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Research Article

The protective effect of *Ginkgo biloba* leaves extract against liver toxicity in comparison with anti-fiberotic drug in male rats

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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 that are responsible for several liver 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 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 CCl4 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 CCl4. Thereafter, liver fibrosis was induced by oral administration of CCl4 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, haematological changes and transmission electron microscopy for the liver sections were investigated to evaluate the protective effect of GbE. **Results:** showed that CCl4 induced a significant (P>0.5) decrease in RBCs count, Hb content, and platelets count, but WBCs count was significantly increased when compared with control group. On the other hand, treatment with GbE caused alterations to all of these parameters when compared with both CCl4-intoxicated group and Legalon group. CCl4-intoxication caused loss of the normal structure integrity while liver sections of animals supplemented with GbE against CCl4-toxicity showed a remarkable improvement in the liver tissue. Conclusion: pre-treatment of the animals with GbE improved the liver damage that induced experimentally by CCl4. The hematological parameters and histopathological studies showed that GbE extract have a hepato-protective effect due to its ability to ameliorate the deleterious effect on the structure of the liver, produced by CCl4.

Keywords: *Ginkgo biloba* leaves extract; Legalon drug; liver toxicity.

INTRODUCTION

The liver is regarded as the most important between the largest organs in the human body; it is responsible for many functions, such as the process of metabolism which is essential for life (Kandimalla *et al.*,, 2016). It's location in the human body make it to be vulnerable to various toxic substances of foreign origin. These xenobiotics pass first through the liver before it's absorption by the intestine that makes it threatened by disease more than any other organ (Stickel *et al.*,, 2002).

Liver disease has become a global burden and every year up to 20000 deaths occur due to liver affections (Latha *et al.*,, 2009). Liver disease can be resulted from various mechanisms. A prevalent type of liver disease is viral infection. Viral hepatitis like Hepatitis B virus and Hepatitis C virus can be vertically transferred during birth through contact with infected blood (Benova *et al.*,, 2014, Komatsu, 2014). Liver fibrosis refers to the response of the liver to various chronic insults like parasitic disease, chronic viral infection (hepatitis B and C), immunologic attack (autoimmune hepatitis), hereditary metal overload, toxic damage, and so forth. Because of the worldwide spreading of these insults, liver fibrosis is prevalent and is correlated with a great number of morbidity and mortality (Chen *et al.*,, 2002, Han, 2002, Shen *et al.*,, 2003).

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Hepatotoxicity stimulated by CCl_4 solvent is regarded as common model used to study hepatoprotective potency of natural products (Lu *et al.*,, 2016).The latter is a chemical solvent that induce liver injury like that generated by viral hepatitis (Ponmari *et al.*,, 2014). CCl_4 arrived to the liver as a foreign toxin and it converted into two free radicals, which are trichloromethyl and trichloromethyl peroxy, through the microsomal system dependent on monooxygenaseP-450. Furthermore, these two free radicals elicited the beginning of the oxidation of unsaturated lipids (Zhao *et al.*,, 2016). CCl_4 also cause the oxidation of unsaturated lipids leading to generation of hepatic injury (Buege and Aust, 1978). Moreover many compounds are known with their crucial role against liver damage induced by CCl_4 by producing their protective action either by reducing CCl_4 derived free radicals or by their antioxidant activities (Hewawasam *et al.*,, 2004).

Ginkgo biloba extract is popularly marketed to populate as it has the ability to provide useful improvements in memory impairments, stroke, edema, inflammation, Alzheimer's dementia and vasooclusive disorders (Diamond *et al.*,, 2000). *Ginkgo biloba* leaves extract contains several chemical constituents that have been isolated and identified, comprising diterpenes (e.g., ginkgolides A, B,C, and J), sesquiterpenes (e.g., bilobalide), ginkgo flavonol glycosides (e.g., the glycosides of kaempferol, quercetin, and isorhamnetin), triterpenes (e.g., sterols), organic acids, and polyprenols (Beek *et al.*,, 1998).

Therefore, the present study has been carried out to investigate the possible ameliorative effect of a plant extract (*Ginkgo biloba* leaves extract) and antifiberotic drug (Legalon) against liver toxicity induced experimentally by carbon tetrachloride in male rats.

MATERIALS AND METHODS

Chemicals

CCl_4, Legalon drug, and *Ginkgo biloba* leaves extract were purchased from Sigma chemicals, Egypt. 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 were received GbE orally at a dose (100 mg/kg) day after day for 8 weeks (Welt *et al.*,, 2007). **Group III:** Legalon control group that received Legalon at a dose (100mg/kg) day after day for 8 weeks(Roy and Das, 2010). **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(Fischer-Nielsen *et al.*,, 1991). **GroupV:** 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 withdrawing blood. The blood samples were collected on EDTA for hematological studies. The liver of each rat was removed then cut into parts and stored in osmium tetroxide (4%) for transmission electron microscopy.

Measurement of Haematological Parameters

Haematological parameters including the count of red blood corpuscles (RBCs), white blood cells (WBCs), haemoglobin (Hb) concentration and platelets count were conducted using haematological analyzer (Sysnex Ts-21) Japan (Dacie and Lewis, 1991).

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 Graph pad prism. Differences were considered significance at $p \leq 0.05$**(Snedecor and Cochran, 1980).**

Histological and Ultrastructural Methods

Transmission electron microscopy for liver sections was performed as described Masyuk *et al.*, (2014) .

RESULTS

Evaluation of blood cell indices

Table (1) showed a significant ($P<0.05$) decrease in RBCs count, platelets count and haemoglobin content in CCl_4-intoxicated group as compared with control group while, a significant ($P<0.05$) increase was observed in WBCs count. On contrary, pretreatment with *Ginkgo biloba* leaf extract resulted in improvement in all of blood cell indices when compared with CCl_4-intoxicated group.

Table (1): Hematological parameters in control and different treated animal groups:

Parameters	Animal Groups						
	Control	GbE	L	CCl4	GbE+CCl4	L+CCl4	GbE+L+CCl4
RBCS (10^6/mm^3)	5.598±0.4197\	6.140±0.4044	4.700±0.2249	2.463^{a}±0.1963	4.713^{b}±0.2052	$3.965^{a,b}$±0.175	$4.000^{a,b}$±0.2394
WBCS (10^3/mm^3)	5.935±0.2384	6.548±0.2442	7.330±0.1945	11.03^{a}±0.4669	$8.470^{a,b}$±0.4218	9.440^{a}±0.4104	$8.948^{a,b}$±0.4777
Platelets count (10^3/mm^3)	740.7±21.5	618.1±21.53	575.7±21.53	373.9^{a}±21.53	$560.4^{a,b}$±21.53	$476.3^{a,b}$±21.53	$498.0^{a,b}$±21.53
Hb content (g/dl)	12.64±0.2031	12.92±0.2031	10.97±0.2031	9.160^{a}±0.2031	10.90^{ab}±0.2031	9.978^{a}±0.2031	$10.09^{a,b}$±0.2031

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.

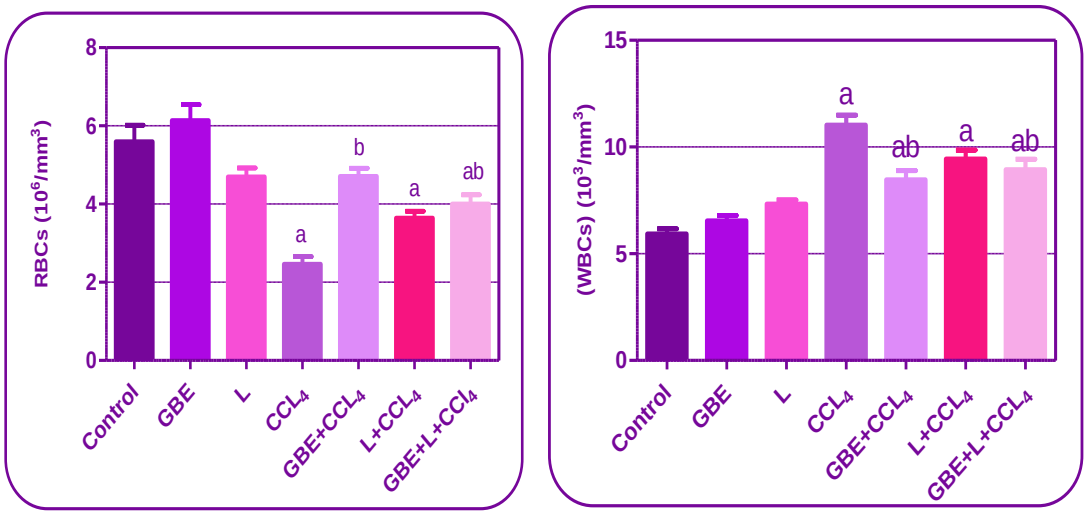

Figure (1): (RBCs) 10^6/mm^3 count and (WBCs) (10^3/mm^3) count of control and different animal groups.

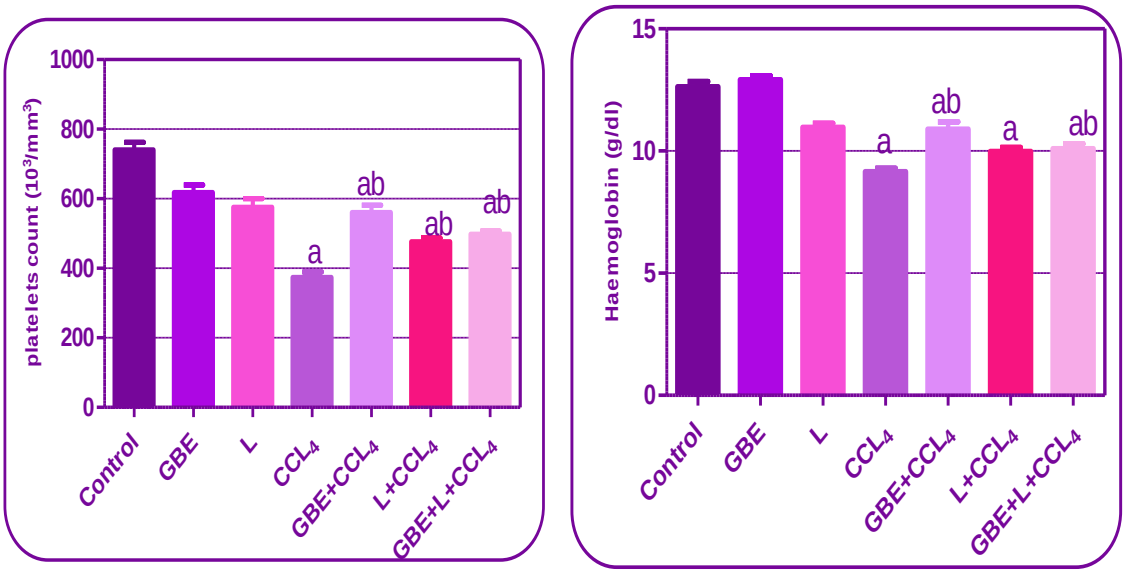

Figure (2): Platelets count (10^6/mm^3) and Haemoglobin (Hb) content (g/dl) of control and different animal groups.

Transmission Electron Microscopy Observations:

The liver sections of the control group showed hepatocytes possess a spherical nucleus with a vascular nucleolus, as well as cell organelle including numerous mitochondria contained medium density, homogenous matrices and many fine cristae. Endoplasmic reticulum confined to numerous small stakes is detected scattered through the cytoplasm Fig. A. On contrary, hepatocyte

of CCl_4-induced liver damage showed shrunken of hepatocytes, margination of chromatin, Fatty degeneration, kupffer cells, and clumping of mitochondria Fig. B. Liver sections of GbE treated group showed uniform mitochondrial distributions, regeneration of rough endoplasmic reticulum and vacuolations in cytoplasm Fig. C. Legalon treated group showed mild migration of chromatin, condensed cytoplasm, and regeneration of rough endoplasmic reticulum Fig. D.

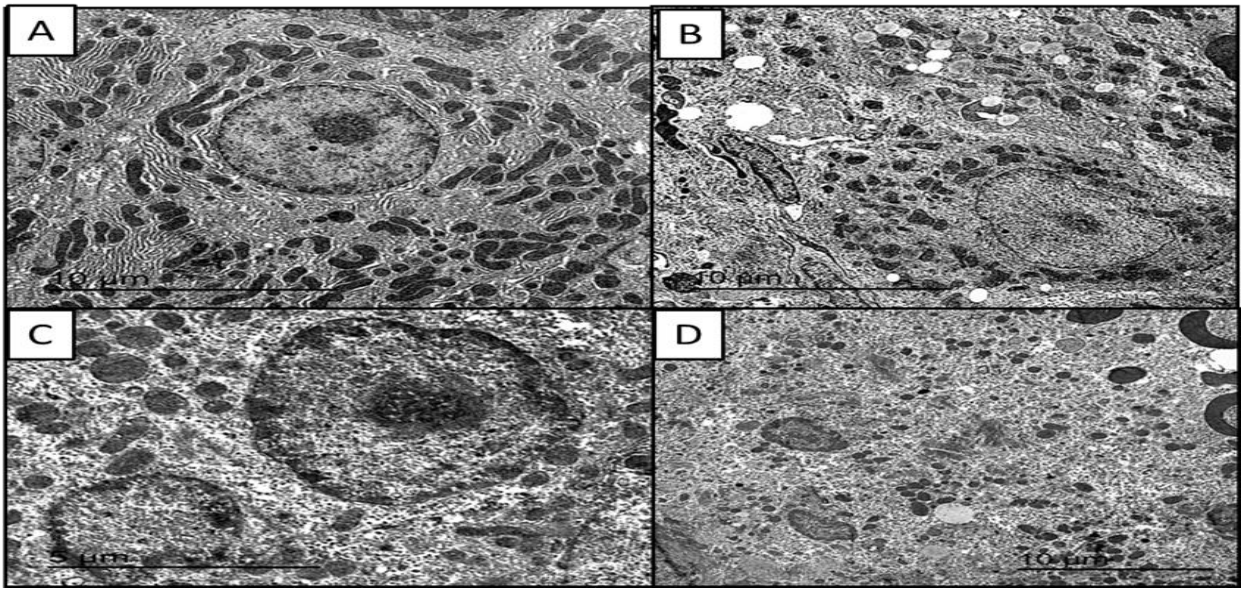

Figure 3: Transmission electron microscopy of liver sections of the different groups including (A)control group/ (B)CCl4 intoxicated group / (C) the group pretreated with GbE against CCl4 intoxication /(D)the group pretreated with Legalon against CCl4.

DISCUSSION

Hepatotoxicity is known as liver dysfunction or liver damage that is related to high concentrations of drugs or xenobiotics (Navarro and Senior, 2006). The chemicals that induce liver injury are known as hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical importance and may comprise high doses of definite medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett *et al.*,, 2004).

On the cellular level, liver fibrosis is generated by aggregation of extracellular matrix (ECM) proteins such as collagen and fibronectins, synthesized by hepatic stellate cells (HSC) (Bataller and Brenner, 2005). These activated HSC are featured by increased mRNA expression of transforming growth factor beta (TGF-β), alpha smooth muscle actin (α-SMA), and collagen. Subsequently, the anti-fibrotic therapies are based on targeting phenotypically activated HSC (ur Rehman *et al.*,, 2018). Carbon tetrachloride CCl_4 toxicity leads to oxidative stress which causes not only hepatocytes damage but also causes generation of cytokines such as TGF-β. TGF-β induces rat HSC and helps them to differentiate into myofibroblast-like cells that cause accumulation of ECM and collagen (Jeong *et al.*,, 2005). The HSC stimulated by ROS and different cytokines in an accelerated fashion elicited liver fibrosis (ur Rehman *et al.*,, 2018).

A small number of drugs are effective in the treatment of chronic liver disease (Muriel and Rivera-Espinoza, 2008). But, their cost is very high and has unavoidable side effects (Fried, 2002). Therefore a great concern is directed to herbal medicines due to their economy, facility of accessibility and little or no side effects. Herbal remedies are widely used by one quarter of hepatic patients (Stickel and Schuppan, 2007). *Ginkgo biloba* (Ginkgoaceae family) contains various phytoactive compounds like flavonoids, terpenetrilactones, proanthocyanidins, ginkgolic acids, biflavone, and ginkgotoxins (Mahady, 2001). Flavonoids in GbE have the ability to attenuate the tissue damage induced by oxygen-derived free radicals, therefore attenuation of lipid peroxidation (Aljadaani *et al.*,, 2016).

It has been observed in our study that oral administration of CCl_4 for 8 weeks significantly affected all hematological parameters including a significant decrease in RBCs count, Hb content, and platelets count, but WBCs count was significantly increased. Our findings are in agreement with that reported by (ur Rehman *et al.*,, 2018).

In the present study, it was found that oral administration of *Ginkgo biloba* leaf extract against toxicity induced by CCl_4, caused amelioration in all hematological parameters, where we observed increasing in the RBCs count, Hb content and platelets count, while WBCs count was reduced. Our results are

in accordance with several results published previously elsewhere (Wang *et al.*,, 2015, Aziz *et al.*,, 2018).

In the present work Transmission electron microscopy liver sections of animals intoxicated with CCl_4 showed shrunken of hepatocytes, margination of chromatin, Fatty degeneration, kupffer cells, and clumping of mitochondria. While liver sections of rats supplemented with GbE against CCl_4-intoxication showed uniform mitochondrial distributions, regeneration of rough endoplasmic reticulum and vacuolations in cytoplasm.

CONCLUSION:

Ginkgo biloba leaves extract caused a protective effect against CCl_4-induced liver necrosis and fibrosis and exhibited clear liver hepatic recovery characterized by noticed regeneration of hepatocytes and the hepatic tissue appeared more or less normal in most sections, compared with the CCl_4-induced liver fibrosis group.

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