HEPATOPROTECTIVE EFFECTS OF GINKGO BILOBA AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC INJURY IN RATS

K. ASHOK SHENOY*, S. N. SOMAYAJI**, K. L. BAIRY*

* Department of Pharmacology, Kasturba Medical College, Manipal, Karnataka.
** Department of Anatomy, International Centre for Health Sciences, Manipal, Karnataka.

Objectives: To assess the protective activity of Ginkgo biloba (GB) against CCl4 induced hepatotoxicity in rats and probe into its mechanism of action.

Methods: Liver damage was induced in Wistar rats by administering (150-250 g) CCl4 (0.5 ml/kg, i.p.) once daily for 7 days. GB (50 mg/kg, i.p.) was given for one week. Silymarin (200 mg/kg, p.o.) was given as a reference drug. Levels of marker enzymes (AST, ALT, ALP) and total proteins (TP), albumin (Alb) were estimated in serum. A probe into the mechanism of action was attempted by estimating thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels in liver homogenates in order to evaluate the degree of lipid peroxidation. Histopathological studies were also done to confirm the biochemical changes.

Results: The mean ± SEM serum AST, ALT, ALP levels in control animals were 66.8 ± 4.2, 31.1 ± 2.0 and 445.3 ± 23.1 IU/L respectively whereas in CCl4 treated rats, the level rose to 319.6 ± 22.7, 192.8 ± 16.0 and 809.3 ± 65.3 IU/L respectively. GB reduced the AST, ALT and ALP levels to 55.5 ± 5.3, 36.5 ± 3.6 and 489.6 ± 43.9 IU/L respectively. Silymarin reduced AST, ALT and ALP levels to 51.8 ± 5.2, 30.8 ± 3.4 and 437.8 ± 35.7 IU/L respectively. There was a significant decrease in serum TP and Alb levels after CCl4, which was reversed by GB and silymarin. The tissue mean ± SEM values of TBARS and GSH in control animals were 3.1 ± 0.1 nmol of malondialdehyde/g of wet tissue and 1.9 ± 0.1 mg/g of wet tissue respectively. In CCl4 treated animals, the TBARS and GSH levels were 3.91 ± 0.41 and 1.97 ± 0.11 respectively. GB reduced TBARS to 2.4 ± 0.09 nmol of MDA/g of wet tissue and increased GSH level to 2.4 ± 0.1 mg/g of wet tissue. Silymarin reduced TBARS to 2.1 ± 0.2 nmol of MDA/g of wet tissue and increased GSH level to 2.5 ± 0.17 mg/g of wet tissue.

Conclusion: GB has protected the liver from CCl4 damage. Probable mechanism of action is by protection against oxidative damage produced by CCl4.

KEYWORDS Hepatoprotective effect Ginkgo biloba carbon tetrachloride silymarin malondialdehyde glutathione

INTRODUCTION
The Ginkgo biloba (GB) tree, also known as maidenhair tree is the sole representative of once flourishing botanical division, that of the so called Ginkgophytes. The leaves are recommended as being beneficial to the heart and lungs; inhalation of decoction of leaves is used in bronchial asthma1. Extracts of the leaves of GB have been used for cerebrovascular insufficiency due to degenerative or vascular causes, to improve learning and memory, peripheral vascular diseases, as cardioprotective and many other diseases2-4. GB exhibits a variety of interesting pharmacological properties such as oxygen free radical scavenging activity, cyclonucleotide phosphodiesterase inhibition, membrane stabilising effect, increase in blood fluidity and improvement in cognitive function2,3,5,6.

Among the various mechanisms involved in hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generation7,8 and antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous
substances. GB has antioxidant properties\textsuperscript{4,5,9-12}. Hence the objective of the study was to evaluate the effect of GB on CCl\textsubscript{4} induced hepatotoxicity.

**MATERIALS AND METHODS**

**Drugs and chemicals:** Ginkgo biloba dry extract (containing 0.24 mg of ginkgo flavonolglycosides/g of dry extract) was a kind gift from Ranbaxy Laboratories, Delhi. Carbon tetrachloride was obtained from S.D. Fine-Chem Ltd., Boisar. Silymarin was obtained from Indena s.p.a., Milan, Italy. Thiobarbituric acid (TBA), 5,5'-Dithio-bis 2-Nitrobenzoic acid (DTNB) and Glutathione (GSH) were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were obtained from local sources and were of analytical grade.

**Animals:** Twenty-four locally bred adult male albino rats (150 - 250 g) of Wistar strain were used. They were housed in clean polypropylene cages and fed with commercial pelleted rat chow (M/S Hindustan Lever Ltd., Mumbai) and water \textit{ad libitum}. Before starting the experiment, permission from Institutional Animal Ethics Committee was obtained.

**Experimental procedure:** A total of 24 animals were equally divided into 4 groups (n=6 in each group). Group I, which served as normal control, received distilled water intraperitoneally (\textit{i.p.}). Group II received CCl\textsubscript{4} 0.5 ml/kg, \textit{i.p.} once daily for 7 days\textsuperscript{13}. Group III received CCl\textsubscript{4} 0.5 ml/kg, \textit{i.p.} and GB 50 mg/kg, \textit{i.p.}\textsuperscript{14} simultaneously for 7 days. Group IV received CCl\textsubscript{4} 0.5 ml/kg, \textit{i.p.} and silymarin 200 mg/kg, \textit{p.o.}\textsuperscript{15} simultaneously for 7 days. At the end of the treatment, blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes \textit{viz.}, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total proteins (TP) and albumin (Alb). The rats were sacrificed by cervical dislocation, the livers were removed immediately, washed with ice-cold saline and a 50% homogenate prepared in 0.05M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 700 x g for 10 minutes at 4°C and the supernatant was used for the estimation of malondialdehyde (MDA), the end product of lipid peroxidation and glutathione (GSH).

**Enzyme assays:** The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed in serum using standard kits from Lupin Laboratories and Pointe Scientific respectively. The results were expressed as units/litre (IU/L).

**Protein estimation:** The levels of TP and Alb were estimated in serum of experimental animals by biuret method and brom cresol green method respectively\textsuperscript{16}. Standard kits from Ranbaxy Laboratories, Delhi were used for these estimations.

**Lipid Peroxidation:** The quantitative measurement of lipid peroxidation was done by measuring the concentration of TBARS in liver using the method of Yagi\textsuperscript{17}. The amount of malondialdehyde (MDA) formed was quantitated by reaction with Thiobarbituric acid (TBA) and used as an index of lipid peroxidation. The results were expressed as nmol of MDA/g of wet tissue using molar extinction coefficient of the chromophore (1.56 x 10\textsuperscript{5} M\textsuperscript{-1} cm\textsuperscript{-1}).

**Glutathione estimation:** GSH was estimated in the liver homogenate using DTNB by the method of Buetler\textsuperscript{18}. The absorbance was read at 412 nm and the results were expressed as mg GSH/g of wet tissue.

**Histopathologic examination:** Animals were sacrificed on the day of withdrawal of blood and liver was removed, sliced and washed in saline. Liver pieces were preserved in 10% formaldehyde solution for histopathologic study. The pieces of liver were processed and embedded in paraffin wax. Sections made were about 4-6 µm in thickness. They were stained with hematoxylin and eosin and photographed.

**Statistical analysis:** The statistical analysis were carried out by One-way Analysis of Variance (ANOVA). P values <0.05 were considered significant.

**RESULTS**

**Biochemical parameters:** There was a significant (p<0.05) increase in the serum hepatic enzyme levels and significant (p<0.05) decrease in the TP and Alb levels after CCl\textsubscript{4}, which was reversed with GB (Figures 1 and 2). These effects were comparable to silymarin.

In order to probe the possible mechanism by which GB prevents hepatic damage caused by CCl\textsubscript{4},
Figure 1. Effect of *Ginkgo biloba* (GB) and silymarin on serum marker enzymes in CCl₄ induced hepatic damage (mean ± SEM). n = 6 in each group.

![Graph showing the effect of Ginkgo biloba (GB) and silymarin on serum marker enzymes in CCl₄ induced hepatic damage. The x-axis represents groups: I (Control), II (CCl₄), III (CCl₄ + GB), IV (CCl₄ + Silymarin). The y-axis represents IU/L for each enzyme (AST, ALT, ALP).](image)

- a p<0.05 Vs Group I
- b p<0.05 Vs Group II

Figure 2. Effect of *Ginkgo biloba* and silymarin on serum total proteins (TP) and albumin (Alb) in CCl₄ induced liver damage (mean ± SEM). n = 6 in each group.

![Graph showing the effect of *Ginkgo biloba* and silymarin on serum total proteins (TP) and albumin (Alb) in CCl₄ induced liver damage. The x-axis represents groups: I (Control), II (CCl₄), III (CCl₄ + GB), IV (CCl₄ + Silymarin). The y-axis represents g/dl for TP and Alb.](image)

- a p<0.05 Vs Group I
- b p<0.05 Vs Group II
Figure 3. Effect of *Ginkgo biloba* on thiobarbituric acid reactive substances (TBARS) levels in liver in CCl$_4$ induced liver damage (mean ± SEM). *n* = 6 in each group.

![Graph showing TBARS levels in liver with groups labeled: I (Control), II (CCl$_4$), III (CCl$_4$ + GB), IV (CCl$_4$ + Silymarin).](image)

*P <0.05 Vs Group II*

Figure 4. Effect of *Ginkgo biloba* on glutathione (GSH) levels in liver in CCl$_4$ induced liver damage (mean ± SEM). *n* = 6 in each group.

![Graph showing GSH levels in liver with groups labeled: I (Control), II (CCl$_4$), III (CCl$_4$ + GB), IV (CCl$_4$ + Silymarin).](image)

*P <0.05 Vs Group II*
Figure 5. Liver tissue of control rats showing normal histology.

Figure 6. Liver tissue of CCl₄ treated animals showing hydropic changes and steatosis. Congestion of central vein and sinusoids and mild portal chronic inflammatory cell infiltrate is seen.

Figure 7. Liver tissue of rats treated with CCl₄ and GB showing almost normal histology. There is no evidence of parenchymal injury.

Figure 8. Liver tissue of rats treated with CCl₄ and silymarin showing almost normal histology. There is no evidence of parenchymal injury.

Histopathologic examination: In control animals, liver sections showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and central vein (Figure 5). In CCl₄ treated animals the sections showed hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils. Congestion of central vein and sinusoids were seen with acute and chronic inflammatory cells infiltrating sinusoids mainly in the central zone. The midzonal and periportal hepatocytes showed mild to moderate degree of fatty change (Figure 6). Pretreatment with GB showed mild fatty change and mild sinusoidal congestion investigation on levels of TBARS and glutathione were carried out. TBARS were found to be elevated after the administration of CCl₄, which was significantly (p<0.05) reversed by GB (Figure 3). There was a significant (p<0.05) rise in GSH content of liver after treatment with GB (Figure 4). The effects of GB were comparable to that of silymarin.
HEPATOPROTECTIVE EFFECTS OF GINKGO BILOBA 265

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases\(^\text{19}\). The hepatotoxic effects of CCl\(_4\) are largely due to its active metabolite, trichloromethyl radical\(^\text{19,20}\). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give products like malondialdehyde (MDA) that cause damage to the membrane. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl\(_4\)\(^\text{21,22}\). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT and ALP. GB has significantly reduced these liver enzyme levels. Further, GB has increased the level of total proteins and albumin in the serum, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells\(^\text{23}\).

Since GB has increased the glutathione content of liver, it may also be useful in hepatotoxicity induced by other agents. However, glutathione levels were not affected in CCl\(_4\) treated groups (Figure 4).

In our study, elevation in the levels of end products of lipid peroxidation in liver of rats treated with CCl\(_4\) were observed. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Pretreatment with GB significantly reversed these changes. Hence it is possible that the mechanism of hepatoprotection of GB is due to its antioxidant effect.

Histopathological studies showed that CCl\(_4\) caused steatosis and hydropic degeneration of the liver tissue. GB pre-treatment exhibited protection, which confirmed the results of biochemical studies. All the effects of GB were comparable with those of silymarin, a proven hepatoprotective.

The results of our study indicate that simultaneous treatment with GB protects the liver against CCl\(_4\) induced hepatotoxicity.

REFERENCES

13. Rao PGM, Rao SG, Kumar V. Effects of Hepatogard against...


---

**ISOLATED TISSUE**

If you stretch it or let it dry
dear little tissue is sure to die
For if the temperature is too high
then may be it will fly
What to do to make it ply
that I'll discover by & by
It will respond, or it won't
but give up, I don't
Though it has a mind of its own
this much I too have sworn
Frustrated, I shall not be
for, in few years I'll be free

PG Students of Pharmacology
PGIMER, Chandigarh,
(Sent in by Dr. Dinesh Kumar Badyal,
Department of Pharmacology,
Christian Medical College,
Ludhiana)