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ANTIOXIDANT EFFECT OF *GINKGO BILOBA* IN RATS

U.C. GUPTA
Dept. of Zoology, Matsya P.G. College Bansur (Alwar) 301402 (India)
Corresponding author : E-mail:uttamkhandelwal.shp@gmail.com

Ginkgo biloba is one of the important traditional medicines mentioned in the Chinese pharmacopoeia as an antiasthmatic against polyuria and for the treatment of cardiovascular diseases. The present study was undertaken to investigate antioxidant efficacy of extract of *Ginkgo biloba* leaves. In cholesterol fed wistar rats. Result of the study showed a significant ($P < 0.01$) increase in lipid peroxidation (TBARS level) with a concomitant decrease in glutathione (GSH) content ($P < 0.01$) and catalase ($P < 0.05$) activity in liver of cholesterol fed rats . Simultaneous treatment of *Ginkgo biloba*leaves extract at 10, 25 and 50 mg/kg b.wt./day doses, orally for 90 days along with cholesterol feeding significantly prevented the rise of liver, TBARS level and improved the levels of glutathione and catalase in liver. The results of the present study suggest that extract of *Ginkgo biloba*significant antioxidant activities in Wistar rats.

Keyword: Antioxidant; Catalase ;Cholesterol; Glutathione; *Ginkgo biloba*; Lipid peroxidation; Liver; Rat.

Introduction

Cardiovascular diseases remain the leading cause of morbidity and mortality all over the world[1]. *Ginkgo biloba*isone of the important traditional medicines mentioned in the Chinese pharmacopoea as an antisthmatic against polyuria and for the treatment of cardiovascular diseases. Now a day's acetone water extract from ginkgo leaves has taken a prominent place in phytotherapy in several countries [2-3].

Ginkgo biloba contains flavonoids (ginkgo flavone glycosides) and terpenoids (ginkgolides and bilobalide) which are responsible for most of the pharmacological action. Flavonoids are able to scavenge free radicals that may be generated to a higher exent under pharmalogical condition. Due to membrane stabilizing properties, the flavonoids may protect erythrocytes against hemolysis. Myricetine and quercetin have been shown to induce oxidative metabolism in both resting and Ca^{2+} loaded brain neurons. Such an antioxidant action may be responsible for part of the beneficial effect of *Ginkgo biloba* on brain neurons [4].

The therapotic effect of ginkgo extracts in placebo controlled clinical tiles is reportedly similar to currently prescribed drugs such as tracrine and donepezil with minimum side effects [5-6].

In *in-vitro* and *in-vivo* assessment ginkgo extract has shown antioxidant properties which may be useful in numerous skin diseases especially inflammatory reaction and photosenesence.

In the present investigation antioxidant effect of *Ginkgo biloba* extract was evaluated in cholesterol fed rates.

Materials and Methods

Ginkgo biloba: Tablets containing standardized extract of *Ginkgo biloba* leaves (Ginkocer, Glaxo India) were purchased from the medical store.

Cholesterol Powder: Cholesterol powder was purchased from Himedia Laboratories Ltd. (India).

Animals: Colony bred, adult, healthy, male Wistar albino rats weighing 175-210 gram were utilized for these experiments. The rats were housed in groups in polypropylene cages under controlled conditions of temperature and light and provided balanced pallet diet (Lipton, India Pvt. Ltd.). The animals were maintained as per guidelines of the committee for the purpose of control and supervision of experimental animals (CPCSEA) regulations. The study was approved by the institutional ethical committee, Department of Zoology, University of Rajasthan, Jaipur, India. The ras were randomly divided into following groups each having seven rats:-

Group I : Rats fed on normal pallet diet and distilled water (0.5 ml / rat) as vehicle.

Group II : Rats orally administered with cholestrol (500 mg/kg. b. wt/day) dissolved in coconut oil (0.5 ml / rat) and distilled water as vehicle.

Group III : Rats orally administered with cholestrol (500 mg/kg. b. wt/day) + *Ginkgo biloba extract* (10 mg/kg. b. wt/day) suspended in distilled water (0.5 ml / rat).

Group IV : Rats orally administered with cholestrol (500 mg/kg. b. wt/day) + *Ginkgo biloba extract* (25 mg/kg. b. wt/day) suspended in distilled water (0.5 ml / rat).

Group V : Rats orally administered with cholestrol (500 mg/kg. b. wt/day) + *Ginkgo biloba extract* (50 mg/kg. b. wt/day) suspended in distilled water (0.5 ml / rat).

Autopsy: At the end of experimental period the rats were deprived of food overnight, sacrificed under mild ether anesthesia. Liver lobes were removed, cleaned and weighted on electric balances. Liver sample refrigerated for bio-chemical analysis.

Tissue Biochemistry:

Lipid Peroxidation and Antioxidant Defense System

Lipid peroxidation in liver was estimated by employing the thiobarbituric acid reactive substance (TBARS) assay [7].

Glutathione (GSH)[8] and catalase activity [9] were also determined in liver sample.

Statistical Analysis:

The data obtained after biochemical estimations of control and treated rats were averaged, standard error of the mean was calculated and compared by applying Student ‘t’ test.

Result

Cholesterol fed rats depicted a highly significant increase in the level of lipid peroxidation (TBARS) in liver ($P < 0.001$) as compared to the normal rats.

Oral administration of *Ginkgo biloba* extract of 10,25 and 50 mg/ kg b. wt. / day doses along with cholesterol exhibited a significant fall in level of LPO in liver ($P<0.05$ to $P<0.001$) as compared to the cholesterol fed controlled group. Cholesterol fed group showed highly significant decrease ($P< 0.01$) in the level of glutathione in liver as compared to the normal rats. There was no significant change in the level of glutathione in liver in *Ginkgo biloba* 10 mg / kg b. wt. treated group as compared to the cholesterol fed group. But *Ginkgo biloba* 25 and 50 mg/ kg b.wt.treated groups showed a significant increased the content of glutathione in liver ($P < 0.01$) as compared to the cholesterol fed controlled group .

Table 1. Effects of *Ginkgo biloba* extract on Liver, TBARS, and Antioxidant Defense Parameters.

Group/ Treatment	Lipid Peroxide (TBARS) (Mole/mg Tissue)	Glutathione (GSH) µmole/g tissue	Catalase n mole of H_2O_2 decomposed/min/mg Protein
Group: 1 (Normal control Rats)	1.41 ± 0.17	3.21 ± 0.22	54.39 ± 3.12
Group : II (Cholesterol fed Rats)	4.52 ± 0.43***	1.78 ± 0.20**	40.36 ± 3.27*
Group : III Cholesterol + *G. biloba*(10 mg / kg)	2.95 ± 0.24*	2.76 ± 0.19 **	52.67 ± 4.37^{ns}
Group : IV Cholesterol + *G. biloba*(25 mg / kg)	2.07 ± 0.20 ***	3.13 ± 0.26**	60.75 ± 3.96**
Group : V Cholesterol + *G. biloba*(50 mg / kg)	1.63 ± 0.17 ***	3.29 ± 0.17***	63.11 ± 3.63 **

Levels of Significance:
ns= non significant; *=p<0.05 ; ** = p < 0.01 ; *** = p < 0.001 ; when group III,IV,V compared with group II and group II compared with group I.

There was a significant deduction (P< 0.05) in the level of catalase in cholesterol fed rats as compared to the normal rats. Rats receiving *Ginkgo biloba* extract 10 mg/ kg b. wt. / day along with cholesterol showed a non significant change in the level of catalase in liver as compared to the cholesterol fed controlled group. *Ginkgo biloba* 25 and 50 mg/ kg b.wt. / day fed group showed a significant rise (P < 0.01) in the level of catalase in liver as compared to the cholesterol fed controlled rats.

Discussion

Lipid peroxidation occurs mainly in membranes where the content unsaturated fatty acid is relatively high. Peroxidation of membrane lipids arising out of oxidative damage of intact cells results in decreased fluidity. Inactivation of membrane bound enzymes and receptors and change in nonspecific ion permeability[10].

Chemical compounds and reactions capable of generating potential toxic oxygen species / free radicals are referred to as pro-oxidants. On the other hand compounds and reaction disposing of these species. Scavenging them or suppressing their formation or opposing their actions are called antioxidants. In a normal cell there is an appropriate pro-oxidant; anti-oxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when the levels of antioxidants are diminished. This state is called oxidative stress and can result is serious cell damage. Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases [11-12.]

In present investigation administration of cholesterol (500 mg/kg. b. wt/day) caused a significant increase in the activity of lipid peroxidation (TBARS) in liver as compared to the normal rats. These results get support from the report of Manimeglai *et al.,* 1993[27], who observed that high fat diet intake caused a significant increase in the level of lipid peroxide (TBARS) in liver as compared to the normal rats.

Hypercholesterolemia condition in general increased the lipid peroxidation in all the tissues [13-15].

Rats fed with cholesterol diet showed significant increases of lipid peroxidation (TBARS level) with a concomitant decline in the activity of catalase and glutathione (GSH). Lipid peroxidation is regarded as one of the basic mechanism of cellular damage caused by free radicals[16]. The relationship between lipid peroxidation and hypercholestremia is well recognized, a cholesterol rich diet results in increased lipid peroxidation rate by induction of free radical production[17]. Hypercholestremia and the process of lipid peroxidation are believed to be critically involved in the development of atherosclerotic lesions[18-19]. Parallel to present finding, other workers have also recorded significant increase in TBARs level in aorta of high cholesterol diet fed animals [20-24]. The decrease observed in the levels of catalase and glutathione in liver of animals cholesterol fed rats in the present study suggest deficiency of antioxidant molecules which might be resulted due to their over utilization to alleviate free radicals generated during hyperlipidemic state. Earlier studies have clearly shown that hypercholesterolemia diminishes the antioxidant defense system by elevating lipid peroxidation in aorta resulting in oxidative stress [22,25].

Oral administration of *Ginkgo biloba* extract along with cholesterol caused a significant decrease in the level of lipid peroxidation (TBARS) in liver as compared to the cholesterol treated rats. These results suggest reduced oxidative stress. These results are in agreements with the finding of [26], who also observed a significant decrease in the level of lipid peroxidation in plasma after feeding of *Ginkgo biloba* extract in high fat diet fed rabbits. The extract of *Ginkgo biloba* (Egb-761) is a mixture of flavonoid glycosides and other natural compounds. Flavonoid glycosides and proanthocyanidine present in *Ginkgo biloba* have significant oxygen free radical scavenging activity [28].

Several studies suggest the naturally occurring flavonoids and polyphenols scavenge free radicals including hydroxyl and superoxide anions, inhibit lipid peroxidation and improve lipid profile [29-30].

Glutathione (GSH) is the most abundant thiol, synthesised in the liver and acts as a substrate for glutathione peroxidase enzyme. This also serves as a scavenger of different free radicals. Catalase is a tetrameric enzyme present in most of the cells and acts by catalysing the decomposition of H_2O_2 to water and oxygen [12].

In present investigation administration of cholesterol caused a reduction in the level of glutathione and catalase in liver

Decrease in glutathione might be due to increased utilization of it to scavenge increased generation of free radicals resulted due to cholesterol feeding. Similar decrease in the level of glutathione [27]and catalase[13, 25]in liver of hyperlipidemic rats has been reported.

Oral administration of *Ginkgo biloba* extract along with cholesterol caused a rise

in the level of hepatic catalase and glutathione as compared to cholesterol fed rats.

Increase in the activity of catalase and glutathione in liver in *Ginkgo biloba* extract treated group might be due to the flavonoid content in *Ginkgo biloba* extract which acts like antioxidants.

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