



## CCl<sub>4</sub> INDUCED LIVER INJURY;

### AQUEOUS EXTRACT OF GINKGO BILOBA (GKBE) AMELIORATES CYTOPLASMIC AND MITOCHONDRIAL ENZYMES

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## INTRODUCTION

Ginkgo biloba, a famous natural herb, is commonly used medicinal remedy. The medicinal use of Ginkgo biloba (Gkb) dates back to centuries since time immemorial. Gkb is recognized because of its efficacy and cure of various diseases over many centuries back.<sup>1</sup> Biochemical analysis of Gkb extract (GkbE) shows various ingredients such as kaempferol, isorhamnetin, quercetin and organic acids such as the 4-hydroxybenzoic acid. Gkb contains diterpene lactones include the bilobalide and Ginkgolides (A, B, C, M and J). It also contains biflavones which include the ginkgetin, isoginkgetin, bilobetin. These ingredients are reported of pharmacological efficacy.<sup>2,3</sup> GkbE exerts powerful biological anti oxidant activity. Reported therapeutic effects of Gkb include the; anti diabetic potential, cardioprotective effects, neuromodulatory and memory enhancing effects,

**ABSTRACT... Objectives:** Evaluate the mitigating effect of aqueous extract of Ginkgo biloba (GkbE) on liver enzymes and histology in carbon tetrachloride (CCl<sub>4</sub>) induced liver injury in albino rat. **Study design:** Experimental study. **Setting and Duration:** Animal house, Bhitai Dental and Medical College Mirpurkhas and Agriculture University Tando Jam from Animal house from May 2015 - August 2016. **Subjects and Methods:** Sixty rats were equally divided into 3 groups Group 1- Controls (0.9% isotonic saline), Group 2- (CCl<sub>4</sub> CCl<sub>4</sub> 1.0mg/kg intraperitoneal) and Group 3- (CCl<sub>4</sub>+ GkbE). Blood samples were collected at end of experiment from tail veins. Liver was obtained after rat sacrifice by cervical dislocation. Tissue was fixed in formaldehyde and embedded in paraffin. Microscopy of 3μ tissue sections was performed after H & E staining. Statistix 10.0 (USA) software was used for data analysis at 95% confidence interval. **Results:** Four weeks GkbE administration in CCl<sub>4</sub> rat showed significant amelioration of liver enzymes and improved liver histology (p=0.0001). In GkbE treated rats, the histological changes of degeneration, fatty change, inflammatory cell infiltration, sinusoid congestion and necrosis was minimal (p=0.0001). GkbE was proved of mitigating the hepatocellular injury inflicted by carbon tetrachloride. **Conclusion:** GkbE mitigates the carbon tetrachloride induced liver injury in rat model. GkbE may be used in drug and chemical induced liver injury.

**Key words:** Ginkgo biloba extract, Carbon tetrachloride, Liver enzymes, Liver histology.

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decrease of mental fatigue, increased physical and mental activity. Clinically, Gkb is now most frequently prescribed herb in neuromedicine practice.<sup>4,5</sup> GkbE diminishes the senile cerebral insufficiency. It is prescribed for the treatment of dementia, defective cognition and Alzheimer's type dementia.<sup>6</sup> GkbE has been reported to augment the inhibition of platelet activation and increased blood flow in vivo studies. GkbE scavenges oxygen derived free radicals and protects the cell injury. GkbE has been used in the cardiac injury, infarction, brain infarction, ischemic reperfusion induced injury.<sup>2,7</sup> Efficacy against the ethanol induced gastric mucosal injury,<sup>8</sup> and chemical induced oxidative stress and liver fibrosis have been reported.<sup>9</sup>

Keeping in view the background literature, the protective effect of GkbE against carbon

tetrachloride injury was revisited. The present study was prospectively planned to observe the mitigating effects of GkbE against the carbon tetrachloride hepatocellular injury. Effects of GkbE against carbon tetrachloride injury were evaluated in terms of both liver enzymes and microscopic examination in experimental rat model.

## MATERIALS AND METHODS

Prior written permission was taken from the ethical review and animal ethics committee. A sample of sixty male Wistar albino rats was obtained. The rats were housed at the Animal house, Bhitai Dental and Medical College Mirpurkhas and Agriculture University Tando Jam from Animal house from May 2015 - August 2016. Male Wistar albino rats of 150- 200 grams were the inclusion criterion. Female rats, sick male rats, and rats feeding not well were excluded from the study protocol. Care was taken of optimal temperature (25°C) and humidity was maintained at 55-60%. Animals were environmental protected by 12 hour light-dark cycles. Chaw diet was provided ad libitum and clean water was available all the time.

Sixty rats were equally divided into 3 groups

- Group 1- Controls (0.9% isotonic saline),
- Group 2- CCl<sub>4</sub> 1.0mg/kg intraperitoneal and
- Group 3- CCl<sub>4</sub> (1.0mg/kg intraperitoneal) and GkbE 50/kg orally)

Treatment was given for 4 weeks consecutively

### Aqueous extract of *G. biloba* (GkbE)

GkbE was prepared from leaves (100 grams) soaked in approximately 1 liter boiling water. It was homogenized in distilled water. Magnetic stirrer was used for stirring at 40° C for 1 hour. The solution was filtered through 2-layer of cheese cloth. 17.561 gm GkbE was obtained. Residue was re-extracted within the fresh boiling distilled water. Aqueous Gkb was put in rotary evaporator in vacuum. After lyophilization, it was stored at 4 °C.<sup>1</sup>

### Carbon tetrachloride (CCl<sub>4</sub>) and Induction of DM

Purchase of CCl<sub>4</sub> (Sigma Chemical Co. St. Louis, MO, USA) was ordered from scientific drug

store, sponsored in Pakistan by Biodiagnostic Co. Analytical grading of chemical was checked for high laboratory purity. CCl<sub>4</sub> 1mg/kg b.w was administered intraperitoneal for induction of diabetes mellitus.<sup>10</sup>

### Animal sacrifice

Rats were sacrificed by cervical dislocation (Ketamine and Xylazil) as per Nayak et al.<sup>11</sup>

### Estimation of liver enzymes

Blood samples were collected at end of experiment from tail veins. Blood was centrifuged at 3000 rpm for 10 minute time period to separate sera. Liver enzymes were estimated on Roche Hitachi Chemistry analyzer.

### Liver tissue processing

Tissue was fixed in formaldehyde (10%) and embedded after in paraffin. Tissue was processed according to standard procedure. Microscopic study of 3μ tissue sections was observed after H & E staining. Histological liver injury was graded as; 0 = no abnormal findings observed, +1 = mild injury observed, +2 moderate injury observed and +3 severe injury observed.<sup>12</sup>

### Data analysis

Data was analyzed on Statistix 10.0 (USA) at 95% confidence interval (P≤0.05). Continuous variables were analyzed by ANOVA and post Hoc Duncan test and results were presented as mean± SD. Categorical variables were analyzed by Chi-square test.

## RESULT

The GkbE significantly mitigated the CCl<sub>4</sub> induced hepatocellular injury as observed by improvement in the liver aminotransferases (ALT, AST, ALP), serum GGT and LDH (p=0.001). Group 2 rats treated with CCl<sub>4</sub> showed severe hepatocellular injury as marked rise in liver enzymes was noted. The liver enzymes in Group 3 (CCl<sub>4</sub> +GkbE) showed significant decrease in liver aminotransferase and liver histology (p=0.001). The GkbE treated rats showed a decrease in hepatocyte cytoplasmic enzymes (e.g. ALT) and mitochondrial enzymes (e.g. AST) as shown in Table-I and Figure-1.

Histological examination of liver tissue (graded as; inflammation, vacuolar degeneration, fatty change, sinusoid congestion and hepatocyte necrosis) was significantly improved by GkbE treatment as shown in Table-II. Histological examination of liver tissue is shown in Figure-1 to 3 respectively. Liver tissue in CCl<sub>4</sub> treated rats show severe hepatocellular injury (Figure-2), the

tissue details are lost with distortion. CCl<sub>4</sub> treated liver tissue reveals inflammatory infiltrates, fatty change, sinusoid congestion, vacuolar degeneration and hepatocyte necrosis (Figure 2). All these destructive changes were mitigated by GkbE treated rats as shown in Figure-3 (CCl<sub>4</sub> + GkbE group).

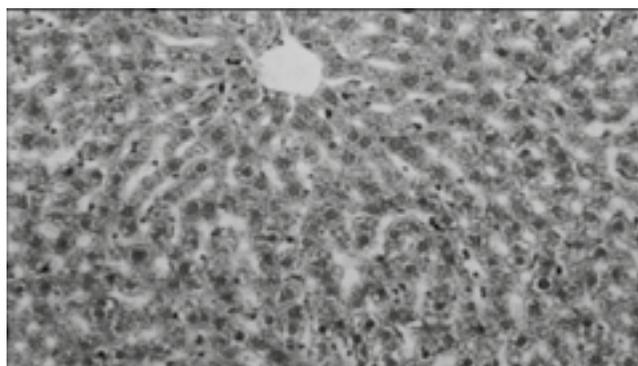
	ALT (iu/L)	AST (iu/L)	LDH (iu/L)	GGT (iu/L)	ALP (iu/L)
Group.1. controls	40.5± 9.2	54.1±11.5	513.5±37.5	23.4±7.5	100.5± 23.0
Group.2. CCl <sub>4</sub>	232.5±23.7	323.7 ±18.5	2058.9±107.6	158.0±17.6	214.1± 38.0
Group.3. CCl <sub>4</sub> + GkbE	150.9±21.6	213.5±21.5	1948.3±121.5	68.3±23.5	153.7±27.1
P-value	0.0001	0.002	0.0001	0.003	0.0001

CCl<sub>4</sub> – Carbon tetrachloride, GkbE- Ginkgo biloba extract

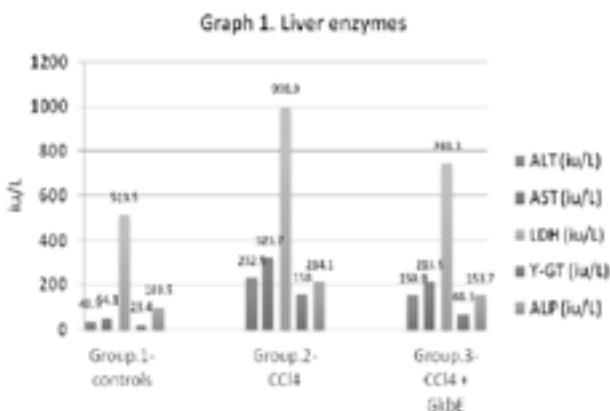
**Table-I. Liver enzymes in controls and experimental animals**

Histological Parameter	Group 1	Group 2	Group 3
Inflammation	0	+4	+3
Vacuolar degeneration	0	+4	+2
Fatty change	0	+4	+4
Sinusoid congestion	0	+4	+3
Necrosis	0	+4	+2

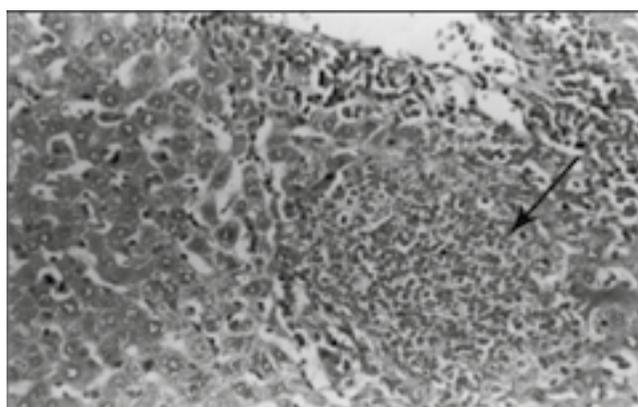
**Table-II. Histological grading of liver injury**  
\*Carbon tetrachloride, † Ginkgo biloba extract



**Figure-1. Liver tissue microscopy in control rats (H& E 40x)**



**Graph-1. Liver enzymes**

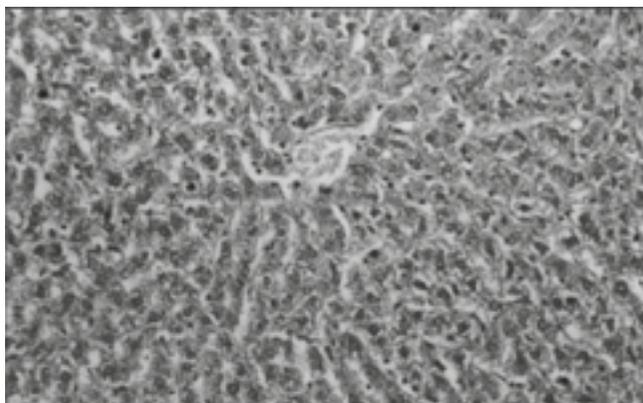


**Figure-2. CCl<sub>4</sub> treated liver tissue. Microscopy examination reveals severe tissue injury (H& E 40x)**

**DISCUSSION**

The present study is the first report on the mitigating effects of aqueous extract of Ginkgo biloba (GkbE) against carbon tetrachloride (CCl<sub>4</sub>) liver injury as indicated by hepatocyte cytoplasmic

and mitochondrial enzymes, and liver histology in albino rat. Drug and chemical induced liver injury is frequently observed in clinical emergency practice and there is need to evaluate easily available herbal remedies.



**Figure-3. GkbE + CCl<sub>4</sub> treated liver tissue. It shows hepatocyte cords with minimal tissue injury (H& E 40x)**

The GkbE showed significant mitigation of carbon tetrachloride induced chemical injury of liver (Table I and II). GkbE improved both cytoplasmic and mitochondrial enzymes and liver histology. These findings are in agreement with previous study.<sup>13</sup> They reported similar results of cytoplasmic and mitochondrial enzymes amelioration in CCl<sub>4</sub> treated animal model.<sup>13</sup> In carbon tetrachloride treated group the liver cell cytoplasmic and mitochondrial enzymes were raised because of severe cell membrane and mitochondrial injury mediated by free radicals generated by carbon tetrachloride.<sup>14</sup> The group 2 animals treated by CCl<sub>4</sub> showed similar pattern of liver enzymes as reported by above study, hence the findings are in keeping with.<sup>14</sup> The CCl<sub>4</sub> induces hepatocyte rupture with release of liver enzymes; AST, ALT, ALP and LDH which are the best markers of cell injury.<sup>15,16</sup> Alanine transaminase is a surrogate marker of hepatocyte cytoplasmic injury which was raised in present study and finding is in keeping with previous studies.<sup>17,18</sup>

In present study, the GkbE proved of its hepatoprotective potential as it mitigated the liver injury markers and liver histology, the results tally with the previous studies.<sup>19-21</sup> The underlying mechanism of how GkbE mitigates the cellular injury needs further studies at molecular level. However, the findings of present study support previous studies.<sup>1,22,23</sup>

The experience of CCl<sub>4</sub> was very much serious

as the rats of group 2 showed seriously rise in liver enzymes compared to control. The CCl<sub>4</sub> is proved hepatotoxic agent used in experimental studies, the findings of CCl<sub>4</sub> induced liver damage supports the previous studies.<sup>24-26</sup> AST is a marker of mitochondrial injury which was found elevated in CCl<sub>4</sub> treated rats; the finding is in agreement with previous studies.<sup>26-28</sup> Liver aminotransferases (ALT, AST, ALP), serum GGT and LDH were restored by GkbE co administration in group 3 (p=0.001). The present study suggests the GkbE may protect against free radical mediated injury by a direct anti oxidant effect, stabilizing cell and mitochondrial membranes which needs further studies. The findings are in agreement with previous studies.<sup>29-32</sup> Moreover; a previous study<sup>7</sup> reported the GkbE protects the vascular endothelial cells and microcirculation also. However, this seems to be not more than a speculation. The present study suggests the GkbE has potential of mitigating the carbon tetrachloride induced chemical injury, and most probably it be having activity against other chemicals and drugs, hence it may be used as herbal remedy for poisoning cases. The GkbE mitigates the carbon tetrachloride induced liver injury and ameliorates the cytoplasmic and mitochondrial enzymes and liver histology. The major limitation of present study is short duration of four weeks, small sample size and molecular phenomena were not evaluated because of non availability of research facilities and funding issues.

## CONCLUSION

The present study concludes the Ginkgo biloba (GkbE) has potential of mitigating chemical induced liver injury as carbon tetrachloride (CCl<sub>4</sub>). Ginkgo biloba (GkbE) ameliorated both liver cellular enzymes and liver histology. Ginkgo biloba (GkbE) may be used as an easily available and inexpensive home herbal remedy in cases of chemical and drug induced liver injury.

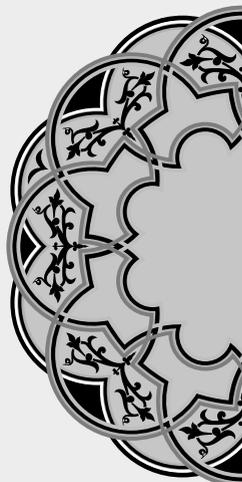
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*“Life is the most difficult EXAM.  
Many people fail because they try to copy others.  
Not realizing that everyone has a different question paper.”*

**Unknown**

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2	Dr. Faisal Irshad	Concept of study, data collection, perform designing.	
3	Dr. Hina Mawani	Concept of study, data collection, perform designing, consent form, manuscript write and proof.	