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Comparative analysis of the antihepatotoxic effects of Ginkgo biloba leaf extract and Legalon using histological and biochemical techniques

Abstract

Drugs, alcohol, and poor nutrition all contribute to the overproduction of free radicals, which linked to numerous diseases and resulted in a high number of cases of liver injury. Antioxidants have shown to play a significant role in reducing the harm caused by these compounds in recent studies. Treatment of liver disease with plants from the natural world has received considerable attention for quite some time.

This study compared *Ginkgo biloba* leaf extract (GbE) with a commonly used drug in Egypt called Legalon for treating liver disorders, in order to assess GbE's hepato-protective effect against hepatotoxicity induced experimentally by CCl_4.

Before the first dose of CCl_4, animals given GbE (100 ml/kg) and Legalon drug (100 ml/kg) orally, once a day, for a week. After that, CCl_4 given orally at a dose of (2.5 ml/kg) in olive oil daily for 8 weeks to induce liver fibrosis, and the administration of GbE and Legalon maintained at the same dose and duration. The protective effect of GbE was determined by observing the result of the experiment, which included a shift in biochemical indictors and the outcomes of histopathological studies.

In comparison to the control group, CCl_4 significantly ($P<0.5$) increased the levels of ALT, AST, ALP, MDA, and lipid profile. In contrast, markers of oxidative stress, including TP, ALB, HDL, TAC, GSH, GPx, CAT, and SOD, were significantly lower in the study's experimental group than in the control group. Nevertheless, GbE treatment led to differences across the board when compared to the CCl_4-intoxicated and Legalon groups. With the help of the histopathological investigations, all of these findings verified.

Conclusion: Liver damage caused experimentally by CCl_4 mitigated when the animals pretreated with GbE. Both biochemical and histopathological studies found that GbE acts as a powerful antioxidant, suppressing oxidative stress to reduce hepatotoxicity and slow the development of liver fibrosis.

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Introduction

Most metabolic processes take place in the liver. Hepatic injury can be caused by hepatotoxicants produced in metabolic reactions, which the liver plays a crucial role in detoxifying.[1] Toxic effects on the liver from substances like drugs or foreign chemicals is known as hepatotoxicity.[2] Hepatotoxicity caused by an overdose of a variety of substances, including pharmaceuticals, industrial chemicals, and even naturally occurring chemicals like microcystins, herbal remedies, and nutritional supplements; these substances are collectively referred to as "hepatotoxicants".[3,4]

Hepatotoxicity testing typically involves the use of carbon tetrachloride (CCl_4) to induce liver injury by producing free radicals.[5] Other than the liver, CCl_4 can also damage the lungs, heart, testicles, kidneys, and brain.[6] Lipid peroxidation of liver membranes has been linked to CCl_4-induced liver damage, which is caused by the bioactivation of CCl_4 into the trichloromethyl CCl_3* free radical by the cytochrome P450 system in liver microsomes.[7] Reversible liver fibrosis may progress to irreversible cirrhosis if the early stages of chronic liver injuries, regardless of cause, not treated effectively. Hepatic extracellular matrix (ECM) protein accumulation is a characteristic of liver fibrosis.[8]

In the East, herbal medicines with a long history of success in treating liver disease have used for thousands of years. Herbal medicine has been studied for over 5,000 years, with early examples found in Chinese and Egyptian papyrus writings from around 3,000 B.C.[9] Researchers have found that people in different countries frequently use the same or very similar herbs for the purpose of preventing, diagnosing, enhancing, or treating physical and mental illness. Some estimates place the number of people who use herbal and traditional medicine as their primary health care system at 75 percent.[10] For a drug to be considered hepatoprotective, it must be able to either counteract the toxic effects of the disease or restore normal hepatic physiological functions.[11] Supplements derived from natural sources have become increasingly popular in recent years due to their potential to increase a drug's therapeutic efficacy or lessen its potentially harmful side effects in the treatment of a variety of diseases.[12]

Leaf extract from the *ginkgo biloba* tree, also known as GbE or gbiloba, has been used as a supplement in traditional Chinese medicine (TCM) for centuries.[13] It prevents liver damage by increasing the expression of genes responsible for producing antioxidant enzymes and decreasing lipid peroxidation and glutathione (GSH) depletion.[14] There are many different types of phytoactive compounds in *Ginkgo biloba* (family Ginkgoaceae), including flavonoids, terpenetrilactones, proanthocyanidins, ginkgolic acids, biflavones, and ginkgotoxins.[15] Ginkgo toxin, which has been linked to a structure similar to that of vitamin B_6, is also present.[16] In light of this, the current study sought to examine whether an antifiberotic drug (Legalon) and a plant

extract (*Ginkgo biloba* leaves extract) could help reduce the liver toxicity caused by carbon tetrachloride in male rats. Many different biomarkers and histopathological analyses helped to confirm this.

Materials and methods

Chemicals

According to the study by Şener, G. et al., and colleagues, CCl_4 was purchased from the Egyptian distributor of Sigma Chemicals, Legalon was purchased from a local pharmacy, and an extract of *Ginkgo biloba* leaves was prepared in the lab of natural products in chemistry department of the science faculty.[17] The other reagents in this study were also of a high quality and analytical grade.

Animals and experimental protocol

Eight-week-old male albino rats weighing 100-120 g were subjected to a photoperiod of 12 hours of light followed by 12 hours of darkness, with the lights on from 6 to 18 hours per day. They kept at a comfortable temperature in stainless steel cages with climate control (22-25°C). Throughout the duration of the experiment, the animals were fed a standard laboratory diet of 40% crushed corn, 30% feed paddy, 20% ground soybean, 10% barley, and molasses, and had access to water ad libitum. After two weeks of adaptation, eight rats randomly assigned to one of seven groups.

Animal grouping

The 56 animals split up into seven groups. Group I: the control group, which consisted of untreated animals. Group II: Animals in this group received GbE orally every day for eight weeks at a dose of 100 mg/kg.[18] Group III: A group that received Legalon at a dose (100mg/kg) every day for 8 weeks.[19] IV rats received CCl_4 dissolved in olive oil (V/V) every day for eight weeks at a dose of 2.5 ml/kg body weight.[20] Animals in group V were given the same doses of GbE and CCl_4 as before. Rats in group VI received CCl_4 and Legalon. Animals in group VII received GbE, Legalon, and CCl_4 at the same doses as before.

Sampling

After an overnight fast, rats sacrificed at the end of the experimental period (8 weeks), and blood samples taken, allowed to clot, and then centrifuged at 2000 rpm for 20 min for biochemical analysis. For further analysis and projections, blood sera carefully separated and stored at -20°C. 24 hours after the last treatment, the rats sacrificed, and their livers removed and divided into two parts: one was stored in neutral formalin (10%) for histopathological investigation, and the other frozen for biochemical evaluation.

Preparation of liver homogenate

Labeled samples of liver tissue were weighed, homogenised in distilled water to create a 10% (w / v) homogenate, and then stored frozen at -20°C for later use.

Assessment of biochemical parameters

AST and ALT activities in serum and liver were measured according to the methods of Reitman and Frankel.[21] The activity of ALP and GGT were measured according to the methods of Belfield and Goldberg (1971) and respectively Persijn and Van der Slik.[23] We also measured the level of total bilirubin according to the method of Walters and Gerarde.[24] Additionally, we used the serum and liver homogenate for determination the level of total proteins,[25] albumin,[26] globulin,[27] total cholesterol,[28] HDL,[29] LDL,[30] VLDL,[31] and triglycerides.[32] Lipid peroxidation (as measured by malondialdehyde, MDA) was also assessed by preparing liver homogenate from the rats in each group and applying the method described by Okhawa et al.[33] Furthermore, antioxidants including GSH,[34] GPx,[35] SOD,[36] and CAT[37] were measured in the liver homogenate.

Histological methods

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

Statistical analysis

Results displayed using means±SE. One-way analysis of variance (ANOVA) used to determine the statistical significance, and then the LSD test used to compare the results using GraphPad Prism. At $p \leq 0.05$, differences were deemed significant.[40]

Results

Evaluation of liver damage

When compared to the normal control group, the data from table (1) for the CCl_4-intoxicated group showed a significant rise ($P < 0.05$) in the activities of hepatic and serum liver enzyme markers like ALT, AST, ALP, and GGT as well as a rise in the level of total bilirubin. The level of serum and hepatic total protein, albumin, and globulin in the CCl_4-intoxicated group was found to be significantly lower ($P<0.05$) than in the normal control group. As opposed to both the CCl_4-intoxicated group and the Legalon administered group, oral administration of *Ginkgo biloba* leaf extract against CCl_4-intoxication improved all of these outcomes.

Evaluation of lipid profile

When CCl_4 was administered orally, total cholesterol, triglycerides, LDL cholesterol, and VLDL-C concentrations increased significantly ($P < 0.05$) compared to the control group, but HDL- C levels decreased significantly ($P < 0.05$), as shown in the table 3. On the other hand, as shown in table, rats protected with *Ginkgo biloba* leaf extract against CCl_4-intoxication displayed a significant improvement in the lipid profile (2).

Evaluation of lipid peroxidation and the antioxidant activities

Data shown in table 3& Figure 3 demonstrated that, in comparison to healthy rats, the CCl_4-intoxicated group showed a significantly higher level of MDA (the final by-product of lipid peroxidation), which led to tissue damage. As opposed to the CCl_4-intoxicated group, pretreatment with GbE resulted in a significant ($P < 0.05$) decrease in the level of hepatic MDA as well as a significant ($P < 0.05$) increase in the level of hepatic GSH, GPx, CAT, and SOD, indicating the elevated antioxidant capacity of GbE.

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

Upon histological examination, normal lobular architecture with normal hepatocytes arranged around the central vein in strands and

separated by clear blood sinusoids seen in the control liver's H& E-stained sections. Figure 4A The liver section of CCl_4-intoxicated animals showed absence of normal liver architecture with several histopathological changes 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. Figures 4B& C Hepatocytes with cytoplasmic vacuolization and karyolitic nuclei, necrosis, and connective tissue hyperplasia, as well as swollen hepatocytes seen. Figure 4D Pre-treatment with *Ginkgo biloba* leaves extract (GbE) prevented hepatocyte denaturation and necrosis from being visible in the liver sections of CCl_4-intoxicated rats. Hyperplasia of connective tissue and liver fibrosis were also reduced. Figure 4E The liver tissue showed a remarkable improvement compared to CCl_4-intoxicated rats in the histopathological observations of the liver section caused by co-administration of GbE & Legalon in combination against intoxication for 8 weeks, especially the density of the lobular fibrosis that also decreased. Figure 4G.

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

Parameters Animal groups	S.ALT (U/L)	S.AST (U/L)	S.ALP (IU/L)	S. GGT (U/L)	S.Total Bilirubin (mg/dl)	H.ALT (U/g)	H.AST (U/g)
Control	51.5±2.58	89.3±1.27	127.5±1.99	13.5±0.429	0.49±0.032	58.20±1.10	57.62±2.655
GbE	52.5±1.06	85.33±1.273	169.0±1.813	12.64±0.3110	0.523±0.0189	55.83±1.230	60.41±1.063
L	59.8±0.886	91.59±1.133	175.3±2.635	13.38±0.2661	0.578±0.0125	71.86±4.287	71.07±2.393
CCl_4	199.9^{a}±3.37	246.5^{a}±1.133	295.0^{a}±23.39	26.75^{a}±0.3848	0.725^{a}±0.0144	105.4a±3.215	122.6^{a}±2.503
GbE+CCl_4	88.6^{a,b}±4.19	111.9^{b}±9.610	153.2^{b}±2.745	18.73^{a,b}±0.52	0.56^{b}±0.024	80.28^{a,b}±1.15	84.61^{a,b}±2.05
L+CCl_4	137.1^{a,b}±3.54	146.2^{a,b}±5.533	186.4^{a,b}±3.129	21.23^{a,b}±0.595	0.67^{a}±0.01958	91.02^{a,b}±2.059	91.54^{a,b}±±2.68
GbE+L+CCl_4	126.6^{a,b}±4.16	150.6^{a,b}±7.97	179.5^{a,b}±3.75	20.09^{a,b}±0.537	0.66^{a}±0.014	87.09^{a,b}±1.66	87.21^{a,b}±1.73

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

Parameters Animal groups	S.Total protein (g/dl)	S.Total Albumin (g/dl)	S.Total Globulin (g/dl)	H.Total protein (g/g tissue)
Control	5.598±0.42	4.02±0.038	2.08±0.022	5.26±0.113
GbE	6.140±0.2410	3.99±0.05935	2.268±0.0427	5.063±0.0551
L	5.450±0.1963	3.485±0.0718	1.973±0.0206	4.90±0.06745
CCl_4	2.463^{a}±0.196	1.803^{a}±0.1211	1.655^{a}±0.0239	3.145^{a}±0.0366
GbE+CCl_4	5.213^{b}±0.106	3.938^{b}±0.177	2.035^{b}±0.010	4.74^{a,b}±0.168
L+CCl_4	3.715^{a,b}±0.144	3.300^{a,b}±0.136	1.718^{a}±0.0296	4.12^{a,b}±0.0591
GbE+L+CCl_4	3.695^{a}±0.186	3.29^{a,b}±0.063	2.045^{b}±0.014	4.55^{a,b}±0.186

Data is shown as means+SE (n = 8 rats in each group).

(P<0.05), a = significance in comparison to the control group, b = comparison to the CCl_4 group. H=hepatic, S=serum.

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

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

Data is shown as means±SE (n = 8 rats in 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	MDA (nmol/ mg)	Total antioxidant capacity(ng/ml)	GSH (ng/ml)	GPX (ng/ml)	Catalase (ng/ml)	SOD (IU/mg)
Control	0.098±0.0026	0.254±0.0045	0.24±0.00540	0.204±0.0020	0.288±0.0018	11.41±0.3534
GbE	0.089±0.0045	0.317±0.00868	0.2910±0.0086	0.219±0.00168	0.357±0.00625	12.13±0.2241
L	0.17±0.01486	0.194±0.0132	0.247±0.0098	0.149±0.0108	0.307±0.0032	10.99±0.4251
CCl_4	0.40^{a}±0.00299	0.119^{a}±0.0073	0.061^{a}±0.0194	0.097^{a}±0.0064	0.145^{a}±0.0036	6.248^{a}±0.2805
GbE+CCl_4	0.175^{b}±0.0187	0.21^{ab}±0.0036	0.205^{b}±0.0023	0.187^{b}±0.0032	0.29^{b}±0.00598	9.613^{a,b}±0.369
L+CCl_4	0.31^{a,b}±0.0351	0.16^{a,b}±0.0072	0.167^{a,b}±0.005	0.109^{a}±0.0087	0.2^{a,b}±0.00512	8.723^{a,b}±0.491
GbE+L+CCl_4	0.23^{a,b}±0.01755	0.187^{a,b}±0.0056	0.18^{a,b}±0.00575	0.1205^{a}±0.0068	0.21^{a,b}±0.00405	8.885^{a,b}±0.3756

Data is shown as means±SE (n = 8 rats in 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.

Citation: El-Shabasy EA, Amer MAA, Keshk FA, et al. Comparative analysis of the antihepatotoxic effects of *Ginkgo biloba* leaf extract and Legalon using histological and biochemical techniques. *J Microbiol Exp.* 2022;10(6):229–236. DOI: 10.15406/jmen.2022.10.00378

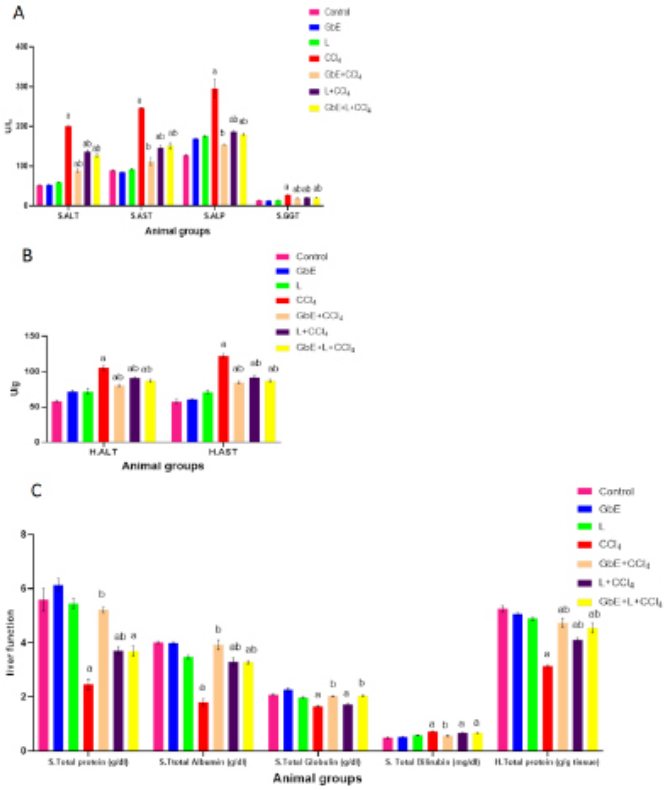


Figure 1 Serum and hepatic biochemical parameters in the control and different treated animal groups.

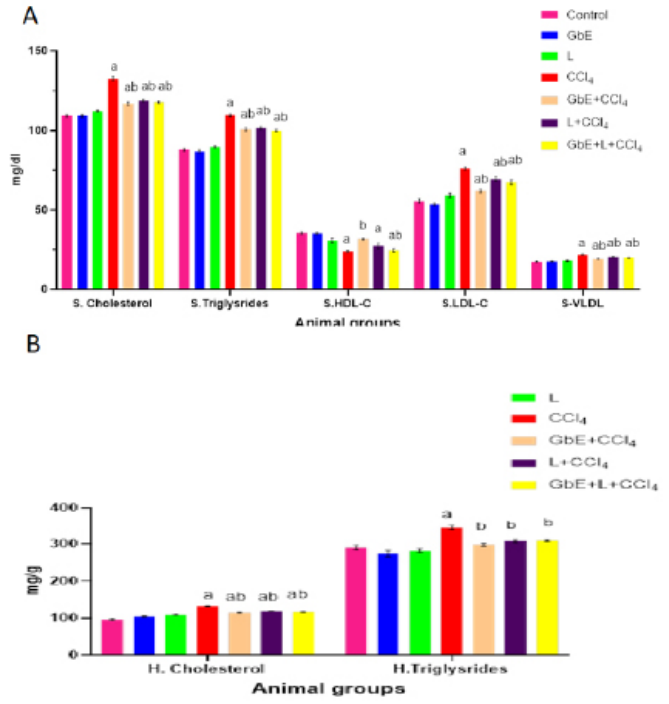
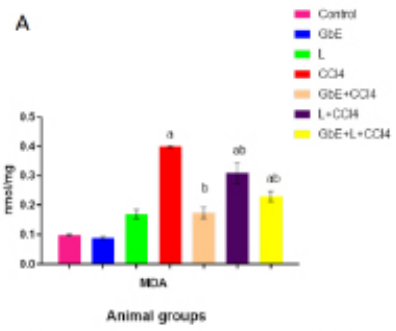
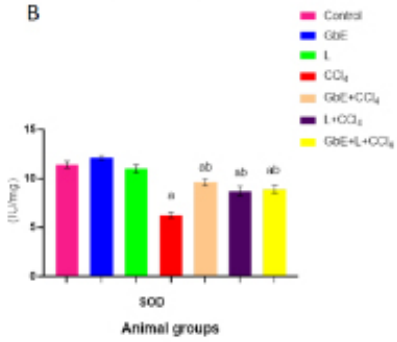


Figure 2 Lipid profile (serum& liver) in control and different treated animal groups.





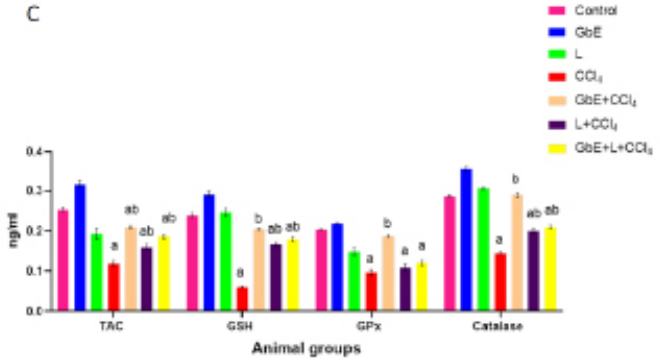


Figure 3 Lipid peroxidation product (MDA) levels and antioxidant parameters in control and different animal groups.

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 Figure 5A. When compared to the healthy control group, liver samples from rats given CCl_4 showed extensive connective tissue accumulation that led to the development of continuous interlobular septa, obvious alterations and dilations in the central vein, and marked inflammation demonstrated by severe neutrophilic infiltration, extensive fatty changes, and severe centrilobular necrosis. These findings shown in Figure 5B. Examination of liver sections of rats pretreated with *Ginkgo biloba* leaves extract (GbE) against CCl4-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 Figure 5C.

Citation: El-Shabasy EA, Amer MAA, Keshk FA, et al. Comparative analysis of the antihepatotoxic effects of *Ginkgo biloba* leaf extract and Legalon using histological and biochemical techniques. *J Microbiol Exp.* 2022;10(6):229–236. DOI: 10.15406/jmen.2022.10.00378

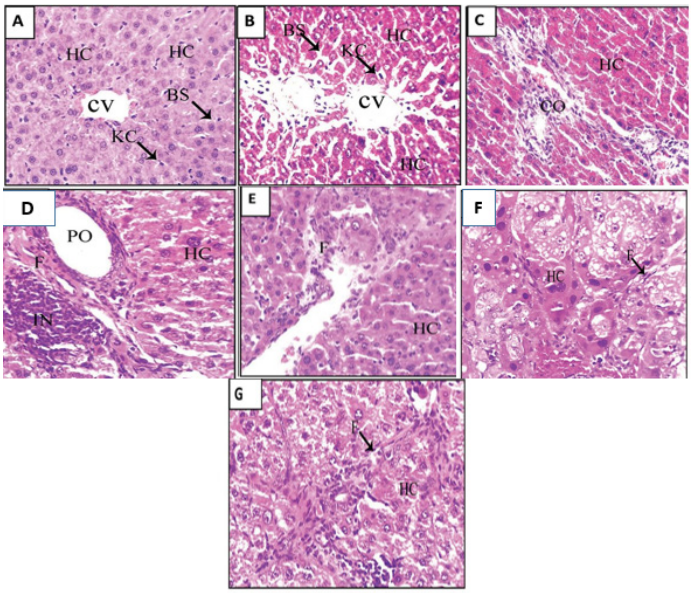


Figure 4 Photomicrographs of liver sections stained with Hematoxylin and Eosine (H&E) including: (A) control group/ (B) positive control GbE/ (C) positive control Legalon/ (D1) CCl4 intoxicated group/ (E) rat livers suffered from hepatic toxicity and received GbE/ (F1)) rat livers suffered from hepatic toxicity and received Legalon/ (G) rat livers suffered from hepatic toxicity and received a combination of GbE and Legalon.

Figure (4) Photomicrographs of liver sections stained with Hematoxylin and Eosine (H&E):

Figure (A): Control liver showing normal and binucleated hepatocytes (HC) arranged in hepatic strands radiating from a central vein (CV) and separated by blood sinusoids (BS) with their kupffer cells (KC).

(HX&E×400)

Figure (B): Normal rat liver received GbE showing central vessel outlined with thin collagen coat, hepatocytes (HC) with centrally located nuclei and kupffer cells (KC) blood sinusoids (BS).

(HX&E×400)

Figure (C): Normal rat liver received Legalon showing small patches of collagen deposition (CO) and hepatocytes (HC). (HX&E×400)

Figure (D): Hepatic toxicity induced by CCl4 showing loss of the normal liver architecture, inflammation (IN), fibrosis (F), hepatocytes (HC) and portal area (PO).

(HX&E×400)

Figure (E): Rat livers suffered from hepatic toxicity and received GbE, showing small periportal fibrosis (F) and hepatocytes (HC). (HX&E×400)

Figure (F): Rat livers suffered from hepatic toxicity and received Legalon, showing active fibroblasts distributed in between the blood sinusoids, fibrosis (F) and necrotic hepatocytes (HC).

(HX&E×400)

Figure (G): Histopathological observation of the liver sections of rats suffering from liver toxicity but received a combination of Legalon and GbE showing appearance of thin collagen distribution with in liver tissues, fibrosis (F) and hepatocytes with fatty changes (HC). (HX&E×400)

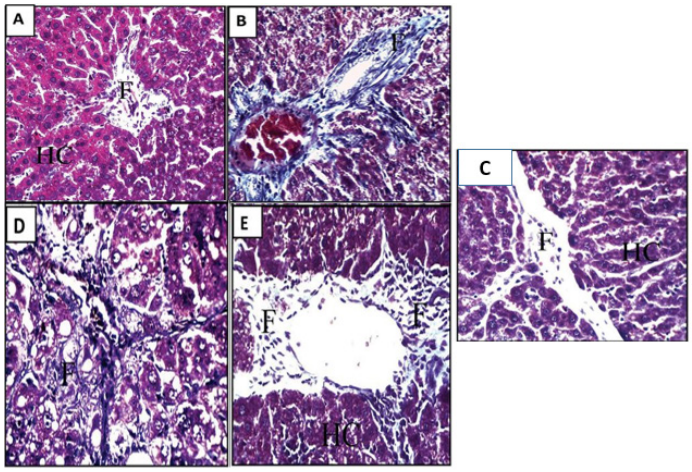


Figure 5 Photomicrographs of liver sections stained with Masson trichrome (MT) including: (A) control group/ (B) CCl4 intoxicated group / (C1) rat livers suffered from hepatic toxicity and received GbE / (D) rat livers suffered from hepatic toxicity and received Legalon / (E) rat livers suffered from hepatic toxicity and received a combination of GbE and Legalon.

Figure (5) Photomicrographs of liver sections stained with Masson trichrome (MT):

Figure (A): Section of liver of control rat showing normal architecture with tiny amount of collagen fibers. (MT×400)

Figure (B): Liver section of rats intoxicated with CCl4 displaying a significant amount of collagen fibers around the central vein and other hepatic blood capillaries, the central vein and sinusoids were congested with acute and chronic inflammatory cells and blood, leukocyte infiltration, extensive fibrosis and fibrous bands, indicating very high collagen accumulation leading to nodule formation. (MT×400)

Figure (C1): Section in liver of rats supplemented with GbE against CCl4 intoxication showing mild collagen deposition. (MT×400)

Figure (D): Section in liver of rats pre-treated with Legalon against CCl4 intoxication showing apparent increase of collagen fibers and activated fibroblasts. (MT×400)

Figure (E): Section in liver of rats pre-treated with a combination of GbE and Legalon showing increased fibrosis (F) and blue collagen fibers seen among hepatocytes (HC) and central vein. (MT×400)

Discussion

Liver fibrosis is a pathological response that can develop from chronic exposure to specific drugs or from inflammatory liver diseases. That which heals liver tissue after repeated or prolonged trauma.[41] With minimal inflammatory response and extracellular matrix deposition, hepatocytes regenerate, replace necrotic, and apoptotic cells after an acute injury. However, in the case of chronic injury, the ability to regenerate is diminished, and hepatocytes are compelled to undergo apoptosis; this, in turn, results in the activation of hepatic stellate cells (HSCs), proliferation, and increased production of extracellular matrix (ECM).[42]

The public and medical community have come to widely accept herbal medicine as a viable treatment option as our understanding of the herbs' positive effects on health and quality of life has increased.[43]

The effectiveness of a hepatoprotective drug is typically measured by its capacity to either maintain normal physiological function or to reduce the worth effect induced by hepatotoxic agents.[11,44] Many believe that the *ginkgo biloba* tree is the oldest living tree species. Although *Ginkgo biloba* was originally from China, it has since become a popular landscaping choice in many other countries.[45] It now known that ginkgo leaf extracts can help with a variety of medical issues, including memory loss, Alzheimer's disease and other forms of dementia, and even symptoms of intermittent claudication.[46-48] These extracts also used to treat multiple sclerosis, tinnitus, and sexual dysfunction, among other conditions.[47,49-51] *Ginkgo biloba* leaf extract found to be more effective than Legalon in the current study due to its nontoxicity, lack of side effects, and lack of mortality in the Ginkgo group, while the Legalon group showed a number of deaths and weight loss. Biochemical parameters and histopathological studies both corroborated these findings.

It founded that AST, ALT, ALP, and GGT serum and liver activities significantly increased after oral administration of CCl_4. In addition, total bilirubin levels in the serum were significantly higher than in the healthy control group. That suggests serious dysfunctional changes and cellular damage in the liver. Similar findings were obtained by Eidi, Mortazavi,[52] Wu Li, [53] Bouhrim Ouassou,[54] and Essawy Abdel-Wahab[55]. In addition, Okpara Atiku,[56] an enzyme found in the liver, skeletal muscles, and myocardial cells, showed that CCl_4 administration significantly increased its activity. The increased release of AST into the peripheral circulation may be evidence of hepatic or muscle damage induced by CCl_4. An elevated level of ALP activity also reported to induced by CCl_4 in the same study. Elevated ALP activity is linked to pathological alterations in the bone, kidneys, bile duct, and testes,[57,58] and it is not just a result of liver damage. This suggests that alterations in any of these organs[56] were responsible for the increased ALP activity seen in those who had consumed CCl_4.

On the other hand, the results of liver function markers showed that pretreatment of rats with *Ginkgo biloba* leaves extract reduced liver fibrosis process and tissue damage induced by CCl_4 administration. This shown by a significant decrease in total bilirubin, transaminases (ALT, AST), phosphatase ALP, and GGT in comparison to the CCl_4 intoxicated group. Findings are consistent with those of several prior studies, such as in.[59,60] However, when comparing the CCl_4-intoxicated group with the group that treated with Legalon, there was no statistically significant difference. Together with previously published data from hematoxylin and eosin (H&E) and Masson's trichrome staining and transmission electron microscopy examination, these findings suggest that GbE administration for 8 weeks has inhibitory effects on the levels of key liver function indicators. Since this is the case, GbE can prevent liver damage.

Declines in total protein, albumin, and globulin after CCl_4 intoxication taken as evidence of liver injury. The inability of the liver to synthesise proteins, as well as a general decrease in protein levels, are both symptoms of liver damage.[55] Compared to healthy rats, total protein and albumin were significantly lower in the current study due to hepatotoxicity caused by CCl_4-intoxication. These findings are consistent with those of several prior studies.[61-63] However, the present study showed that total protein and albumin concentrations were increased after pre-treatment with *Ginkgo biloba* leaves extract against CCl_4-intoxication.[64]

Table 3's findings showed that when CCl_4-intoxicated rats compared to the control group, serum cholesterol, triglycerides, and LDL cholesterol rose while HDL cholesterol fell. These outcomes are consistent with a number of earlier studies.[65,66] Contrarily, GbE administration resulted in a significant drop in a lipid profile marker, which was consistent with research by Abdel-Zaher, Farghaly,[67] and Huang, Zhang.[68]

The metabolization of radical scavengers and chain terminators like vitamin C and E, antioxidants like GSH, and redox regulatory enzymes like CAT, SOD, and glutathione peroxidase are just a few examples of the body's multiple defense mechanisms against free radicals.[69] In rats supplemented with CCl_4, which may result in cell, tissue, or organ damage, the results of the current study showed a significant increase in the markers of oxidative stress while the levels of antioxidants significantly reduced. The findings also revealed a marked increase in MDA levels, which thought to be the byproducts of the lipid peroxidation process and serve as indicators of the oxidative degradation of polyunsaturated lipids, which can eventually result in cell death.

The current findings are consistent with earlier research, which demonstrated that polyunsaturated fatty acid degradation by reactive oxygen species (ROS) damages membrane structurally and/or functionally.[56,70-72] Furthermore, according to[73] ROS disrupts antioxidant defense mechanisms and weakens the effects of SOD-induced liver damage, cirrhosis, and hepatocarcinoma. In contrast, oral administration of *Ginkgo biloba* leaf extract to protect against toxicity induced by CCl_4 and its reactive metabolites led to a decrease in oxidative stress markers and an increase in antioxidant enzyme levels. The MDA level also seen to be decreasing, which led to the inhibition of lipid peroxidation and the prevention of cell obstruction, ultimately protecting the liver from ROS-induced damage.

These findings concur with those of Þener, Kabasakal[74] who examined the antioxidant and antifiberotic effects of long-term administration of *Ginkgo biloba* extract on liver fibrosis induced by bile duct ligation (BDL) and scission in Wister male albino rats. They claimed that *Ginkgo biloba* protected the rats' livers from BDL's oxidative damage. This action most likely includes the suppression of lipid peroxidation and neutrophil infiltration.

Conclusion

The findings of the current study demonstrated that *Ginkgo biloba* leaf extract offers significant protection against long-term liver damage brought on by CCl4 and may be a useful agent in preventing liver damage by stifling oxidative stress in the experimental animals. Additionally, it appeared that Legalon alone or in combination with GbE had a worse hepatoprotective effect than GbE. Due to its antioxidant properties, ability to prevent lipid peroxidation, and ability to replenish glutathione levels, GbE can shield the liver from harm. The advantage might be similar to Legalon's (derived from silymarin). Further research is necessary, though, to determine whether GbE alone can treat liver damage.

Acknowledgments

None.

Conflicts of interests

The authors declare that they have no conflict of interest.

Ethical approval

All deals with animals in this study were carried out according to international valid guidelines of experimental animal studies and research protocol was approved by the local ethical committee of the faculty of Science, Mansoura University with code number Sci-Z-P-2022-117.

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