QUALITY ASSESSMENT OF *GINKGO BILOBA* IN DIABETIC NEPHROTOXICITY

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Summary

The World Health Organization estimates that about 80% of the populations living in developing country rely on traditional system of medicine. In this study, the antioxidant potential of *Ginkgo biloba* preparation was assessed in diabetes induced nephrotoxicity by examining first line defense system of the body like superoxide dismutase (SOD), catalase (CAT), reduced glutathione. Furthermore the histopathological study was carried out to determine the extent of damage to the kidney. The results indicated that *Ginkgo biloba* can be projected as an antioxidant in diabetes to minimize the risk of free radical induced complications associated with diabetes.

Keywords: Diabetes, Nephrotoxicity, Free radicals, Antioxidant

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Introduction

Diabetes mellitus have a lot of evidence of increased oxidative stress with depleted antioxidant enzymes and vitamins (1). This is due to the appropriate balance of pro-oxidant: antioxidant shifted towards pro-oxidant, when generation of oxygen species increases or levels of antioxidant diminishes in the cell. This state is an oxidative stress and if it is prolonged, it causes serious cell damage. In diabetes mellitus, free radicals are generated due to high glucose concentration via glucose metabolism and auto-oxidation (2). Other responsible mechanisms are protein glycation (3) and activation of protein kinase C (4).

Diabetic nephrotoxicity is one of the severe microvascular complications of diabetes mellitus. Clinical course of nephrotoxicity starts from microalbuminurea to proteinurea and finally renal failure. It is the major cause of morbidity and mortality in diabetes mellitus and the leading cause of end stage renal disease (5). The human body although continuously produces free radicals, it possesses several defence system, which are constitutes of enzymes and radical scavengers. These are called "first line antioxidant defense system" but are not completely efficient because almost all components of living bodies, tissues and cells undergo free radical destruction. The enzymatically potential antioxidants are superoxide dismutase, catalase and glutathione peroxidase while non enzymatic category contains vitamin C, E and A. β-caretenoids, uric acid and ubiquinone. The antioxidant enzymes together with the substances that are capable of either reducing reactive oxygen species or preventing their formation, form a powerful reducing buffer, thereby form the protective mechanisms, which maintain the lowest possible levels of reactive oxygen species inside the cell.

In traditional medicine for degenerative disorders, the extract of *Ginkgo biloba* has been widely used (6). Major constituents of *Ginkgo biloba* are flavonoid glycosides like quercitine, isorhamnetin, kaempherol and some substances of terpenic group (7). The terpens are known potent inhibitors of platelet activating factor and flavonoids are free radical scavenger in both enzymatic and non-enzymatic system.

Materials and methods

Animals

Albino rats of either sex weighing between 200-250 gms procured from National Toxicological Centre, Pune were used for the investigation.

Chemicals

Thiobarbituric acid, tris buffer, pyrogallol, methanol and hydrogen peroxide were purchased from Loba Chemie, trichloroacetic acid from Merck and ethylene diamine tetra acetic acid from BDH. All other chemicals and reagents used were of analytical grade.

Induction of diabetes

The rats were divided into five groups each containing five animals and maintained on normal diet and water *ad libitum*

Group I : Normal control

Group II : Diabetic control, received alloxan 120 mg/kg intraperitoneally to induce diabetes (8) at the interval of 14 days.

Group III : Diabetic + GB-I, received alloxan 120 mg/kg intraperitoneally at the interval of 14 days for 2 months and GB-I (Ginkgocer tablet, Ranbaxy) 300 mg/kg orally, daily for 2 months (9).

Group IV : Diabetic + GB-II, received alloxan 120 mg/kg intraperitoneally at the interval of 14 days for 2 months and GB-I (Bilovas tablet, German Remedies) 300 mg/kg orally, daily for 2 months.

Group V : Diabetic + Vit. E, received alloxan 120 mg/kg intraperitoneally at the interval of 14 days for 2 months and Vit. E as a standard 150 mg/kg orally, daily for 2 months (10).

The blood glucose level of each rat was measured from time to time throughout the protocol by GOD-POD method (11).

Kidney function test

Serum creatinine, serum urea and serum uric acid were determined by alkaline picrate, diacetyl monoxime and Henry-Caraway's method respectively for proper functioning of kidney (12).

Lipid peroxidation assay

For determination of lipid peroxidation, the blood was withdrawn from retro-orbital plexus and was taken in the centrifuge tube containing anticoagulant. From this 5% suspension of RBC in 0.1M phosphate buffer saline was prepared. To the 2 ml of this 5% suspension, 2 ml of 28% trichloroacetic acid was added and centrifuged. After centrifugation the supernatant was separated. To the 4 ml supernatant 1 ml of 1% thiobarbituric acid was added, heated in boiling water for 60 min. and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm (13). The lipid peroxidation was expressed in terms of nanomoles of MDA/g Hb.

Antioxidant enzyme assay

Superoxide dismutase assay

The activity of superoxide dismutase enzyme (SOD) was determined in the erythrocyte lysate prepared from the 5% RBC suspension. To 50µl of the lysate, 2 ml of 75mM of tris HCl buffer (pH 8.2), 0.6 ml of 30mM of EDTA and 0.3 ml of 2mM of pyrogallol were added. An increase in the absorbance was measured at 420 nm for 3 min. using spectrophotometer. The activity of SOD is expressed in unit/mg protein (14).

Catalase assay

The activity of catalase enzyme was determined in erythrocyte lysate. 50µl of the lysate was taken and added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and then 1 ml of 30 mM of H_2O_2 was added to it. The decrease in was measured at 240 nm for 1 minute using spectrophotometer. One unit of activity is equal to one milimoles of H_2O_2 degraded per minute and is expressed as units/mg protein (15).

Histopathological study

After the withdrawal of blood for biochemical parameters and antioxidant enzymes assay, the rats were sacrificed by deep ether anesthesia. The kidney was isolated and preserved in 10% formaldehyde solution for fixation. Then the organ was washed in running tap water for 6-7 hours to remove excess of formalin. Then the organ in capsule was kept in ascending grades of alcohol (70, 80, 90 and 95% for 12-15 hours and absolute alcohol for 6 hours) to remove water from tissue. The clearing of tissue was done by keeping the capsule in xylene solution for 10-15 minutes; the tissue was then embedded in paraffin wax by boiling for 2 hours. Then the tissue was enclosed in paraffin block. Finally the

sections of 4-6 microns were cut on microtome machine. They were stained by haematoxylin and eosin method and photographed (16).

Results and Discussion

Kidney function test

The results of serum creatinine, serum urea and serum uric acid (Table 1) indicated that there was increase in the level of these parameters in diabetic group as compared to normal control. However, the GB-I and GB-II treatment in diabetic subjects reduced the elevated levels of creatinine, urea and uric acid as compared to diabetic control group. These results suggested that *Ginkgo biloba* formulations have marked protective effect on tubular epithelial cells of glomeruli which were affected as a result of oxidative stress.

Table 1: Effect of GB-I and GB-II on kidney function parameters

Group	Serum creatinine (mg/dl) Mean±S.D.	Serum urea (mg/dl) Mean±S.D.	Serum uric acid (mg/dl) Mean±S.D.
Normal control	0.42±0.02	21.6±0.1	3.82±0.03
Diabetic control	1.5±0.03[a]	34±2.7[a]	4.86±0.02[a]
Diabetic + GB-I	0.94±0.02[b]	28±2.3[b]	4.68±0.04[b]
Diabetic + GB-II	1.16±0.09[b]	28.6±2.0[b]	4.28±0.02[b]
Diabetic + Vit. E	0.7±0.02[b]	25±1.8[b]	4.04±0.04[b]

[a]$p<0.05$ when compared to normal control.
[b]$p<0.05$ when compared to diabetic control group.

Lipid peroxidation assay

The results of lipid peroxidation (Table 2) indicated that there was marked increase in the level of lipid peroxidation (MDA) which is a marker of generation of free radicals. In normal control group the value

of MDA was 260.4 (±20.33) while in diabetic control it was 515.4 (±21.81). In *Ginkgo biloba* treated groups the level of malondialdehyde was 390.4 (±11.54) and 402.1 (±9.86) when compared to Vitamin E treated group which was 325 (±11.18). These results indicated that the level of MDA was lowered in diabetic subjects treated with GB-I and GB-II.

Antioxidant enzyme assay

From the results obtained (Table 2), it was observed that the level of endogenous antioxidant enzyme system i.e. SOD and catalase was decreased in diabetic control group while it was increased in the diabetic subjects treated with GB-I and GB-II. Increased level of lipid peroxidation and decreased level of SOD and catalase in diabetic subjects indicated the generation of free radicals. In diabetic subjects treated with *Ginkgo biloba*, there was decreased level of lipid peroxidation and increased level of SOD and catalase because *Ginkgo biloba* have free radical scavenging activity and this may be attributed to the increase in the level of endogenous antioxidant enzyme system.

Table 2: Effect of GB-I and GB-II on Lipid peroxidation, SOD and catalase

Group	Lipid peroxidation (nm MDA/g Hb) Mean±S.D.	Superoxide dismutase (Units/mg protein) Mean±S.D.	Catalase (Units/mg protein) Mean±S.D.
Normal control	260.4±20.33	32.26±2.73	290.4±20.55
Diabetic control	515.4±21.81	24.76±1.78[a]	220.6±18.36[a]
Diabetic + GB-I	390.4±11.54	28.38±2.36[b]	232.2±18.86[b]
Diabetic + GB-II	402.1±9.86	27.2±2.56[b]	240.7±18.46[b]
Diabetic + Vit. E	325±11.18	30.06±2.46[b]	250±19.82[b]

[a]$p<0.05$ when compared to normal control.
[b]$p<0.05$ when compared to diabetic control group.

Histopathological study

The results of histopathological study indicated that haemorrhage was observed in normal control group. In diabetic control group haemorrhage in medullary area and infiltration of leukocytes around the glomerules and interstitial spaces, cloudy and sloughy swelling of tubular epithelial cells and necrosis of tubular epithelium was observed. *Ginkgo biloba* treated groups showed mild haemorrhage, infiltration of leukocytes around the glomerules and cloudy and sloughy swelling of tubular epithelial cells and necrosis of tubular epithelium while vitamin E treated group showed extensive haemorrhage in cortical area. This suggested that the kidney was more affected in diabetic control group than normal control group. Diabetic subjects treated with GB-I, GB-II and Vit. E was considerably less affected as compared to diabetic control group. This exhibited the protective effect on *Ginkgo biloba* on kidney which is due to its free radical scavenging activity. The photographs of the study are given in figure 1-5.

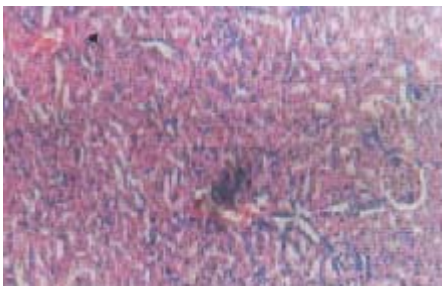

Fig. 1 Normal control group

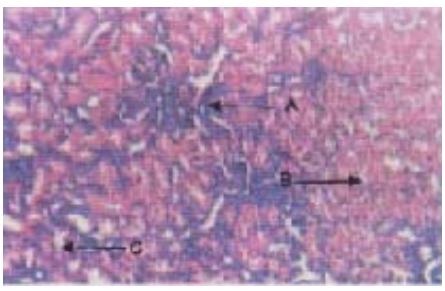

Fig. 2 Diabetic control group

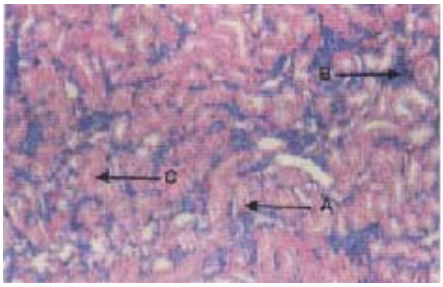

Fig. 3 Diabetic + GB-I

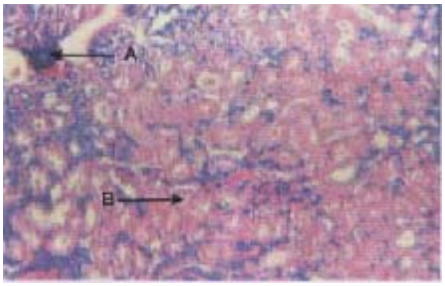

Fig. 4 Diabetic + GB-II

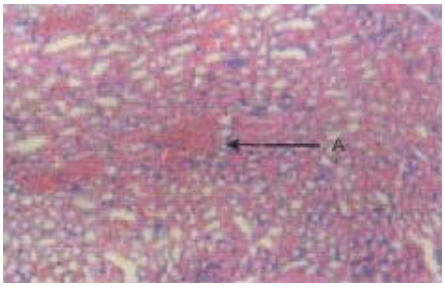

Fig. 5 Diabetic + Vit. E

Conclusion

From the results obtained it can be concluded that *Ginkgo biloba* have potent antioxidant activity and it can be projected in the treatment complications due to generation of free radicals associated with diabetes like diabetes nephrotoxicity, hepatotoxicity, cataract and neurotoxicity.

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