Possible Protective and Antioxidant Effects of Ginkgo Biloba on Experimentally Induced Hepatic Toxicity in Rats

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Abstract

Background: The analgesic acetaminophen (AAP) causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. It was reported that these toxic effects of AAP are due to oxidative reactions that take place during its metabol- olism. Ginkgo Biloba (GB) has been used in traditional medicine to treat circulatory disorders and enhance memory. Ginkgo contains two types of chemicals (flavonoids and terpenoids) believed to have potent antioxidant property which is claimed to be one of the mechanisms of hepatoprotective effect.

The aim of the current article was to investigate the possible protective and anti-oxidant effects of Ginkgo Biloba on experimentally induced hepatic toxicity in rats. Silymarin was used in the current study as a standard hepatoprotective agent for comparison with Ginkgo biloba.

Material and Methods: Adult male albino rats were randomly divided into 4 groups: Group (1) normal control group, group (2) treated with acetaminophen 500mg/kg, IP to induced hepatotoxicity, group (3) Ginkgo Biloba treated hepatotoxic group (50mg/kg/IP/10 days, three days before and seven days after acetaminophen administration), group (4) silymarin treated hepatotoxic group (200mg/kg/PO/d/10 days, three days before and seven days after acetaminophen administration). At the end of the study period, blood samples and liver tissues were collected and subjected to the biochemical and histopathological examination. Liver functions and oxidative stress markers in liver tissues were assessed. In addition, histopathological examination of liver was also carried out.

Results: Acetaminophen induced hepatotoxicity was manifested by a significant elevation of activity of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and alkaline phosphatase (ALP). There was significant decrease in serum albumin. At the same time, there was a significant increase of lipid peroxidation measured as malondialdehyde (MDA), decrease of antioxidant superoxide dismutase (SOD) and glutathione (GSH) contents in liver tissue homogenate. Results of the present work revealed that the rats treated with Ginkgo biloba or silymarin showed significant improvement of the liver function, lipid peroxidation and antioxidant impairment when compared with acetamini- phen treated rats. The biochemical observations were supported by histopathological examination of liver.

Conclusion: Ginkgo Biloba is as effective as sylimarin as hepatoprotective agent. The therapeutic benefits may be attributed to its antioxidant effect.

Key Words: Ginkgo biloba (GB) – Acetaminophen (AAP) – Serum alanine aminotransferase (ALT) – Serum aspartate aminotransferase (AST) – Alkaline phosphatase (ALP) – Malondialdehyde (MDA) – Glutathione (GSH) – Superoxide dismutase (SOD).

Introduction

GINKGO Biloba is one of the oldest living tree species and its leaves are among the most extensively studied in Europe and the United States. Ginkgo is widely used supplements among the best-selling herbal medications. It consistently ranks as a top medicine prescribed in France and Germany [1]. Ginkgo Biloba is used for a variety of indications as cerebral insufficiency [2] and to treat certain peripheral vascular diseases [3]. GB exhibits a variety of interesting pharmacological properties such as oxygen free radical scavenging activity, cyclonucleotide phosphodiesterase inhibition, membrane stabilizing effect, increase in blood fluidity and improvement in cognitive function [4,5,6]. More than 40 components isolated from the Ginkgo tree leaves have been identified, but only two components are believed to be responsible for its medicinal effects; Flavonoids and terpenoids, believed to have potent antioxidant properties [7]. Laboratory and animal studies have shown that flavonoids protect the nerves, heart, blood vessels and retina from damage. Terpenoids (such as ginkgolides) improve blood flow by dilating blood vessels and reducing the stickiness of platelets [7].

Antioxidants are substances that scavenge free radicals compounds in the body that damage cell membranes, tamper with DNA and even cause cell death [8]. Free radicals occur naturally in the body and grow in number as we age [9]. Antioxidants
such as those found in GB may help neutralize free radicals and may reduce or even help in preventing some of the damage they cause.

Silymarin, a standardized extract obtained from seeds of Silybum marianum, is widely used in treatment of liver diseases of varying origins [10]. Seeds of S. marianum have been shown to treat liver and gall bladder disorders, including hepatitis, cirrhosis and jaundice and to protect the liver against poisoning from chemicals, snake bites, environmental toxins, insect stings, mushroom poisoning and alcohols [11].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases [12]. More attention has been paid to the protective effects of natural antioxidants against drug induced toxicities especially whenever free radical production is involved [13].

The purpose of this work to investigate the potential hepatoprotective effect of well know cerebral protective agent Ginkgo biloba based on its antioxidant effect. Silymarin is being used in the current study as a standard agent, due to its proven hepatoprotective and antioxidant properties.

Material and Methods

Animals:

Adult male albino rats, with initial body weight ranging from (150-200g) were used. Rats were purchased from Experimental Animal Breeding Farm, Helwan. All animals were housed in a controlled laboratory conditions at 20-25°C in a 12h light/dark cycle and had free access to food and water. They were allowed for one week acclimatization period before to their use in the experiment at Pharmacology Department, Faculty of Medicine, Banha University from 2010-2011.

Drugs and chemicals:

• Silymarin (Sedico, Egypt) as powder.
• Ginkgo Biloba (Pharco Pharma CO, Egypt) as powder.
• Acetaminophen (Sterling, USA) supplied as powder dissolved in saline. Other drugs were dissolved in water.

Experimental groups:

Rats were divided randomly into 4 groups; each group consisting of 10 rats. Group (1) served as a normal control group received saline. They received no drugs, only drug vehicle (saline was received both orally and intraperitoneally in volume (1ml) comparable to that of administrated drugs in other groups. Group (2) served as hepatotoxic group treated with a single injection of acetaminophen 500mg/kg, IP dissolved in saline to induce hepatotoxicity [14]. Group (3) Ginkgo Biloba treated group (50mg/kg/IP/10 days, three days before and seven days after acetaminophen administration [15] (Naik and Panda 2007). Group (4) Silymarin treated group (200mg/kg, PO/d/10 days, three days before and seven days after acetaminophen [15].

Experimental parameters:

Hepatic parameters:

Twenty four hours after the end of the experimental period, blood samples were collected from retro-orbital plexus. The blood were allowed to stand for about 15 minutes to clot. Serum was separated by centrifugation for spectrophotometric assessment of liver function tests, rats were sacrificed and livers were dissected out and washed with ice cold saline. Parts of three major lobes of each liver were fixed in 10% formalin and embedded in paraffin for histopathological studies, the remaining were homogenized and the homogenates were used for determination of hepatic oxidative stress.

Biochemical parameters:

• Determination of serum aminotransferase: Alanine transaminase and aspartate transaminase was determined by Reitman and Frankel [16].
• Determination of serum alkaline phosphates (ALP) activity by colorimetric method of Donald and Ralph [17].
• Determination of serum albumin concentration by calorimetric method of Doumas, et al. [18].

Determination of lipid peroxidation and antioxidant enzymes in the liver homogenates: Lipid peroxidation was determined by the formation of malondialdehyde and was measured by the thiobarbituric acid reactive method according to Ohkawa, et al. [19]. Antioxidant enzymes: Glutathione (GSH) was determined by the method of Ellman [20] and superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich [21].

Histopathological examination: Paraffin sections of 5-7 µm thickness were prepared and subjected to Hematoxylin and Eosin (Hx&E) staining according to Drury and Wallington [22].

Statistical analysis:

The results were expressed as mean ± SE. Statistical analysis was performed using one way analysis of variance (ANOVA) test. Statistical significance between individual groups was deter-
mined using unpaired student t-test. The differences were considered to be statistically significant when $p \leq 0.05$ [23]. The data was coded and entered using computer program SPSS (Statistical Program for Social Science) software, version 13 for windows.

**Results**

**Biochemical analysis:**

The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular injury and necrosis.

I- **Serum determinations results:**

Table (1) & Fig. (1) show significant ($p<0.05$) liver injury evidenced by elevation of the mean values of serum alanine transaminase and aspartate transaminase and serum alkaline phosphates in group received acetaminophen compared with normal control group indicating hepatocellular damage.

Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) or Silymarin (200mg/kg po/d/10days) on average serum liver enzymes on acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats showed significant reduction ($p<0.05$) of ALT, AST and ALP parameters in comparison to acetaminophen hepatotoxic group Table (1) Figs. (1,2,3). Also, GB showed significant elevation ($p<0.05$) in ALT, AST in comparison to normal control group. Regarding to the comparison between the two tested drugs, there is no statistical significance difference.

II- **Determination of lipid peroxidation and antioxidant enzymes in the liver homogenates:**

Determination of enzymatic oxidative stress markers results: Table (2) Figs. (5,6,7) show a significant ($p<0.05$) increase MDA, and also, showed significant ($p<0.05$) decreased in liver GSH and SOD in acetaminophen administered group (group 2) compared with normal control group. Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) (group 3) or Silymarin (200mg/kg, PO/d/10 days) (group 4) showed a significant ($p<0.05$) decrease in MDA and significant ($p<0.05$) increase GSH & SOD levels in comparison to acetaminophen induced hepatotoxic group and GB showed significant elevation in MDA and significant decrease in glutathione in a comparison to normal group. Regarding to the comparison between the two tested drugs, there is no statistical significance difference.

The present work also showed that there was a significant reduction in serum albumin concentration of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats as compared with normal control group indicating decrease in protein synthesis. Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg, PO/d/10 days) on serum albumin concentration showed significant elevation in comparison to acetaminophen hepatotoxic group and GB showed significant decrease to normal Table (1) Fig. (4). Regarding to the comparison between the two tested drugs, there was no statistical significance difference.

Data are represented as Mean ± SE (n=10).

* Significant compared with control group.

** Significant compared with acetaminophen administered group (hepatotoxic group).

Fig. (1): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10days) on serum avarge of ALT in acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Data are represented as Mean ± SE (n=10).

* Significant compared with control group.

** Significant compared with acetaminophen administered group (hepatotoxic group).

Fig. (2): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on serum avarge of AST in acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.
Possible Protective & Antioxidant Effects of Ginkgo Biloba

Fig. (3): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on serum average of ALP in acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Fig. (5): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on MDA level in liver of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Fig. (4): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on serum Albumin of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Fig. (6): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on GSH level in liver of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Fig. (7): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on SOD level in liver of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

Data are represented as Mean ± SE (n=10).
* Significant compared with control group.
** Significant compared with acetaminophen administered group (hepatotoxic group).
Histopathological examination:
Histopathological examination of the liver sections of control normal group showed normal cellular architecture with radiating hepatic cords, normal sinusoidal spaces and central veins (Fig. 8A). While the liver specimens obtained from the rats administrated acetaminophen alone (group 2) revealed a preservation of liver architecture, hepatocytes showed marked hydropic changes some necrotic inflammatory foci, dilated congested central veins and portal tract inflammation indicating hepatocellular damage (Fig. 8B). The liver specimens obtained from the rats treated with GB (group 3) or silymarin (group 4) resulted in apparent amelioration of necrosis and inflammatory cellular infiltration. The hepatocytes show no hydropic changes, congested central veins and portal tract inflammation (Fig. 8C, D).

Table (1): Effect of Ginkgo Biloba (50mg/kg/IP/d/10 days) and Silymarin (200mg/kg po/d/10 days) on average (Mean ± SE) serum liver enzymes, alanine transaminase, aspartate transaminase, alkaline phosphatase and serum albumin of acetaminophen (500mg/kg/IP) induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALT (U/ml)</th>
<th>AST (U/ml)</th>
<th>ALP (KAU)</th>
<th>Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td></td>
<td>45.83±0.95</td>
<td>34.00±3.58</td>
<td>11.66±1.33</td>
<td>3.55±0.06</td>
</tr>
<tr>
<td>Non treated Acetaminophin induced hepatotoxic group</td>
<td>90.33±2.46*</td>
<td>89.16±3.08*</td>
<td>36.00±2.97*</td>
<td>2.97±0.05*</td>
<td></td>
</tr>
<tr>
<td>Ginkgo Biloba treated acetaminophin induced hepatotoxic group</td>
<td>55.66±2.39**</td>
<td>48.00±2.65***</td>
<td>16.33±0.95**</td>
<td>3.02±0.02***</td>
<td></td>
</tr>
<tr>
<td>Silymarin treated acetaminophin induced hepatotoxic group</td>
<td>53.91±4.2**</td>
<td>43.12±3.1**</td>
<td>13±1.02**</td>
<td>3.49±0.03**</td>
<td></td>
</tr>
</tbody>
</table>

Data are represented as Mean ± SE (n=10).
* Significant compared with control group
** Significant compared with acetaminophen administrated group (hepatotoxic group).
### Parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g wet tissue)</th>
<th>GSH (mol/mg)</th>
<th>SOD (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>55.89±1.27</td>
<td>10.16±0.5</td>
<td>134.65±12.43</td>
</tr>
<tr>
<td>Non treated Acetaminophin induced hepatotoxic group</td>
<td>124.01±6.2*</td>
<td>3.15±0.21</td>
<td>92±3.358*</td>
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<tr>
<td>Ginkgo Biloba treated acetaminophin induced hepatotoxic group</td>
<td>73.08±3.29***</td>
<td>8.27±0.18***</td>
<td>125.13±10.7**</td>
</tr>
<tr>
<td>Silymarin treated acetaminophin induced hepatotoxic group</td>
<td>69.54±3.61**</td>
<td>8.92±0.41**</td>
<td>128.11±9.7**</td>
</tr>
</tbody>
</table>

Data are represented as Mean ± SE (n=10).
* Significant compared with control group.
** Significant compared with acetaminophen administered group (hepatotoxic group).

### Discussion

In the assessment of liver damage by acetaminophen, the determination of enzyme levels such as alanine aminotransferase, aspartate aminotransferase is largely used. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury [24,25].

A conventional hepatoprotective drugs used for the treatment of such case are often inadequate.

Therefore, efforts to explore hepatoprotective effect of any natural products carry a great clinical significance against acetaminophen induced hepatotoxicity.

In the present study, the rise in the serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in acetaminophen administrated rats has been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm and released into the circulation after cellular damage. This was evidenced by both biochemical and histopathological evidences [26,27,28]. Histopathological study of the above mentioned non treated group received acetaminophen showed profound acute liver cell damage as evidenced by the presence of centrilobular necrosis, venous congestion and inflammatory cell infiltration. Ginkgo Biloba seems to preserve the structural integrity of the hepatocellular membrane as evident from significant reduction in acetaminophen induced rise in serum enzymes in rat. This may be due to the prevention of leakage of the intracellular enzymes by its membrane stabilizing activity and regeneration of hepatocytes which was supported by limited extent of histological change [29]. These levels significantly not returned to normal by GB.

Serum alkaline phosphatase, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [30]. In this work, alkaline phosphatase elevation was observed in acetaminophen received group, this confirmed by [31] who reported that the necrotic liver cell due to acetaminophen press on portal tract and bile canaliculi which may cause venous congestion and release of bile epithelial located enzyme namely alkaline phosphatase in the serum. Our results using acetaminophen induced hepatotoxicity in rats demonstrated that Ginkgo Biloba or silymarin caused significant control of alkaline phosphatase activity points towards an early improvement in the secretory mechanism of hepatic cell. This level significantly not returned to normal by GB.

The observed significant decrease in serum albumin in acetaminophen treated group may be attributed to deterioration of acetaminophen induced damaged liver cells to synthesize albumin [15,25,32]. In the present study, Ginkgo Biloba or silymarin significant elevated serum albumin indicating improvement in the synthetic function of hepatic cell. Stimulation of protein synthesis has been advanced as a contributor hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells. This is in agreement with [25] who reported that the decreased serum level of ALP due to hepatic toxicity was significantly reversed by treatment with Ginkgo.
It is established that covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, with sulphhydryl groups of protein in cell necrosis and lipid peroxidation in the liver [33]. In addition, NAPQI can increase the formation of reactive oxygen species such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite, respectively. Excess levels of reactive oxygen and nitrogen species can attack biological molecules such as DNA, protein and phospholipid, which leads to lipid peroxidation, nitrilation of tyrosin and depletion of antioxidant enzymes as superoxide dismutase that further result in oxidative stress [34]. In accordance with previous findings, in our study, we observed a high free radical markers namely MDA with concomitant decrease in free radical scavengers namely superoxide dismutase and glutathione in the hepatic tissue during acetaminophen toxicity. These were in agreement with [35,36] who reported that GSH is non enzymatic biological antioxidant, widely distributed in cells. GSH is an intracellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. This may be explained by the profound oxidative effect of N-acetyl metabolite of acetaminophen which over seeded the limited capacity of natural antioxidants namely glutathione [26]. In our study, the decreased level of glutathione has been observed in acetaminophen received group, whereas significantly found to be increased in Ginkgo Biloba or silymarin treated group. The SOD participating with other antioxidant enzymes, in the enzymatic defense against superoxide radical and hydrogen peroxide [37]. Therefore, in this study, Ginkgo Biloba treatment was observed to exhibit hepatoprotective effect as demonstrated by enhanced activities of antioxidant enzyme (as superoxide dismutase) and glutathione which diminish amount of lipid peroxide against acetaminophen induced hepatotoxicity animals. In accordance with this result, [25,38] reported that Ginkgo Biloba or silymarin has increased the glutathione content of liver, it may also be useful in hepatotoxicity induced by acetaminophen and other agents. The levels of MDA and glutathione levels significantly not returned to normal by GB. [18] emphasized that Ginkgo Biloba and silymarin elicited significant hepatoprotective activity by decreasing the activities of serum marker enzymes and lipid peroxidation and elevated the levels of GSH, SOD, CAT, GPX, GR, Alb and TP.

In previous studies Silymarin has shown hepatoprotective activity. The cytoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavenging properties [39]. The effects of GB were comparable to that of silymarin.

Our study revealed that either silymarin or Ginkgo biloba ameliorated acetaminophen hepatotoxicity in rats as evidenced by improvement of the markers of biochemical tested and decreasing formation of free radicals. This was associated with partial improvement of histopathological tested picture. This is in agreement with the commonly accepted view that serum levels of aminotransferases return to significant level according to healing of hepatic parenchyma and the regeneration of hepatocytes. This may indicate the vivo protective effects against paracetamol induced liver damage [40]. Ginkgo biloba may decrease free radicals through its terpenoids and flavonoid contents. A number of scientific reports indicated certain flavonoids and terpenoids which present in Ginkgo biloba have protective effect on liver due to their antioxidant properties [7,15,25]. The present work in accordance with [41] who demonstrated that administration of GB or silymarin significantly decreased lipid peroxidation and increased endogenous antioxidants, such as SOD, and GSH causing hepatoprotective. This may imply that free radical scavenging effect plays a crucial role in the hepatoprotective effect of tested drugs. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by toxins. GB significantly reducing the harmful effect of acetaminophen with hepatoprotective effect.

**Conclusion:** From the above mentioned results, one may conclude that either Ginkgo biloba or silymarin effective in protection against acetaminophen induced hepatotoxicity by their antioxidant properties but silymarin more normalized the hepatic cells. Nevertheless, further study is required to ensure the therapeutic effective of the proposed combination and another comparative study to evaluated side effect of each drug on different organs.

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