ORIGINAL ARTICLE

Ginkgo Biloba Protects Against Carbon Tetrachloride Induced Liver Injury in Albino Rat

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ABSTRACT

Objective: To investigate hepatoprotective effect of Ginkgo biloba aqueous extract (GkB) against carbon tetrachloride (CCl_4) induced liver injury in albino rat model.

Study design: Experimental/Analytical study

Place and Duration: Animal House, Isra University Hyderabad campus, from March to July 2014.

Subjects and Methods: Forty five albino rats were divided into three groups; Group I. controls received 0.9% isotonic saline, Group II. received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group III. received the CCl_4+ GkB. Blood samples were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 4% formaldehyde, was embedded in paraffin. Tissue sections of 5μ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on Statistix 8.1 using one-way analysis of variance using post Hoc testing. A p-value of = 0.05 was taken statistically significant.

Results: The liver biochemical and histological findings reveal statistically significant differences among the controls, CCl_4 and CCl_4+ GkB groups (p=0.0001). Liver enzymes and histology were deranged significantly in CCl_4 group compared to controls and CCl_4+ GkB group (p=0.0001).The CCl_4+ GkB group showed lesser rise in liver enzymes and derangement of liver histology compared to CCl_4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis were found more pronounced in CCl_4 group.

Conclusion: The Ginkgo biloba protects against oxidative damages caused by carbon tetrachloride induced liver injury in albino rat.

Key words: Ginkgo biloba, Carbon tetrachloride, Liver injury

INTRODUCTION

Ginkgo biloba (GkB). (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.[1] Chemically, the active constituents of GkB 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.[2,3] The extract of GkB leaves have been proved to be an effective antioxidant and found to possess cardioprotective, anti-asthmatic, anti-diabetic, and potentiates central nervous system activities, like enhancement of memory, concentration, mental alertness and decrease in mental fatigue.[4,5] 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.[6]

This extract has been shown several in vivo

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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.[2-7] The herb in addition posses other important pharmacological actions. GkB extract decreased gastric injury caused by ethanol,[8] protected against chemically induced oxidative injury and fibrosis.[9] Therefore, the purpose of this study was designed to investigate the protective effect of GkB on carbon tetrachloride injury induced acute liver injury in rats.

MATERIAL AND METHODS

The present experimental study included forty five albino rats at animal house of Isra University from March to July 2014. Albino male rats of 250-300 grams were included while female rats and rats weighing <250 grams or >300 grams were excluded from the study. The Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw like fresh alfalfa and clean water are provided freely.

Experimental animals:

Forty-five albino rats were purchased and housed at Isra University animal house. Animals were housed in plastic cages, fed on standard casein diet 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 animals were divided into following groups;

Group I. (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group II. (n=15) Rats were given CCl_4 orally mixed in olive oil for three successive weeks.

Group III. (n=15) Rats received 12% concentrated prune juice extract (GkB) + CCl_4 for three successive weeks.

Drugs and chemicals:

Carbon tetrachloride was purchased from scientific drug store (Sigma Chemical Co. St. Louis, MO, USA). Carboxymethyl 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. Pakistan. All chemicals used were analytical grade of the highest laboratory purity.

Preparation of Aqueous Extract of G. Biloba

GkB leaves (100 gm dried powder) were soaked in 1 liter boiling distilled water. After 2 hours it homogenized in the same distilled water, stirred by using magnetic stirrer at 40° C for 1 hour, 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 °C until further use according to as referenced.[1] Lyophilization was conducted 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.

Induction of Hepatotoxicity with CCl4:

Animals were injected intraperitoneally (i.p) with CCl_4 (1mL/kg b.w., 1:1 v/v mixture of CCl_4 and liquid paraffin) every 72 h for 14 days according to as referenced[10].

Blood Collection and Serum Separation:

Twenty four hours after the end of experimental period, blood samples were collected from peripheral veins. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes.

Animal Sacrifice:

The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006)[11] and liver was removed promptly for histological study.

Liver enzyme assays were used for determining of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase

(ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Each sample of liver obtained was washed in normal saline and tissues were fixed in previously marked containers, containing 10% formaldehyde as preservative. The tissues were embedded in paraffin, cut into 5 um thick sections and stained with Hematoxylin-Eosin (H & E) and Masson`s trichrome staining for histological examination. The histological criteria included vacuolar degeneration, inflammatory cell infiltrate, congestion and necrosis. The histological parameters were graded as follows;

- 0 = no abnormal findings,
- + = mild injury,
- ++ = moderate injury
- +++ = severe injury.[12]

The data was analyzed on Statistix 8.1 (USA). The continues variables were presented as mean±SD and range. The categorical variables were analyzed by Chi-square test. While the continues variables among and between groups were calculated by one-way analysis of variance (one-way ANOVA) and post hoc Tukey`s HSD testing. A p-value of = 0.5 was taken statistically significant.

RESULT

In present study, we observed major differences in liver enzyme assays among groups. The ALT, AST, ALP and LDH in serum of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant p-value for all variables (p=0.001) The CCl_4+ GkB group showed a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The GkB when mixed with CCl_4 showed significant reduction in the liver enzyme elevation in blood sera. The finding shows significant hepatoprotection by the GkB in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Different parameters of histological score of liver injury are shown in Table. II. The Liver sections from control group showed intact central portal venules and compact hepatocytes arrangement. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm were seen in control group. (Figure. 1). The CCl_4 group showed derangement of hepatocytes cords, hydropic changes with congestion of central venules and sinusoids, and abundant inflammatory cell infiltration (Figure.2). The centrilobular hepatocytes showed hydropic changes and necrosis, while midzonal and peripheral hepatocytes showed vacuolar degeneration and fatty changes in CCl_4 group. (Figure.3.). In CCl_4+ GkB animals, liver tissue sections revealed least derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group.

Table-1. Liver Enzyme Levels in Controls, *CCl_4 & CCl_4 + GkB† Extract Groups

Groups	ALT (IU/l)	AST (IU/L)	LDH (IU/L)	ALP (IU/L)
Group. I (Controls)	48.7±3.29	92.1±15.91	713.5±47.8	95.5±7.98
Group. II (*CCl_4)	185.7±10.97	493.7±19.9	2768.9±137.6	171.1±7.02
Group. III (*CCl_4 + GkB†)	88.9±16.98	169.9±20.3	2148.6±141.3	135.8±17.5

*Carbon Tetrachloride

† Ginko Biloba

Table-2. Histology of Liver Injury of Controls, *CCI4 & CCI4 + GkB† Extract Groups

Groups	Congestion	Inflammation cell markers	Vacuolar Degeneration	Necrosis
Group. I (Controls)	0	0	0	0
Group. II (*CCl_4)	++++	++++	++++	++++
Group. III (*CCl_4 + GkB†)	++	++	++	++

*Carbon Tetrachloride

† Ginko Biloba

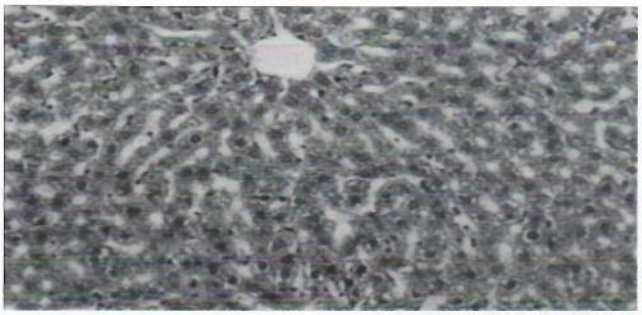

Figure.1. Liver slide of control group shows normal looking hepatocytes arranged in cords. Central vein is shown separated by sinusoids

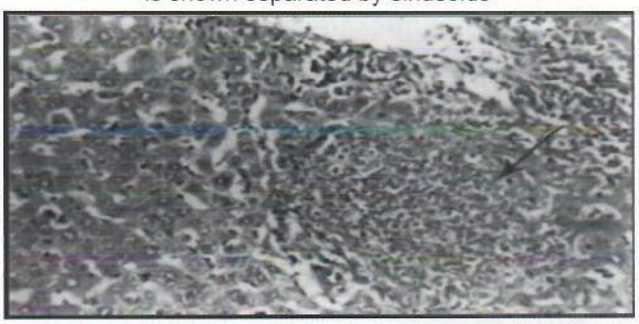

Figure.2. CCl_4 group showing hydropic degeneration, inflammatory cell infiltrate & necrosis

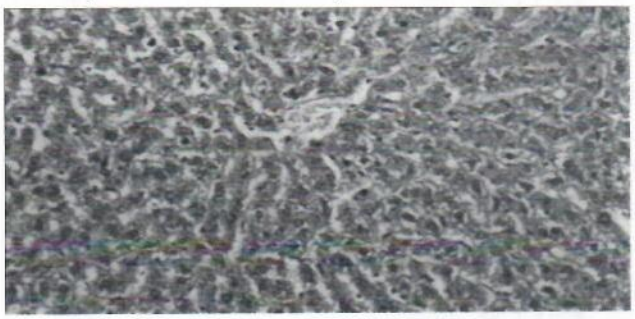

Figure.3. Ginkgo extract & carbon tetrachloride group showing normal hepatocyte arranged in cords with congested sinusoids, and few lymphocytic infiltrations

Discussion

In the present study, GkB was evaluated for the hepatoprotective activity using CCl_4 induced acute hepatotoxicity in rats. The Hukkeri proved elevation in the plasma level of cytoplasmic and mitochondrial enzymes due to liver injury induced by CCl_4 in animal models.[13] Increased blood levels of liver enzymes indicate rupture of the cell membrane and damage of hepatocytes sufficient to release cytoplasmic enzymes into blood circulation.[14] In the present study, damage of liver caused by CCl_4 was evident by the rise in serum marker enzymes beside the histological changes in liver tissue. Administration of CCl_4 significantly increased the serum levels of liver enzymes; AST, ALT, ALP and LDH, which are indices of liver cell damage and leakage of enzymes from cells.[15,16] It is reported that rise in ALT is almost always due to hepatocellular damage; accompanied by rise in AST and ALP.[17] The carbon tetrachloride is found to produce free radicals, which affect cellular permeability of hepatocytes leading to elevated levels of liver enzymes.[18]

In this study, results showed that rats group intoxicated with CCl_4 revealed significant reduction in body weight compared to control group. These results were in agreement with previous studies.[19-21] Pretreatment of rats with GbE showed a significant increase in weight gain percent, and food intake 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 to previous study.[22] Guo et al.[1] 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, and food intake as compared to control group, this confirmed its safe use and agree with Dias et al. [23] who found no significant alterations 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.[24,25] Elevated levels of serum liver marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver[26], since ALT is thought to be one of the indices of the degree of cell membrane damage and ALT is an indicator for mitochondrial damage; mitochondria contain 80% of this enzyme. 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[27]. 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[28]. 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[29]. 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[30].

This agrees with previous studies also.[30,31] 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 rise 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.[32] 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.[7] 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. Treatment with GkB significantly reduced effects of carbon tetrachloride induced hepatocellular damage and it was evidenced by the decreased level of liver enzymes and restoration of hepatocellular architecture. The findings are consistent with our current work. The present study reveals that GkB has hepatoprotective potential against oxidative damages caused by carbon tetrachloride. The GkB may be used as an effective protector against chemical induced liver damages.

CONCULSION

The present study concludes that Ginkgo biloba shows hepatoprotective potential against oxidative damages caused by carbon tetrachloride. Ginkgo biloba may be used as an effective protector against chemical induced liver damages.

REFERENCES

1. Guo WY, Ni ZJ, Wang ZX, Fu ZR, Li RD. The protective effects of Ginkgo leaf extract on CCl4-induced liver injury in mice. J. Medicinal Plants Res. 2011;5(11):2361-4.
2. Ahlemeyer B, Krieglstein J. Neuroprotective effects of Ginkgo biloba extract. Cell Mol. Life Sci. 2003; 60:1779-92.
3. Boonkaew T, Camper ND. Biological activities of Ginkgo extracts, Phytomedicine 2004; 12: 31823.
4. Naik SR, Pilgaonkar VW, Panda VS. Neuropharmacological evaluation of Ginkgo biloba phytosomes in rodents. Phytother. Res. 2006;20:901-5.
5. Naik SR, Panda VS. Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int.2007;27:393-9.
6. Kwon YS, Ann HS, Nabeshima T, Shin EJ, Kim WK, Jhoo JH. Selegiline potentiates the effects of EGb 761 in response to ischemic brain injury. Neurochem. Intl 2004;45:157-70.
7. Zhang WR, Hayashi T, Kitagawa H. Protective effect of ginkgo extract on rat brain with transient middle cerebral artery occlusion. Neurol. Res.2000;22:517-21.
8. Wang Q, Zhao WZ, Ma CG. Protective effects of Ginkgo biloba extract on gastric mucosa. Acta. Pharm. Sin. 2000;21:1153-6.
9. Ding J, Yu J, Wang C, Hu W, Li D, Luo Y. Ginkgo biloba extract alleviates liver fibrosis induced by CCl4 in rats. Liver Intl. 2005;25:1224-32.
10. Karthikeyan M & Deepa K. Hepatoprotective effect of Premna corymbosa (Burm. f.) Rottl. & Willd. leaves extract on CCl4 induced hepatic damage in Wistar albino rats. Asian Pacific J Tropical Medicine. 2010;3(1):17-20.
11. Nayak S, Nalabothu P, Sandiford S, Bhogadi V, and Adogwa A. Evaluation of wound healing activity of Allamanda cathartica L. and Laurus nobilis. L. Extracts on rats. BMC Complementary and Alternative Medicine. 2006;6:12.
12. Murat-Bilgin H, Atmaca M, Deniz-Obay B, Ozekinci S, Tasdemir E & Ketani A. Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. Exp Toxicol Pathol. 2011;63(4):325-30.
13. Hurkkeri VI, Jaiparkash B, Lavhale RV, Karadi RV and Kuppast IJ. Hepatoprotective activity of Anthus Excelsa Roxb leaf extract on experimental liver damage in rats. J Pharmacogn. 2002;11:120-28.
14. Shaarawy SM, Tohamy AA, Elgendy SM Elmageed ZY, Bahnasy A, Mohamed MS, et al. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci. 2009;5(6):549-57.
15. Rajesh M and Latha M. Preliminary evaluation of anti-hepatotoxic activity of Kamilari, a polyherbal formulation. J Ethnopharmacol. 2004;91:99-104.
16. Bashandy S & Al-Wasel S. Carbon tetrachloride-induced hepatotoxicity & nephrotoxicity in rats: Protective role of vitamin C. J Pharm Toxicol. 2001;16(30):283-92.
17. Ravikumar V, Shivashangari K & Devak IT. Hepatoprotective activity of Tridax procumbens against d-galactosamine/ lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol 2005; 101: 55-60.
18. Kumar P, Sivaray A, Elumalai E & Kumar B. Carbon tetrachloride-induced hepatotoxicity in rats - protective role of aqueous leaf extracts of Coccinia grandis. Int J Pharm Tech Res. 2009;1(4):1612-5.
19. Venukumar MR, Latha MS. Hepatoprotective effect of the methanolic extract of Curculigo Orchioides in CCl4-treated male rats. Indian J Pharmacol 2002, 34:269-275.
20. Chang HF, Lin YH, Chu CC, Wu SJ, Tsai YH, Chao JC. Protective effects of Ginkgo biloba, Panax ginseng, and Schizandra chinensis extract on liver injury in rats. The Am J of Chinese Medicine 2007; 35 (6): 995-1009.
21. Balamurugan G, Muthusamy P. Observation of the hepatoprotective and antioxidant activities of Trianthema decandra Linn. (Vallai sharunnai) roots on carbon tetrachloride-treated rats. Bangladesh J Pharmacol. 2008; 3(2):83-89.
22. Farooq S, Ahmed I, Pathak GK. In vivo protective role of Koflet (an ayurvedic preparation) against cellular toxicity caused by CCl4. J Ethnopharmacol. 1997;58(2):109-16.

23. Dias MC, Rodrigues MAM, Reimberg MCH, Barbisan LF. Protective effects of Ginkgo biloba against rat liver carcinogenesis. Chemico-Biological Interactions. 2008; 173:32-42.
24. Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacol. 2005; 5(2):1-8.
25. Anand KV, Anandhi R, Pakkiyaraj M, Geraldine P. Protective effect of chrysin on carbon tetrachloride (CCl4)-induced tissue injury in male wistar rats. Toxicol Ind Health. 2011; 27(10):923-33.
26. Drotman RB, Lawhorn GT. Serum enzymes are indicators of chemically induced liver damage. Drug Chem Toxicol. 1978;1(2):163-71.
27. Achliya GS, Kotgale SG, Wadodkar AK, Dorle AK. Hepatoprotective activity of Panchgavya Gritha in carbon tetrachloride induced hepatotoxicity in rats. Indian J Pharmacol. 2003;135:311.
28. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J Appl Toxicol. 1992; 12(6):439-42.
29. Thabrew M, Joice PA. A comparative study of the efficacy of Pavetta indica and Osbeckia octanda in the treatment of liver dysfunction. Planta Med. 1987;53(3):239-41.
30. Itil T, Martorano D. Natural substances in psychiatry (Ginkgo biloba in dementia). Psychopharmacol Bull. 1995;31:147-58
31. Cha´vez-Morales RM, Jaramillo-Jua´rez F, Posadas del Rý´o FA, Reyes-Romero MA, Rodrý´guez-Va´zquez ML, Martý´nez-Saldan˜a MC. Protective effect of Ginkgo biloba extract on liver damage by a single dose of CCl4 in male rats. Human and Experimental Toxicology. 2010;30(3):209-16.
32. Suresh KSV, Sujatha C, Syamala J, Nagasudha B, Mishra SH. Hepato-protective activity of extracts from Pergularia daemia Forsk against carbon tetrachloride induced toxicity in rats. Phcog Mag. 2007; 3:11.