Role of *Ginkgo biloba* Extract, Against Isoproterenol Induced Cardiac Toxicity in Rats

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ABSTRACT

Objective: *Ginkgo biloba* is a potent antioxidant dietary source for human health. Oxidative stress through generation of free radicals damages the myocardium in different experimental condition. The present research was designed to evaluate the cardio protective role of chronic oral administration of *Ginkgo biloba* leaf extract against Isoproterenol induced myocardial injury. Material and methods: Male Wistar albino rats were randomly divided into five groups (n = 6) and treated as per treatment protocol with three different dose of *Ginkgo biloba* extract (125, 250, and 500 mg/kg b.w.) orally for thirty days. At the end of the treatment all the rats (except control rats) were administered with Isoproterenol (85 mg/kg) two consecutive days and subjected to biochemical and histopathological estimation. Results: Isoproterenol (group II) induced the oxidative myocardial damage via alteration in the endogenous antioxidant enzymes and myocardial marker enzymes. *Ginkgo biloba* extract in all three dose (group III, IV and V) shows protective mechanism via decreasing thiobarbituric acid reactive substance (TBARS) and enhancing the endogenous antioxidant enzymes (reduced glutathione (GSH), superoxide dismutase (SOD) and catalase). The extract effect was compared with the reference standard α-tocopherol which also offered similar protection in biochemical and histopathological changes. Conclusion: Thus, the study shows that *Ginkgo biloba* extract exhibits significant antioxidant activity and protect the heart from free radical mediated toxicity of Isoproterenol.

Key words: Antioxidant, Cardiotoxicity, *Ginkgo biloba*, Isoproterenol, Wistar albino rats, α-tocopherol.

INTRODUCTION

Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand.¹ Oxidative stress resulting from increased production of free radicals is associated with decreased levels of antioxidants in the myocardium and plays a major role in cardiovascular diseases.² Damage to the myocardial cells arises due to the generation of toxic reactive oxygen species such as superoxide radical, hydrogen peroxide and hydroxyl radical.³ Isoproterenol (ISO) is an adrenergic agonist and acute administration of ISO in experimental animals causes necrosis to heart muscle.⁴ ISO damages the myocardial via calcium accumulation in cytosolic membrane, generation of reactive oxygen species and procoagulant activity.⁵ ISO causes the patchy pathological changes in the myocardial tissue, which is almost clinically relevant to myocardial infarction of ischemic heart disease.⁶ Phytopharmaceutical are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and less toxic nature. Drugs to enhance the endogenous antioxidant enzymes to protect the heart from stress have been paid more attention. Natural antioxidants play a major role to...
reduce the oxidative stress by scavenging the excess free radicals. Administration of antioxidants during ischemic reperfusion injury ameliorates the severity of IRI through augmentation of endogenous antioxidants, which might be a promising loom to treat heart disease. 

Several natural products have been reported to have protective roles against ISO-induced MI in rats. Ginkgo biloba L. (Family-Ginkgoaceae) is the one of oldest existing seed plants, also known as “living fossils.” It contains Flavonol and flavone glycosides, Ginkgolides, Diterpen lactones, ascorbic acid, Sesquiterpenes, Catechin and Iron-based superoxide dismutase. Ginkgo biloba has been reported to have memory enhancer, antidepressant, antimicrobial, hepatoprotective, anticoagulant, anti-inflammatory, cytotoxic, anti-stress, anti-ulcer, anti-tubercular and anti-aging properties. Ginkgo biloba also known for its antioxidant activity and effective scavenger of oxidative radicals. Ginkgo biloba leaves are popular for their nutritional and medicinal values but have no studies conducted in direction of protective role of ginkgo biloba extract against ISO induced Cardiotoxicity may be via antioxidant system. Hence, the present study was undertaken to find out the cardio protective potency of methanolic extract of Ginkgo biloba leaves.

MATEIRALS AND METHODS

Drugs and Chemicals

Leaves of Ginkgo biloba was collected from Darjeeling, West Bengal, India, and were authenticated by Dr. S. K. Mahajan M Sc, PhD, department of botany, Govt. P. G. Collage, Khargone, M.P, India. All chemicals were of analytical grade purchased from sigma chemicals, USA.

Extract preparation

Dried leaves of Ginkgo biloba were coarsely powdered and 1 kg of this powered plant material was extracted with the help of the soxhlet apparatus using methanol as a solvent. The solvent from the methanolic extract was removed under vacuum distillation; dried material was kept in a desiccators. A suspension of the leaves in 5% Tween 80 (Vehicle) was made daily.

Physicochemical Analysis

For physicochemical analysis fresh plant material was collected and shade dried.

Physical constants were determined following Indian Pharmacopoeia. It includes ash value, extractive value and moisture content.

Preliminary Phytochemical analysis

MEGB was analyzed for the various classes of phytoconstituents such as flavonoids, phenolic acids, anthocyanins, quinones, alkaloids, tannins, and saponins using standard phytochemical methods. Phytochemical tests were carried out following Shah and Quadry and Kokate.

Experimental animals

Male Wistar albino rats of body weight 150-200 g were obtained from the Institute Animal House. The rats were acclimatized in the department animal house at an ambient temperature of 25°C, under a 12 h dark -12 h light, cycle, for the whole period of the study. The animals were fed with a standard pellet diet and water ad libitum. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental on Animals, New Delhi, India and the research protocol was approved by the Institute animal ethical committee (1151/ac/07/CPCSEA).

Experimental Protocol

The rats were divided into 5 groups (6 in each group) and fed with the suspension of Ginkgo biloba leaves extract of three doses (125 mg/kg, 250 mg/kg and 500 mg/kg) by oral gavages once a day for 4 weeks (6 days/week). At the end of the treatment period rats from all groups except control group were administered Isoproterenol (ISO) 85 mg/kg i.p., for two consecutive days to induce myocardial injury. Rats were pretreated with α-tocopherol (60 mg/kg body weight, orally) for a period of 28 days and in addition, received isoproterenol 85 mg/kg body weight) on the 29 and 30 day at an interval of 24 h. After 48 hours of the first dose of ISO the rats were sacrificed, hearts and blood samples were collected and immediately frozen in liquid nitrogen for biochemical estimation.

Treatment protocol

The groups studied were:

Group I: Control, Vehicle + saline injected rats
Group II: Vehicle + ISO treated rats (85 mg/kg)
Group III: 125 mg/kg of MEGB + ISO treated rats (85 mg/kg)
Group IV: 250 mg/kg of MEGB + ISO treated rats (85 mg/kg)
Group V: 500 mg/kg of MEGB + ISO treated rats (85 mg/kg)
Group VI: α-tocopherol (60 mg/kg body weight, orally)
Estimation of biochemical parameters

The following biochemical parameters were studied in the heart homogenate.

Myocardial thiobarbituric acid reactive substances

TBARS levels in the myocardium were determined by the method described by Ohkawa et al (1997). Hearts were homogenized with 10 ml of Trichloroacetic acid (TCA). 0.2 ml of whole homogenate was taken to which 0.2 ml of 8.1% Sodium lauryl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. Volume was made up to 4 ml with double distilled water. It was heated at 95°C for 60 min. After cooling, 1 ml of double distilled water and 5 ml of butanol: pyridine mixture was added and centrifuged at 4000 rpm for 10 min in a cold centrifuge. The organic layer was separated and absorbance was observed at 532 nm in a spectrophotometer.

Myocardial reduced glutathione

Myocardial GSH was estimated by the method of Ellman et al, (1959). The reaction mixture contained 0.1 mL of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH- 8.4), 0.4 ml of double-distilled water and 0.5 ml of 5, 5 dithiobis 2-nitrobenzoic acid. The reaction mixture was incubated for 10 min and the absorbance was measured at 412 nm. Data are expressed as mole per gram wet weight.

Superoxide dismutase

SOD levels in the hearts were determined by McCord and Firdovich method (1969) and modified by Kakkar et al. A sample (100 µl) was added to sodium pyrophosphate buffer (pH-8.3), followed by addition of 0.1 mL of 186 M phenazine methosulfate, 0.3mL of 300 mM nitroblue tetrazolium and 0.2 ml of 780M NADH. The reaction mixture was incubated for 90 second at 30°C and the reaction was stopped by adding 1.0 ml of acetic acid, 4.0 ml of n-butanol was then added and centrifuged at 3000 g for 10 min. The absorbance of the organic layer was measured at 560 nm. Data are expressed as units per mg protein.

Estimation of Catalase

Catalase level was estimated by the method described by Aebi et al. Sample (50 µl) was added to a 3.0 ml cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH 7.0). Then 1.0 ml of 30 mM hydrogen peroxide was added and changes in absorbance were followed for 30 s at 240 nm at an interval of 15 s. Catalase levels are expressed as units per mg protein.
Physicochemical parameters

After estimation of physical constants the results obtained were total ash 10.33% w/w, Acid Insoluble ash 2.12% w/w, water soluble ash 5.33% w/w, water soluble extractive value 40% w/w, Alcohol soluble extractive 72% w/w and moisture content 4.8% w/w.

Phytochemical Investigation

Successful evaluation of botanical phytocompounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Hence our choice is methanol. The result of phytochemical screen showed the presence of saponins, Glycosides, Triterpenes, flavonoids, Carbohydrates and steroids leave of Ginkgo biloba (Table 1).

Pharmacological estimation

The results obtained in the different groups subjected to in-vivo ischemic reperfusion injury are presented below.

Myocardial TBARS

Myocardial TBARS in GII group (68.65±1.28) was significantly higher than that in control group (44.82±1.54). In GIV and GV treated groups there was a significantly lower TBARS (53.76±2.46 and 44.45±1.95) respectively, whereas in the GIII group the TBARS shows no significant change (59.25±1.46) in comparison to GII group (Table 2).

Myocardial GSH

Myocardial GSH level was significantly lower in GII group (217.66±5.81) in comparison to that of the control group (353.85±3.69). There was a significant increase in the levels of GSH in the GV (330.4±4.83), whereas in there was slight increase in the levels of GSH levels GIII (304.5±6.31) and GIV (312.58±6.31); in comparison to the GII group (Table 2). The blood GSH levels of the ISO treated animals showed (64.33±1.11) a significant decrease (p <0.01) as compared to normal control (73.16±1.29) group. Here also MEGB at the dose of 500 mg/kg provided a highly significant (p <0.001) increase (71.8±1.14) in blood GSH as compared to Group II, III, IV (Table 3).

Myocardial SOD

Myocardial SOD activity was significantly lower in GII group (1.31±0.16) than that in control group (2.91±0.14). Myocardial SOD levels showed no signifi-
Table 1: Qualitative Chemical Examination of Various Extracts (Obtained By Successive Solvent Extraction of Ginkgo biloba)

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloid</th>
<th>Carbohydrate</th>
<th>Glycoside</th>
<th>Saponins</th>
<th>Proteins and Amino acids</th>
<th>Phytosterols</th>
<th>Fixed oils and Fats</th>
<th>Phenolic compounds and flavonoids</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzene</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>Methanol</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform water</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

(+) Present,  (-) Absent

Table 2: Effect of MEGB on TBARS, GSH, SOD and CAT in rat heart

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Saline 10mg/kg)</th>
<th>Isoproterenol (85mg/kg)</th>
<th>MEGB (125 mg/kg)</th>
<th>MEGB (250 mg/kg)</th>
<th>MEGB (500 mg/kg)</th>
<th>α-tocopherol (60 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>44.82±1.54</td>
<td>68.65±1.28</td>
<td>59.25±1.46a</td>
<td>53.76±2.46a</td>
<td>47.45±1.95b</td>
<td>45.6±7.71</td>
</tr>
<tr>
<td>GSH</td>
<td>353.85±3.69</td>
<td>217.66±5.81</td>
<td>304.5±7.57b</td>
<td>312.56±6.31b</td>
<td>300.4±4.83c</td>
<td>348±9.62</td>
</tr>
<tr>
<td>SOD</td>
<td>2.91±0.14</td>
<td>1.31±0.16</td>
<td>1.47±0.18a</td>
<td>2.78±0.18b</td>
<td>3.86±0.09c</td>
<td>4.83±1.83</td>
</tr>
<tr>
<td>CAT</td>
<td>49.65±0.81</td>
<td>27.9±1.48</td>
<td>35.0±0.92a</td>
<td>40.15±0.63a</td>
<td>48.25±1.20c</td>
<td>48.6±2.33</td>
</tr>
</tbody>
</table>

Values are mean ± SD (N=6). Values in the same row with different alphabet superscripts are significantly different at a < 0.05, b < 0.01, c < 0.001. Values are obtained by one way ANOVA followed by Dunnett multiple comparison test. MEGB: methanolic extract of Ginkgo biloba; TBARS: thiobarbituric acid reactive substances; GSH: reduced glutathione; SOD: Superoxide dismutase; CAT: catalase;

Table 3: Effect of MEGB on TBARS, GSH, SOD and CAT level in Serum

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Saline 10mg/kg)</th>
<th>Isoproterenol (85mg/kg)</th>
<th>MEGB (125 mg/kg)</th>
<th>MEGB (250 mg/kg)</th>
<th>MEGB (500 mg/kg)</th>
<th>α-tocopherol (60 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>73.16±1.29</td>
<td>64.33±1.11</td>
<td>65.85±1.02a</td>
<td>66.75±1.01b</td>
<td>71.8±1.14c</td>
<td>72.0±7.06</td>
</tr>
<tr>
<td>SOD</td>
<td>139.86±0.6</td>
<td>105.74±2.07</td>
<td>108.44±2.80a</td>
<td>120.12±1.80b</td>
<td>136.3±1.28c</td>
<td>137.6±4.22</td>
</tr>
<tr>
<td>CAT</td>
<td>12.13±0.37</td>
<td>2.28±0.19</td>
<td>3.06±0.15a</td>
<td>4.05±0.11b</td>
<td>7.16±0.10c</td>
<td>9.16±4.16</td>
</tr>
</tbody>
</table>

Values are mean ± SD (N=6). Values in the same row with different alphabet superscripts are significantly different at b < 0.01, c < 0.001. Values are obtained by one way ANOVA followed by Dunnett multiple comparison test. MEGB: methanolic extract of Ginkgo biloba; GSH: reduced glutathione; SOD: Superoxide dismutase; CAT: catalase;
The blood SOD levels of the ISO treated animals showed a significant decrease (105.74±2.07) as compared to normal control group (139.86±0.6). Here the dose of MEGB 500 mg/kg (120.12±1.80) and 1000 mg/kg provided a highly significant (p <0.001) increase (136.3±1.28) in blood SOD as compared to Group II (Table 3).

**DISCUSSION**

The effect of ISO on heart is mediated through beta receptors. Both adrenoceptors mediate the positive inotropic and chronotropic effects to beta adrenoceptor agonist. It has been reported to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle. ISO-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs and cardiac function.31 It is also well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane.32 Extract from the leaves of *Ginkgo biloba* extract, MEGB, contains flavonoids, saponins, glycoside, steroids, carbohydrate and terpenoids, which are the most important active substances in the extract. The most important flavonoids are glycosides of kaempferol, quercetin, and isorhamnetin with glucose or rhamnose. *Ginkgo biloba* extract is well known for its antioxidant property, which may result from its ability to scavenge free radicals, and to neutralize ferryl ion-induced peroxidation.33 Several studies have reported that the antioxidant activity of *Ginkgo biloba* extract could be helpful in the prevention and therapy of diseases and degenerative processes associated with oxidative stress.33-37 However, there have been very limited studies on the cardio protective activity of *Ginkgo biloba* extract. It is reasonably hypothesized that *Ginkgo biloba* extract may be helpful for the therapy of heart failure. Therefore, the purpose of this study was to investigate the cardio protective effect of *Ginkgo biloba* methanolic extract in rats. Extensive literature survey has shown that there are no scientific reports available on the effect of MEGB in cardiotoxicity induced by isoproterinol. In our laboratory, we observed the preventive effect of MEGB on cardiac marker enzymes, TBARS, GSH, SOD and CAT in ISO-induced myocardial infarction in rats. In the present study chronic i.p. administration of *Ginkgo biloba* leaves extract caused significant rise in myocardial endogenous antioxidants (SOD, GSH and Catalase) in the 250 and 500 mg/kg treated groups but not with other baseline treated groups. The increase in TBARS is...
indicative of an enhanced oxidative stress, which in the absence of any evidence of cellular injury (as evidenced by histological studies), may be considered as non-lethal. It is, therefore possible that the increase in oxidative stress was non-lethal and might be responsible for cellular adaptive mechanisms. The principal finding of the present study is that cardio toxicity was associated with oxidative stress, as evidenced by increase in myocardial TBARS and depletion of myocardial endogenous antioxidant status (SOD, GSH and Catalase). Similar observations were made earlier by other studies.\(^{38-41}\)

In this respect, the present study showed for the first time that the leaves of Ginkgo biloba are particularly useful agents, as they could enhance myocardial and blood endogenous antioxidants level without producing any cytotoxic effects. Therefore, the protection against myocardial injury in the treated rats is attributed to enhanced endogenous antioxidant activity. So I concluded that this study of Ginkgo biloba can help for further research area for ayurveda in cardiovascular and another disease which caused due to oxidative stress.

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The authors are highly thankful to management, GRY Institute of Pharmacy to provide the facilities to carry out the research work. Thanks are due to Dr. S. K. Mahajan, Botanist at Govt. PG Collage, Khargone-451001 for authentication of plant.

ABBREVIATION USED

ISO: Isoproterenol; MEGB: methanolic extract of Ginkgo biloba; CPCSEA: Committee for the Purpose of Control and Supervision of Experimental on Animals; TBARS: thiobarbituric acid reactive substances; TCA: Trichloroacetic acid; GSH: reduced glutathione; SOD: Superoxide dismutase; CAT: catalase; NADH: nicotinamide adenine dinucleotide

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