Ginkgo biloba* leaves extract (GbE) observed that hepatocyte denaturation and necrosis were not obvious due to GbE protection, hyperplasia of connective tissue was also decreased as well as liver fibrosis (Fig. E). The histopathological observations of liver section induced by co-administration of GbE &Legalon in combination against CCl_4-intoxication for 8 weeks showed a remarkable improvement in the liver tissue compared with CCl_4-intoxicated rats, especially the density of the lobular fibrosis that also decreased (Fig. G).

Histopathological examination of Masson's trichrome stained sections:

Examination of control liver specimens stained for collagen by masson's trichrome method showed collagen to be as blue fibrils in dense bundles around blood vessels and lesser amount around blood sinusoids as observed in Fig.A. Liver specimens of rats intoxicated with CCl_4 showed extensive accumulation of connective tissue resulting in formation of continuous interlobular septa, evident alterations and dilations in the central vein and marked inflammation illustrated in sever neutrophilic infiltration, extensive fatty changes and sever centrilobular necrosis compared to the normal control group as displayed in Fig. B. Examination of liver sections of rats pretreated with Ginkgo biloba leaves extract (GbE) against CCl_4-intoxication showed less collagen deposition around most hepatic veins. However, in few spots collagen fibrils were more or less thick around further central vein, suggesting that the liver repaired itself as shown in Fig C.

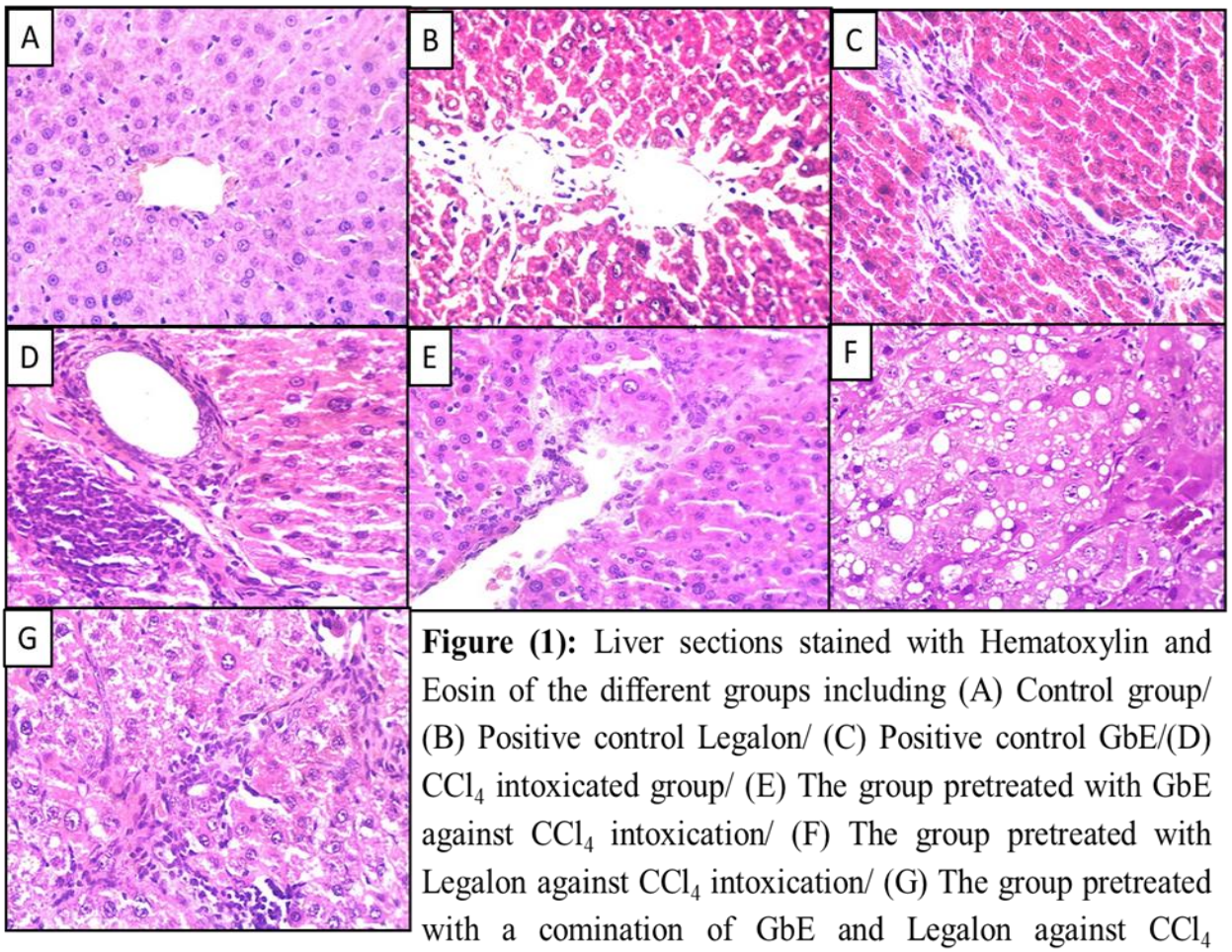

Figure (1): Liver sections stained with Hematoxylin and Eosin of the different groups including (A) Control group/ (B) Positive control Legalon/ (C) Positive control GbE/(D) CCl_4 intoxicated group/ (E) The group pretreated with GbE against CCl_4 intoxication/ (F) The group pretreated with Legalon against CCl_4 intoxication/ (G) The group pretreated with a comination of GbE and Legalon against CCl_4 exposure.

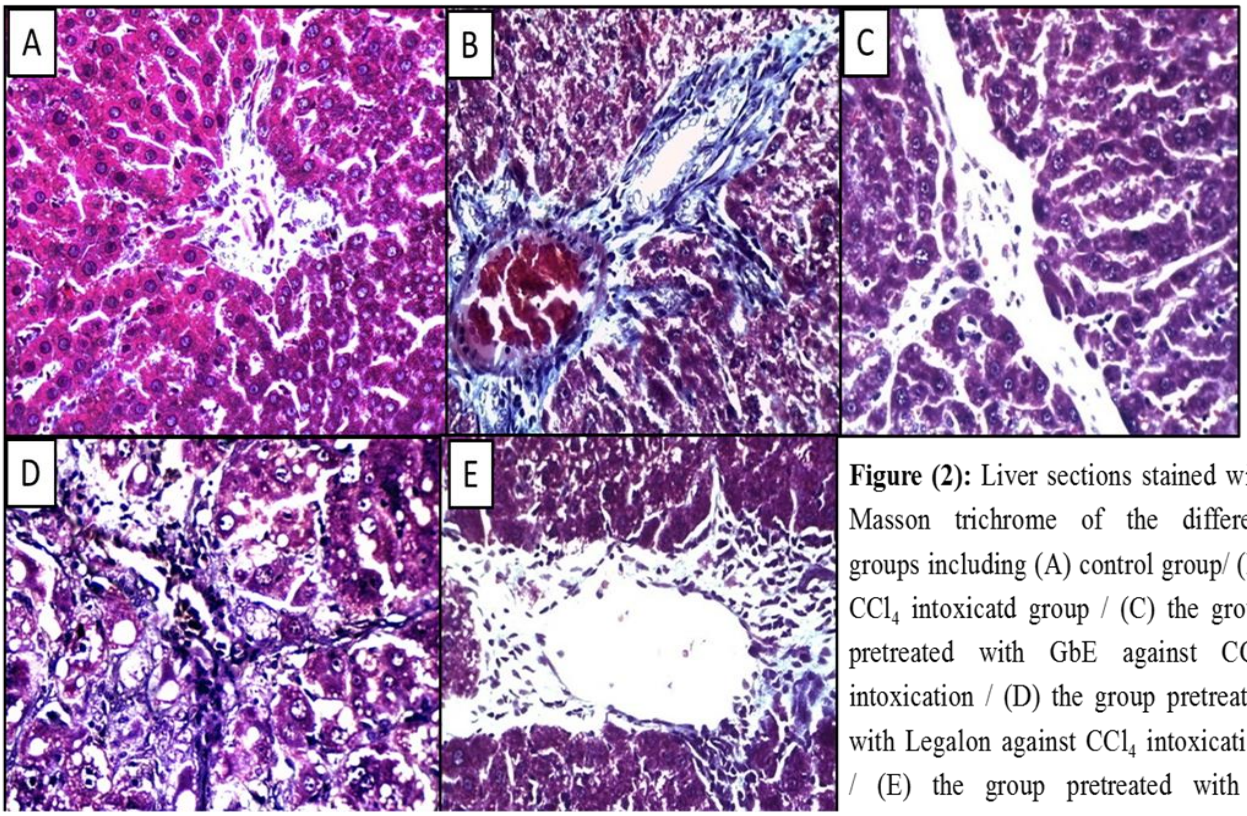

Figure (2): Liver sections stained with Masson trichrome of the different groups including (A) control group/ (B) CCl_4 intoxicatd group / (C) the group pretreated with GbE against CCl_4 intoxication / (D) the group pretreated with Legalon against CCl_4 intoxication / (E) the group pretreated with a

Discussion

Liver fibrosis is considered as a pathological response produced from exposure to certain drugs for long periods or inflammatory liver diseases. It is the method by which wounds in liver tissue are healed during exposure to a frequent or prolonged injury **(41).** After an acute injury, hepatocytes tend to regenerate and replace the necrotic and apoptotic cells; with minimal inflammatory response and extracellular matrix deposition. But in case of chronic injury, the ability to regenerate is decreased and hepatocytes are forced to undergo apoptosis which ultimately resulted inactivation of hepatic stellate cells (HSCs), proliferation and increased production of extracellular matrix (ECM)**(42).**

Improvement in the understanding of the ways by which the herbs positively affect health and quality of life leads to a great acceptance of the herbal medicine from the public and medical researchers **(43).** The efficacy of a hepatoprotective drug usually depends on its ability to preserve standard physiological function or to decrease the worth effect induced by hepatotoxic agents **(11, 44).** *Ginkgo biloba* is regarded as the oldest living tree species in the world. Its origin is from china, *Ginkgo biloba* is now planted in all over the world **(45).** Nowadays, ginkgo leaf extracts have several medical uses such as the improvement of memory, treatment or prevention of Alzheimer disease and other types of dementia, reducing intermittent claudication **(46-48).** Additionally, these extracts are also involved in the treatment of multiple sclerosis, tinnitus, sexual dysfunction, and other health cases **(47, 49-51).**

In the present study *Ginkgo biloba* leaf extract was found to be more effective than Legalon as it is nontoxic, have no side effects and no mortality was observed, but Legalon administered group showed number of mortality cases and weight loss was observed. All of these results were confirmed with the biochemical parameters and histopathological studies.

In the current study, the oral administration of CCl_4 produced a significant rise in the serum and hepatic activities of AST, ALT, ALP and GGT. Also, a significant rise in the serum total bilirubin was observed as compared with normal control group. That is an indicator for the massive alteration of the liver function and cellular damage. The present results are in accordance with those of Eidi, Mortazavi (52), Wu, Li (53),Bouhrim, Ouassou (54) and Essawy, Abdel-Wahab (55). Additionally**,**

Okpara, Atiku (56) demonstrated that administration of CCl_4 produced a significant elevation in activity of AST, an enzyme present in liver, skeletal muscles and myocardial cells. The increased AST activity if considered as evidence to hepatic or muscle damage induced by CCl_4 resulted in increased release of AST into the peripheral circulation. In the same study they reported that CCl_4 also induced a significant increase in ALP activity. Increased activity of ALP is not only resulted from liver damage but also associated with pathological changes in the bone, kidneys, bile duct and testes **(57, 58).** Based on that, elevated activity of ALP in the CCl_4-intoxicated group resulted from disturbances to any of these organs **(56).**

On the other hand, the present results illustrated that pretreatment of rats with *Ginkgo biloba* leaves extract decreased liver fibrosis process and tissue damage induced by CCl_4 administration as confirmed by the results of liver function markers including a significant decrease in total bilirubin, transaminases (ALT, AST), phosphatase ALP and GGT as compared with CCl_4 intoxicated group. These results are in parallel with several previous studies including **(59), (60).**While, rats treated with Legalon as a protective agent against CCl_4 intoxication showed no significance when compared with CCl_4 intoxicated group. Consistent with the previous studies, administration of GbE for 8 weeks produced inhibitory effects on the levels of important liver function indicators, based on these data in combination with the results of hematoxylin and eosin (H&E) and Masson's trichrome staining as well as transmission electron microscopy examination that was published in another paper. So, GbE is able to protect liver damage.

CCl_4-intoxication caused reduction in the levels of total proteins, albumin and globulin that is regarded as affirmation of liver injury. Diminished level of the proteins is an indicator of the liver damage; it also indicates the failure of the liver to synthesize proteins **(55)**. In the present study, CCl_4-intoxication resulted in hepatotoxicity, therefore total protein and albumin were decreased significantly as compared with healthy rats. These results come in parallel with several previous studies **(61-63).** On the other hand, the results of the present study illustrated that pre-treatment with *Ginkgo biloba* leaves extract against CCl_4-intoxication resulted in elevated concentration of total proteins and albumin compared with CCl_4-intoxicated group **(64)**.

Results in table (3) revealed that serum cholesterol, triglycerides, and LDL cholesterol increased while HDL cholesterol decreased in CCl_4 intoxicated rats group compared with control group. These results are in parallel with several previous studies **(65, 66).** On the contrary**,** administration of GbE showing a significant decrease in lipid profile marker that was in agreement with the studies of Abdel-Zaher, Farghaly (67), and Huang, Zhang (68)**.**

It has been established in several previous studies that the body contains several ways of defense against free radicals comprising metabolization of radical scavengers and chain terminators such as vitamin C and E, antioxidants like GSH and redox regulatory enzymes such as CAT, SOD and glutathione peroxidase**(69).** The results of the present study showed a significant increase in the oxidative stress markers while levels of antioxidants were diminished significantly in rats supplemented with CCl_4 that may cause cell, tissue or organ damage. The results also showed a significant elevation in MDA level which considered as the end product of lipid peroxidation process that is an indicator for the oxidative degradation of polyunsaturated lipids subsequently leading to many disorders and diseases ultimately may cause cell death. The present results are in accordance with earlier studies which showed that Products of lipid peroxidation are generated when ROS cause degeneration of polyunsaturated fatty acids, leading to membrane structural and/or functional damage **(56, 70-72).** Additionally**, (73)** reported that ROS disturbs the antioxidant defense mechanisms, reduces the potency of SOD elicited liver injury, cirrhosis and hepatocarcinoma.

On the contrary, oral administration of *Ginkgo biloba* leaf extract to protect against toxicity induced by CCl_4 and its reactive metabolites resulted in reduction in the oxidative stress markers and elevation in the level of antioxidant enzymes. MDA level also was observed to be diminished, subsequently

cause inhibition of lipid peroxidation and prevents cells obstruction, eventually protects liver from damage induced by ROS. These results are in agreement with that of Þener, Kabasakal (74) who evaluated the antioxidant and antifiberotic effects of long term administration of *Ginkgo biloba* extract on liver fibrosis stimulated by bile duct ligation (BDL) and scission in Wister male albino rats. They reported that *Ginkgo biloba* militate against the oxidative damage to the liver by BDL in rats. This action probably includes the inhibition of neutrophil infiltration and lipid peroxidation.

Conclusion: The results of the present study showed that Ginkgo biloba leaves extract provide significant protection against chronic liver injuries induced by CCl_4 and is a potentially contain beneficial agent to reduce liver damage by suppressing the oxidative stress in the experimental animal. Moreover, the hepatoprotective effect of GbE seemed to be better than Legalon only or with GbE. GbE is able to protect the liver from damage since it has antioxidant properties, prevent lipid peroxidation and replenishes the glutathione level. The benefit may be comparable to that of Legalon (derived from silymarin). However, the role of GbE as a therapeutic agent alone in liver damage needs to be further investigated.

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