Antioxidant and hepatoprotective effects of *ginkgo biloba* leave extracts

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ABSTRACT

The present study was undertaken to investigate the in vitro and the in vivo antioxidant and hepatoprotective effects of *Ginkgo biloba* leave extracts. Qualitative analysis of different leaves extracts [ethanol 70% (Et), ethyl acetate (EA), and water (W)] revealed the presence of flavonoids, phenolic compounds, saponins, tannins, terpenes, and carbohydrates. The quantitative analysis of the leaves extracts revealed that the Et extract contains the highest amounts of tannins, saponins, flavonoids and phenolic compounds. The EA extract contains higher amounts of flavonoids and phenolic compounds and lower amounts of tannins and saponins compared to the W extract. The in vitro studies revealed that all the *Ginkgo biloba* leave extracts have a high antioxidant activity. The Et extract showed the highest activity followed by the EA extract then the W extract. Moreover, the in vivo studies revealed that all the *Ginkgo biloba* leave extracts have hepatoprotective activity, which evidenced by the effect of these extracts on the antioxidant enzymes activities [superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), glutathione–S–transferase (GST)], the liver marker enzymes [alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT)], the lipid peroxidation as well as inhibitory effects on nitric oxide (NO) release. The administration of these extracts significantly restored the elevated activities of the liver marker enzymes, and the antioxidant enzymes, which increase during hepatotoxicity. Also, they inhibit the release of NO. The treatment with the Et extract gave the best results for hepatoprotective effects. Moreover, the administration with this extract did not significantly affect the normal values of blood glucose (BG), total proteins (T.P), total lipids (T.L), total cholesterol (T.C), low and high density lipoprotein cholesterol (LDL-c and HDL-c). From this study it can conclude that the treatment with *Ginkgo biloba* leave extracts can inhibit the hepatotoxicity without toxic effect.

INTRODUCTION

*Ginkgo biloba* has been a popular remedy in traditional Chinese medicine for over 4000 years, and it has been a common herbal medicine in Europe since the 1730’s10. The *Ginkgo* tree, the only existing tree in the family *Ginkgoaceae*, is the world’s oldest living tree and is thus
sometimes referred to as a “living fossil”\(^\text{(2)}\). *Ginkgo* trees are now widely planted in China, Japan, Korea, France, Germany, and the United States for both ornamental and medicinal purposes.

Several human diseases have been associated with the overproduction of reactive oxygen species (ROS) and subsequently various antioxidants emerged as potential therapeutic agents that scavenge ROS. Free radical reactions are associated with a range of degenerative diseases and acute ischemic insults affecting liver and other tissues\(^{\text{(3)}}\). The free radical-mediated hepatotoxicity can be effectively managed by administration of such agents.

Kuo, et al.\(^{\text{(4)}}\) demonstrated that *Ginkgo biloba* extract competitively inhibited rat hepatic microsomal CYP1A-mediated enzyme activity, as determined by experiments performed on extracts containing known amounts of terpene trilactones and ginkgo flavonol glycosides. The mechanism of action of *Ginkgo biloba* is not known. However, it appears to possess antioxidant activity\(^{\text{(5)}}\).

The present study was undertaken to investigate chemical composition of various *Ginkgo biloba* extracts and to evaluate their hepatoprotective activity through scavenging peroxynitrite formation and antioxidant activity.

**MATERIALS & METHODS**

**Plant material:**
Leaves samples of *Ginkgo biloba* (*Ginkgoaceae*) were collected in May (2005) from tree cultivated in Orman garden, Giza, Egypt. All leaves were washed then air dried in shade at room temperature for 5–7 days, grinded to fine powder and kept for phytochemical analysis.

**Plant extracts:**
50 g of the air-dried powdered leaves of *Ginkgo biloba* were extracted separately by percolation in three different solvents. The first solvent was ethanol 70% (Et), the second solvent was ethyl acetate (EA) and the third one was water (W). All extracts were filtered, then freeze dried.

**General phytochemical screening:**
Preliminary phytochemical tests were carried out on the different *Ginkgo biloba* leaves extracts. Carbohydrates and/or glycosides, flavonoids, saponins, sterols and/or triterpenes, and phenolic compounds were tested according the methods described by Lewis and Smith\(^{\text{(6)}}\), Geissman\(^{\text{(7)}}\), Shellard\(^{\text{(8)}}\), Balbaa\(^{\text{(9)}}\), Fransworth, et al.\(^{\text{(10)}}\), and Swain and Hillis\(^{\text{(11)}}\), respectively.

**Quantitative determination of the phytochemicals in the different extracts of *Ginkgo biloba*:**
Total tannins, saponins, flavonoids, and phenolic compounds were determined according to the methods described by Balbaa\(^{\text{(9)}}\), Ebrahimzadeh and Niknam\(^{\text{(12)}}\), Zhuang et al.\(^{\text{(13)}}\), and Swain and Hillis\(^{\text{(11)}}\), respectively.

**Free radical scavenging assay:**
The antioxidant activity of the different *Ginkgo biloba* leaves extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydryzyl (DPPH\(^*\)) according to Brand–
Williams et al.\textsuperscript{(14)} and the inhibition percentage of the DPPH\textsuperscript{*} by the samples was calculated according to the formula described by Yen and Duh\textsuperscript{(15)} as follows:
\[
\% \text{Inhibition} = \left[ \frac{(\text{Ac} (0) – \text{Ac} (t))}{\text{Ac} (0)} \right] \times 100
\]
Where: Ac (0) was the absorbance of the control at t = 0 min and Ac (t) was the absorbance of the antioxidant, which varied with the different content.

Superoxide radical scavenging activity by various Ginkgo biloba extracts was determined according to Chung, et al.\textsuperscript{(16)}.

Determination of thiobarbituric acid (MDA):

Lipid peroxidation was measured in the liver according to the method of Burits and Bucar\textsuperscript{(17)}.

Experimental animals:

Forty male Sprague - Dawley rats (175-200g) were housed in the animal house of Research Institute of Ophthalmology. The rats were kept under normal laboratory conditions for two weeks before commencing the experiments.

Route of administration:

Animals were divided into five groups (each group eight rats). Group one represents the negative control, which administered 10% di-methyl sulfoxide (DMSO). Group two represents the positive control, which administered a single oral dose of carbon tetrachloride (CCl\textsubscript{4}) (1:1 in liquid paraffin + 10 % DMSO) to induce liver damage. The single dose was equal 2.0 ml/kg body weight. Groups three, four and five were given with ethanolic, ethyl acetate, and water extracts (100mg/kg/body weight) respectively for 14 days. After six hrs from the last dose of extracts, all groups except group one were administered with a single oral dose of CCl\textsubscript{4} to induce liver damage. Rats were decapitated after 72 hrs from the CCl\textsubscript{4} administration, then blood and liver were taken from various groups and samples were prepared according to each method.

Preparation of Liver tissues for biochemical analyses:-

Liver tissues were washed twice with cold saline solution, placed into glass bottles, labeled, and stored at −80 °C until processing. Liver tissues were homogenized in Tris–HCl buffer 0.1 M (pH 7.4). The homogenates were then centrifuged at 5000×g for 60 min. and the clear supernatant fluid was taken for assaying the enzymes activity. Tissue malondialdehyde (MDA) and nitric oxide levels were determined as describe later.

Biochemical analysis:

Fasting blood glucose (FBG) was estimated by colorimetric method of Trinder\textsuperscript{(18).} Total proteins (T.P) were determined according to the method of Lowry, et al.\textsuperscript{(19)}.

The glutathione peroxidase (GSH-PX) activity was estimated using 1–chloro-2,4-dinitrobenzene (CDNB) as substrate according to the method described by Habig, et al.\textsuperscript{(20)}.

Superoxide dismutase activity (SOD) was measured by the method of Santa-már\textsuperscript{á}, et al., (21). The activity of alkaline phosphatase (ALP) was determined according to Empfehlungen\textsuperscript{(22)}.

Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were estimated according to Reitman and Frankel\textsuperscript{(23).} Hepatic glutathione-S–transferase (GST) activity was
Serum total lipids (T.L), cholesterol (T.C), Low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) were measured by using BioMerie Kits according to the methods described by Zollner and Kirsch (27), Tietz (26), Bergmenyer (27), and Stein (28), respectively.

Serum and liver nitric oxide was estimated using Greiss reagent according to the method of Green, et al. (29).

Statistical analysis:
Statistical analyses were done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

RESULTS

Preliminary phytochemical screening of the different Ginkgo Biloba leaves extracts:-
As shown in table (1), the phytochemical study of the different extracts of Ginkgo biloba leaves [ethanolic extract 70% (Et), ethyl acetate (EA), and water (W) extracts] revealed the presence of flavonoids, tannins, saponins, sterols and/or triterpenes and phenolic compounds. Carbohydrates and/or glycosides were found only in the ethanol and water extracts.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Et1</th>
<th>EA</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates and or glycosides</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Sterols and or triterpenes</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

(+++) High amount (++) moderate amount (+) Low amount (-) Absent.

Chemical analysis of the different Ginkgo biloba leaves extracts:
The obtained data in table (2) showed that the percentage of tannins and saponins compounds were (2.19%, 0.59%, 1.48%) and (1.05%, 0.29%, 0.89%) based on the dry weight, respectively for the ethanolic, ethyl acetate, and water extracts. While the percentage of flavonoids and phenolic compounds were (1.55%, 1.49%, 0.98%) and (2.59%, 1.98%, 1.58 %) based on the dry weight, respectively for the ethanol 70%, ethyl acetate, and water extracts. The highest concentration of tannins, saponins, flavonoids and phenolic compounds were found in the ethanolic extract.
Table (2): Total Tannins, Saponins, Flavonoids and Phenolic Compounds Contents in the Different *Ginkgo Biloba* Leaves Extracts.

<table>
<thead>
<tr>
<th>Compounds Extracts</th>
<th>Total tannins %</th>
<th>Total saponins %</th>
<th>Total flavonoids %</th>
<th>Total Phenolic compounds %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 70% (Et)</td>
<td>2.19</td>
<td>1.05</td>
<td>1.55</td>
<td>2.95</td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>0.59</td>
<td>0.29</td>
<td>1.49</td>
<td>1.98</td>
</tr>
<tr>
<td>Water (W)</td>
<td>1.48</td>
<td>0.89</td>
<td>0.98</td>
<td>1.58</td>
</tr>
</tbody>
</table>

*All determinations based on the dry weight.*

Antioxidant activity of the different *Ginkgo biloba* extracts according to the DPPH radical scavenging method:-

Table (3) shows that rutin (standard) is superior inhibitor of DPPH● compared to *Ginkgo biloba* leaf extracts and gave high percentage inhibition 82.40 % and 91.27 % for 50, 100 µg/ml. The results in table (3) show that the decrease in absorbance of DPPH● radical concerning to all extracts compared with rutin standard. While water extracts showed more decrease in the inhibition effect for all concentrations than other extracts.

Table (3): Comparison of Free Radical Scavenging Activity of different *Ginkgo biloba* leaves extracts with rutin standard expressed as % inhibition.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH● radical decolouration 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*10 (µg/ml)</td>
</tr>
<tr>
<td>Rutin (Standard)</td>
<td>58.1</td>
</tr>
<tr>
<td>Ethanol 70% (Et)</td>
<td>59.47</td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>47.59</td>
</tr>
<tr>
<td>Water (W)</td>
<td>29.57</td>
</tr>
</tbody>
</table>

*Different concentrations of extract. All values mean of 3 replicates.

Antioxidant activity of different extracts from *Ginkgo biloba* leaves according to the Superoxide scavenging activity method:-

Table (4) shows that ethanolic and ethyl acetate extracts of *Ginkgo biloba* leaves showed a good free radical scavenging activity. The activities were increased by increasing the concentration of the extract. The highest superoxide scavenging activity was observed with Et followed by EA extract, and finally the water extract.
Table (4): Superoxide Radical Scavenging Activity of Rutin Standard expressed as % inhibition and Different Ginkgo Biloba Extracts.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Superoxide scavenging activity 100 %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*10 (µg/ml)</td>
</tr>
<tr>
<td>Rutin (Standard)</td>
<td>69.87</td>
</tr>
<tr>
<td>Ethanol 70% (Et)</td>
<td>72.59</td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>64.9</td>
</tr>
<tr>
<td>Water (W)</td>
<td>35.68</td>
</tr>
</tbody>
</table>

* Different concentrations of extracts. All values mean of 3 replicates

Fasting blood glucose, total proteins and MDA contents:-

Table (5) shows the levels of fasting blood glucose (FBG), total proteins (T.P) and malondialdehyde (MDA) in the negative control, the positive control and in the groups treated with the different *Ginkgo biloba* extracts. The obtained data from this table revealed that the highest decrease in the FBG was observed in the rats administrated with the ethanolic extracts compared with the positive control. On the other hand, there was no significant change in the levels of total proteins in the different groups. The level of MDA content in positive control group was significantly increased under oxidative stress. But in the various groups treated with *Ginkgo biloba* extracts there was significant decrease in the levels of MDA content, the lowest content was observed with ethanolic extract.

Table (5): Fasting Blood Glucose (FBG), Total Proteins (T.P) and Malondialdehyde (MDA) in Control and Different *Ginkgo Biloba* Extract Groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>FBG (mg/dl) mean±S.E.</th>
<th>T.P (g/dl) mean±S.E.</th>
<th>MDA (nmol/ml) mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>117.34 ± 15.20 c</td>
<td>6.92 ± 0.41 a</td>
<td>1.60±0.53 c</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>221.61 ± 29.71 a</td>
<td>5.59 ± 0.31 b</td>
<td>2.70±1.61 a</td>
<td></td>
</tr>
<tr>
<td>Ethanol 70% (Et)</td>
<td>128.70 ± 21.01 c</td>
<td>6.12 ± 0.32 b</td>
<td>1.63±0.79 c</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>172.00 ± 23.60 b</td>
<td>6.21 ± 0.19 b</td>
<td>1.70±0.90 c</td>
<td></td>
</tr>
<tr>
<td>Water (W)</td>
<td>200.11 ± 27.10 ab</td>
<td>5.98 ± 0.41 b</td>
<td>1.98±1.19 b</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same column with the same letter are not significant at *P*< 0.05.

Anti-Hyperlipidemic Activity of the different *Ginkgo biloba* Extracts:-

Data in table (6) show a high anti-hyperlipidemic activity of various *Ginkgo biloba* extracts, the highest effect was observed in the group administrated with the Et extract, followed by EA extract and finally by the water extract. On the other hand, the lowest decrease in the lipid profile was observed in the group administrated with the Et extract compared with the other two extracts.
Table (6): Total Lipids (T.L), Total Cholesterol (T.C), Low Density Lipoprotein (LDL-c) and High Density Lipoprotein (HDL-c) in Controls and Different Extracts of Ginkgo Biloba Leaves.

<table>
<thead>
<tr>
<th>Samples</th>
<th>T. L (mg/dl) mean±S.E</th>
<th>T.C (mg/dl) mean±S.E</th>
<th>HDL-c (mg/dl) mean±S.E</th>
<th>LDL-(mg/dl) mean±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>560.54±42.25e</td>
<td>108.23±8.57e</td>
<td>36.78±1.24d</td>
<td>70.70±2.14 e</td>
</tr>
<tr>
<td>Control (+)</td>
<td>890.24±57.15a</td>
<td>230.58±12.24a</td>
<td>25.80±1.98c</td>
<td>188.70±11.14a</td>
</tr>
<tr>
<td>Ethanol 70%(Et)</td>
<td>580.7±32.65 d</td>
<td>135.54±7.89 d</td>
<td>56.35±3.14a</td>
<td>98.96±4.57 d</td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>620.89±28.65c</td>
<td>162.67±11.24c</td>
<td>49.62±2.31b</td>
<td>106.37±6.78 c</td>
</tr>
<tr>
<td>Water (W)</td>
<td>780.98±43.25b</td>
<td>219.38±13.57b</td>
<td>36.46±2.14d</td>
<td>169.6±12.47 b</td>
</tr>
</tbody>
</table>

Means in the same column with the same letter are not significantly at P< 0.05.

The Protective Activity of Ginkgo biloba Extracts Against Hepatotoxicity by its Modulating Effect on Defense Enzymes and Nitric Oxide:-

The obtained data in table (7) show that the Ginkgo biloba extracts caused a significant decrease in the AST and ALT activity compared to the positive control, the same trend was observed with the ALP activity. In addition, the activity of antioxidant enzymes (GSH-PX and SOD) and also nitric oxide were decreased after administration of different Ginkgo biloba extracts.

Table (7): Blood Glutathione Peroxidase (GSH-PX), Superoxide Dismutase (SOD), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Nitric Oxide (NO) in Control and Rats Treated with Different Ginkgo Biloba Extracts.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>AST µ/l mean±S.E</th>
<th>ALT µ/l mean±S.E</th>
<th>ALP µ/l mean±S.E</th>
<th>SOD µ/ml mean±S.E</th>
<th>GSH-PX µm/min/mg mean±S.E</th>
<th>NO µmol/ml mean±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>217.34 ±15.20 c</td>
<td>41.34 ±2.97 a</td>
<td>145.86 ± 6.05 d</td>
<td>30.14 ±9.99 e</td>
<td>0.99 ±0.04 c</td>
<td>53.25 ±2.54e</td>
</tr>
<tr>
<td>Control (+)</td>
<td>321.61 ±29.71 a</td>
<td>96.78 ± 8.21 b</td>
<td>301.75 ±23.27 a</td>
<td>79.42 ±03.71 a</td>
<td>1.59 ±0.03 a</td>
<td>102.21 ±4.58 a</td>
</tr>
<tr>
<td>Ethanol (70)</td>
<td>228.70 ±21.01 c</td>
<td>66.19 ±5.21 d</td>
<td>169.86 ±9.31 d</td>
<td>41.05 ±02.13 d</td>
<td>1.34 ±0.05 ab</td>
<td>76.59 ±4.21 d</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>272.40 ±23.60 b</td>
<td>71.98 ±5.21 c</td>
<td>214.73 ±8.99 c</td>
<td>51.35 ±1.47 c</td>
<td>1.37 ±0.02 ab</td>
<td>85.89 ±3.58 c</td>
</tr>
<tr>
<td>Water (W)</td>
<td>300.11 ±27.10ab</td>
<td>87.32 ±7.81eb</td>
<td>257.95 ±14.01b</td>
<td>63.25 ±4.05 b</td>
<td>1.28 ±0.03 b</td>
<td>89.49 ±5.28 b</td>
</tr>
</tbody>
</table>

Means in the same column with the same letter are not significantly at P< 0.05.
Effect of Different Ginkgo biloba Extracts against Hepatotoxicity of Carbon Tetrachloride in Liver Rats -

The protective effects of various extracts of Ginkgo biloba leaves against liver damage induced by carbon tetrachloride (CCl₄) were evaluated in rats. The results in Table (8) show that CCl₄ administration significantly damages the liver which is evident from the very high activities of liver antioxidant enzymes. Acute CCl₄ administration significantly increased the level of liver injury marker enzymes such as GSH-PX and SOD. It also increased the activity of glutathione-S-transferase, but treatment with different extracts 14 days before CCl₄ treatment offered considerable protection to liver as evidenced from the levels of most biochemical parameters. Administration of Et decreased the activities of GSH-PX, SOD and GST in the liver compared to the positive control.

The data of table (8) revealed that the nitric oxide content was decreased in response to the administration of various Ginkgo biloba extracts and the lowest decrease in the nitric oxide levels was observed with Et, then the E.A. and finally with water extract.

Table (8): Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-PX), Glutathione-S-Transferase (GST), Nitric Oxide (NO), and Malondialdehyde (MDA) Content of the Homogenized Liver Tissues in Controls and Rats Treated with Different Ginkgo biloba Extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD µ/mg protein mean±S.E.</th>
<th>GSH-PX µ/mg protein mean±S.E.</th>
<th>GST µm/mg pt mean±S.E.</th>
<th>NO µmol/mg pt mean±S.E.</th>
<th>MDA nmol/mg mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>91.07 ±6.90e</td>
<td>1611.9 ±99.87c</td>
<td>0.99 ±0.04a</td>
<td>120.89 ±4.58d</td>
<td>1.64 ±0.78c</td>
</tr>
<tr>
<td>Control (+)</td>
<td>277.2 ±19.01a</td>
<td>2781.9 ±159.1a</td>
<td>1.59 ±0.03b</td>
<td>230.17 ±8.24a</td>
<td>2.70 ±1.61a</td>
</tr>
<tr>
<td>Ethanol (70%)</td>
<td>121.3 ±5.2 d</td>
<td>1756.9 ±91.01c</td>
<td>1.40 ±0.03bc</td>
<td>158.95 ±7.54c</td>
<td>1.602 ±0.53c</td>
</tr>
<tr>
<td>Ethyl acetate (EA)</td>
<td>179.38 ±9.02 c</td>
<td>2054.7 ±113.27b</td>
<td>1.37 ±0.02d</td>
<td>188.95 ±6.57 b</td>
<td>1.70 ±0.91 c</td>
</tr>
<tr>
<td>Water (W)</td>
<td>205.85 ±12.73b</td>
<td>2243.16 ±107.91b</td>
<td>1.38 ±0.05e</td>
<td>191.54 ±11.24 b</td>
<td>1.98 ±0.12 b</td>
</tr>
</tbody>
</table>

Means in the same column with the same letter are not significantly at P< 0.05.

DISCUSSION

Ginkgo biloba leaves were used in traditional Chinese medicine, and now grows throughout the world. This popular herbal medicine is extracted from the fan-shaped leaves of the ancient Ginkgo biloba tree. The mechanism of action is not yet well understood. Some studies suggest that Ginkgo biloba may be beneficial for
treating the symptoms of cancer, Alzheimer's disease, learning, memory, attention, or concentration in elderly.\(^{30,31}\) It may have anti-inflammatory, anti-oxidant, anti-coagulant properties and may also increase blood flow to the brain.

Wang, et al.\(^{32}\) found that the contents of flavonoids in the leaves of *Ginkgo biloba* gathered from different producing areas are different. For this reason the current was directed to study the chemical composition of *Ginkgo biloba* in Egypt. The results of phytochemical screening of different *Ginkgo biloba* extracts revealed the presence of carbohydrates and or glycosides, tannins, saponins, flavonoids, sterols and or triterpens and phenolic compounds. Also after extraction with different solvents, the chemical constituents were determined and the results are shown in table 2. Preliminary photochemical screening and chemical analysis of Et (tables 1 and 2) showed the presence of flavonoids which are natural products having significant biological activities including antiradical and antioxidant properties.

The results of Table (3) show that the Et extract showed high percentage inhibition of DPPH\(^*\). These results suggested that the decrease in the absorbance of DPPH\(^*\) radical was due to its reduction by different antioxidants. From these data, the Et1 extract of *Ginkgo biloba* showed good free radical- scavenging activity depending on the concentration used and it contain high significant amount of natural flavonoids and phenolic compounds. These results are agreement with those obtained by Zhang, et al.\(^{33}\) who reported that natural mixtures of phenolic compounds have high antioxidant activity. Based on these experimental results, it is suggested that *Ginkgo biloba* leaf extracts comprise effective potential source of natural antioxidants.

Superoxide radical scavenging activity of the different *Ginkgo biloba* extracts is shown in table 4. Rutin is an important anti–lipoperoxidant agent and also a strong scavenger of hydroxyl and superoxide radicals as reported by Lachman, et al.\(^{34}\), so it was used as a standard in this study. The data of the current study suggested that all *Ginkgo biloba* extracts have superoxide scavenging activity. The most scavenging activity was observed in the Et extract followed by EA extract and lastly the W extract. This may be depends on the flavonoids content of these extracts. The higher flavonoids content the higher scavenging activity. This data was in agreement with Sudheesh and Vijayalakshmi\(^{35}\), who showed that fractions rich in flavonoids obtained from *Punica granatum* extracts have potential anti-peroxidative effect.

The obtained data of table (5) revealed that the highest decrease in the FBG compared with the positive control was observed in the animal administrated with the ethanolic extract. This may be due to the effect of *Ginkgo biloba* on the stimulation of glucose uptake or the activation of insulin receptor as reported by Pinakini, et al.\(^{36}\). There were no significant changes in the total protein concentrations in the groups administrated with the different
extracts. The lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl₄. The obtained data of table 5 revealed that the MDA content was significantly increased in the positive control, but the various Ginkgo biloba extracts treatment caused significant decrease in the levels of MDA content. The lowest content was observed with ethanolic extracts, which may be due to the high antioxidant activity in Et extract compared with other extracts. Also, Ginkgo biloba extract was also found to have a protective effect against lipid peroxidation in hepatic microsomes by inhibiting this lipid peroxidation (37).

Oxidative stress, which involves excess accumulation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide (H₂O₂) and hydroxyl radical, can damage cells by lipid peroxidation and alteration of protein and nucleic acid structure. Data of table (6) showed a high anti-hyperlipidemic activity of Ginkgo biloba extracts. Treated treatment with different extracts indicating the potentiality of Et as antioxidant by preventing the peroxidative damage caused by CCl₄ more than other extracts. This is may be due to high contents of antioxidant compounds and flavonoids resulted in the inhibition of lipid peroxidation. Flavonoids have inhibitory effect on microsomal Cyt P450 enzyme or on lipid peroxidation. It may be interfering with the Cyt P450 and ultimately hindering the biotransformation of CCl₄. At the same time Et may also possess antioxidant activity, which inhibited the deleterious effect of free radicals generated by CCl₄ influencing the membrane rigidity by preventing inhibiting the membrane peroxidation(38). Also the antihyperlipidemic activity may be due to the protective activity of Et extract against induced hepatotoxicity that modulates lipid metabolism and lipid profile to more or less normal levels.

It is thought that the natural antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation (39). The free radical-mediated hepatotoxicity can be effectively managed by administration of such agents possessing antioxidants free radical scavengers. Protective effects of Ginkgo biloba extracts against hepatotoxicity by GSH-PX and SOD defense enzymes modulation are shown in tables (7) in the blood and table (8) in the liver. Damage of the liver cells by CCl₄ cause leakage of cellular enzymes to the blood. CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases (40). Administration of CCl₄ causes oxidative damage through free-radical generation. In the present study, the rats were administered with a single oral dose of CCl₄ to induce liver damage and the rats were killed after 72 hours from the administration of CCl₄. This time is enough to generate a high amount of free radicals and as a result, the activity of antioxidant enzymes increased to overcome these high amounts of free radicals. The liver damage in the positive control was evidenced by an elevation in the serum maker enzymes.
(table 7), namely AST, ALT, and ALP as reported by Johnson and Kroening. Also, administrations of CCl₄ cased a significant increase in the activities of the antioxidant enzymes such as GSH-PX, SOD and GST in the blood (table 7) as well as in the liver. In the present study the crude ethanol, ethyl acetate and water extracts were evaluated for the protective effect against liver damage induced by CCl₄ in rats. The data revealed that *Ginkgo biloba* extracts has decreased the levels of the previous enzymes in the blood but the levels did not reach the normal values, which indicate hepatoprotective activity of these extracts. The most significant decrease in these enzymes was observed in the rats administrated with the Et extract. In addition to the previous results, administration with the Et extract decreased the activities of SOD and GSH-PX enzymes in the blood as well as in the liver more than the other extracts. Generally, the administration of the different extracts showed an improvement in the activities of different antioxidant enzymes. Based on these results, it is clear that the inhibitory effect of Et extract of *Ginkgo biloba* leaves showed the highest inhibitory effect on these enzymes. This may be partly due to its action as free radical scavenger and its ability to chelate ions responsible for the promotion of lipid peroxidation. Moreover, treatment with Et of *Ginkgo biloba* leaves before CCl₄ treatment offered considerable protection to liver as evidenced from the levels of biochemical parameters.

In another explanation, the inhibitory effects of *Ginkgo biloba* extracts on rat hepatic enzyme activity may be due to the inhibition by the flavonoids (kaempferol and quercetin) that detect chemically in *Ginkgo biloba*. Kuo, et al. found that isorhamnetin is a potent inhibitor of rat hepatic microsomal CYP1A. Kaempferol, quercetin, and isorhamnetin all have multiple hydroxyl groups. This result is consistent with the idea that flavonoids possessing hydroxyl groups tend to be inhibitors of these enzymes. Kaempferol has a hydrogen atom and isorhamnetin has a methoxy group, whereas quercetin has a hydroxyl group. Thus, it appears that a hydroxyl renders the flavonol molecule less potent with respect to the inhibition of rat hepatic enzymes. In addition to the flavonoids, *Ginkgo biloba* extracts are known to contain many other chemicals, including carbohydrates and/or glycosides as shown in table (1). The results in the current study suggested that glycoside may be decrease the inhibitory effect on ALP, GHS PX, GST, and SOD as reported in tables (7,8). Based on these results of the phytochemical analysis of *Ginkgo biloba* leaves, it was observed that the highest concentration of glycosides and flavonoids found in the ethanolic extract, which recorded the best results for the *in vivo* inhibition of rat hepatic enzyme activity.

Nitric oxide (NO) is a free radical with many well-known physiological functions; one these are its abilities to act as either a cytoprotective or a cytotoxic agent. It is accepted that the difference between the protective and the deleterious effects of this free radical is determined both by
concentration and time of exposure and by cell type\(^{(44)}\). The effect of Ginkgo biloba extract a on the NO production was studies by Varga, et al.\(^{(45)}\). They found that Ginkgo biloba directly acts as a NO scavenger and concomitantly inhibits the expression of iNOS mRNA. Thus, Ginkgo biloba may act as a potent inhibitor of NO production under the condition of ischemia/reperfusion, improving the recovery of post-ischemic cardiac function. These results showed the lowest decrease in nitric oxide level had been observed in rats administrated with Et extract, then the E.A extract and finally with the water extract. This may be due to the inhibitory effect of Ginkgo flavonoids on the inducible nitric oxide synthetase and scavenge peroxynitrite formation.

In conclusion: the Et extract of Ginkgo biloba has the highest activity against lipid peroxidation and free radical scavenging effect compared to that of ethyl acetate and the water extracts. In addition, it has the most protective activity against liver injury caused by CCl\(_4\) administration. Moreover, it was the most effective extract on the NO. This may be due to its high contents of antioxidant compounds and flavonoids compared to the other two extracts. Finally, the present study can say that Ginkgo biloba has a very wide range of pharmacological actions.

**REFERENCES**


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العالمي من الفلاغونيات، ولذا صمم هذا البحث لدراسة تأثير نشاط مستخلصات نبات الجينكو بيلوبا في حماية الكبد من الشوارع الحرة التي تصدر نتيجة لمعاملة الكبد ببراع كولوريد الكربون ودراسة أسباب و طرق هذه الحماية و ذلك باستخدام التجارب In vitro and in vivo. 

للمركبات الموجودة في كل مستخلص على حدى. أوضح النتائج أن مستخلص الكحلو الإيثيلي يحتوي على أعلى نسبة من المكونات النباتية والفلاغونيات والفتونيات والصابونين. أوضح النتائج تجربة

تشمل المستخلصات الثلاث كمضادات الأكسدة وذلك بإستعمال مادة DPPH

أظهر أعلى المحتويات في إحتواءه على مضادات الأكسدة. أيضاً أظهر نشاط واضح وفعال في تثبيت أكسيد النيتروك و عدم تسبب الوبان السوبر أكسيد

دراسة تأثير المستخلصات السابقة على نشاط الإنزيمات المعضلة للأكدة في الدم و أيضاً الكبد يتم استخدام عدد اربعون فار من فئان التجارب قسمت إلى خمس مجتمعات، المجموعة الأولى تتألف (10% Dimethyl sulphoxide-DMSO) وهي تمت مجموعة الكونتينول الموجب (CCL3 أو مجموعات مثلاً DMSO) وهي تمت الكونتينول السلبي، أما المجموعات الثانوية و الثالثة و الرابعة تتألف كمية 100 مجم/كجم من وزن الجسم من مستخلص الكحلو الإيثيلي و خلايا الأليل

المستخلص المائي على التوالي أيضاً تم إجراء عملية سمية للكبد باستخدام رابع كولوريد الكربون بالنسبة للمستخلصات الثلاث الأخيرة

تم تقدر نسبة أنزيمات السوبر أكسيد ديمبوتيز، الجيلوثيون هم ترافيز الجلوثيون

بوريوكسيديل أكسيد الثيتيك، بالداناديني، الفيتامينات الكلية و نسبة السكر في الدم. وكذلك تم تقدر الدهن ثانية الكولسترول الكلي، بدنات عالية، والدماغة في الكتلة إلخ تحلبيدات الثانوية. قدر أن المستخلص الكحلو يتأثر واضحاً على تقليل الآثار السامة لرابع كولوريد الكربون، بالإضافة إلى مستخلصات الدهون والدهون، وذلك

بأن قل نشاط الإنزيمات المعضلة للأكدة التي تم تقدرها في هذه التجربة. وكذلك كان له القدرة العالية على

تقليل نسبة الملواندييد

وقد استخلص من هذه الدراسة أن المستخلص الكحلو يعبر عن تأثير مضاد للأكدة عالي مقاومة بباحي

الاستخلصات وأن مستخلصات نبات الجينكو بيلوبا ليس لها الآثار سام. 

Osama et al.