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Hypolipidemic Effect of *Ginkgo biloba* Extract in Hypercholesterolemic Rats

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Abstract : The effect of *Ginkgo biloba* (fam. Ginkgoaceae) extract was assessed on serum lipid metabolism in cholesterol fed rats. Oral administration of cholesterol (500 mg/kg b.wt./day) suspended in coconut oil (0.5 ml/rat/day) for 90 days caused a significant ($p<0.001$) elevation in the level of total and LDL cholesterol, triglycerides and phospholipid in serum of rats. Addition of *Ginkgo biloba* extract 25 and 50 mg/ kg b.wt./day showed a significant reduction in concentration of total cholesterol, LDL cholesterol, phospholipid and triglycerides in serum. The HDL cholesterol / total cholesterol ratio was elevated in serum of *Ginkgo biloba* extract treated groups as compared to cholesterol alone fed control rats.

Keywords : *Ginkgo biloba*, Hypolipidemic effect, Cholesterol, Rats

Introduction :

Coronary heart disease resulting from progressive atherosclerosis remains the most common cause of death all over the world (WHO report, 2000; Gupta, 2001). Hyperlipidemia is acknowledged to be a major risk factor for cardiovascular events. The major component of total serum cholesterol that is associated with increased risk is low density lipoprotein cholesterol (LDC-c). In contrast, there is an inverse relationship between symptomatic atherosclerosis and high density lipoprotein cholesterol (HDL-c) levels (Dominiczak, 1998; Report of National Cholesterol Education Program, 2001).

Evidences from lipid lowering trials have clearly shown that reduction of total cholesterol is associated with decreased risk of coronary heart disease (Gotto, 2002). The National Cholesterol Education Program (2001) guidelines state that lowering LDL cholesterol is the primary objective of coronary heart disease risk reduction. Consequently, there is increasing demand for a medical treatment for this problem.

Ginkgo biloba L, commonly known as maidenhair tree is the sole representative of its family, Ginkgoaceae. In Chinese folk medicine the leaves of *Ginkgo biloba* are used for the treatment of cardiovascular diseases and to alleviate asthma. The ripe dry seed of the tree, peikuo, is officially listed in the Chinese pharmacopoeia and has been used in traditional medicine for centuries, as an antiasthamatic drug and expectorant, as well as against polyuria, tuberculosis, leucoorhea and spermatorrhea. Now a days, the extracts of Ginkgo leaves are used to treat cerebral insufficiency,

peripheral arterial vascular diseases, to improve learning and memory as cardio protective and many other diseases (Drieu, 1986; Spegg, 1990; De Feudis, 1991; Oyama *et al*., 1994)

Phytochemical studies have shown that the leaves of *Ginkgo biloba* contains a large number of different metabolites including terpenoids (ginkgolides, bilabolide), flavonoids (catechins, flavones, biflavones) flavonols (kaempferol, quercetin or isorhamnetin), steroids (sitosterol, stigmasterol) and carotenoids (Woerdenbag and Van Beek, 1997). In the present investigation hypolipidemic effects of *Ginkgo biloba* extract have been evaluated in hypercholesterolemic rats.

Materials and Methods

***Ginkgo biloba* :** The tablets containing *Ginkgo biloba* leaves extract (Ginkocer, Glaxo India) were purchased from the medical store. The tablets were suspended in normal saline and required concentrations were made.

Cholesterol powder : Dry, pure cholesterol powder was purchased from Himedia laboratories Ltd. (India).

Animals : Colony bred, adult, healthy male albino rats (Wistar strain) of nearly equal age, weight (175-225 g) were utilized for these experimentation. The rats were housed in groups in plastic cages under controlled conditions of temperature and light and provided balanced pallet diet (Lipton, India Ltd. Bangalore) and water *ad libitum*. The rats were randomly divided into following groups; each having seven rats.

Group I : Rats fed on normal pallet diet.

Group II: Rats orally administered with cholesterol (500 mg/kg b.wt/ day) suspended in coconut oil (0.5 ml/rat) + normal saline (0.5ml)

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

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

Group V : Rats orally treated with cholesterol (500 mg/kg b.wt/ day) + *Ginkgo biloba* extract (50 mg/kg b. wt/day) suspended in normal saline (0.5 ml/rat)

All the rats received treatment for 90 days.

Autopsy : After overnight fasting, rats were sacrificed under mild ether anaesthesia. Blood samples were collected directly from the heart and sera were separated and stored at -20^{o} C for biochemical estimations. Various organs like liver, heart, kidneys, lungs, adrenal glands were removed, cleaned and weighed on electric balance.

Biochemical Analysis : Pooled serum samples were analysed for total cholesterol (Zlatkis *et al.*, 1953), LDL cholesterol (Friedwald *et al.*, 1972), HDL cholesterol (Burnstein *et al.*, 1970), triglycerides

(Gottfried and Rosenberg, 1973), and phospholipid (Zilversmit and Davis, 1950).

Statistical Analysis : The values of body organ weights and biochemical estimations of normal and treated rats were averaged, standard error of the mean was calculated and compared by applying Student 't' test.

Result :

In the present investigation the mean body weight gain in normal rats was 21.93%, in cholesterol fed rats 38.14% and in rats treated with cholesterol + *Ginkgo biloba* extract with doses10, 25 and 50 mg/kg.b.wt/day, 31.22%, 32.52% and 27.25%, respectively.

Administration of cholesterol in rats caused a significant increase in relative weight of liver and kidney ($P<0.05$) and a nonsignificant change in the relative weight of heart, lung and adrenal gland. Simultaneous feeding of *Ginkgo biloba* extract along with cholesterol caused a significant reduction ($P<0.05$) in the relative weight of liver at 25 and 50 mg/kg b.wt./day dose levels. A significant reduction ($P<0.05$) in the relative weight of kidney was also observed in rats receiving 50 mg/kg b.wt./day dose as compared to the cholesterol alone fed control group. The weight of other vital organ viz. heart, lungs and adrenalgland were significantly unchanged when compared with cholesterol fed control rats. No significant difference in the mean diet consumption/rat/day was recorded in rats of various experimental groups. (Table –1)

The concentrations of total cholesterol (Tc), low density lipoprotein cholesterol (LDL-Cholesterol), triglycerides (Tg) and phospholipid in serum, increased significantly ($P<0.001$) after feeding with cholesterol. However the High Density Lepoproteir Cholesterol of serum levels remained significantly unchanged. The HDL cholesterol/total cholesterol ratio (HDL-c : Tc) got declined in cholesterol fed control group. Administration of *Ginkgo biloba* extract at three different doses (10, 25 and 50 mg/kg b. wt/day) along with cholesterol showed a significant dose dependent decease in the serum levels of total cholesterol ($P<0.05$ to $P<0.001$), LDL cholesterol ($P<0.001$) triglycerides ($P<0.01$ to $P<0.001$) and phospholipid ($P<0.05$ to $P<0.01$) but no significant change is noticed in the level of serum HDL cholesterol when compared with cholesterol fed control rats. However the ratio of HDL-c/Tc increased dose dependently. (Table-2)

Discussion :

It is well documented that elevated cholesterol and LDL levels promote atherosclerosis (Dominiczak, 1998; National Cholesterol Education Program report, 2001). Oxidative modification of low density lipoprotein cholesterol (LDL-c) appears to have an important role in coronary artery disease and atherogenesis (Liao, 2000). The agent which can lower serum cholesterol and scavenge the free radicals or inhibit their production have gained wide therapeutic value. Many plant products are increasingly recognised as having protective role in coronary artery disease through several

Table 1 : Effect of *Ginkgo biloba* extract on body and organ weights and feed intake in cholesterol fed rats.

Treatment	Body Weight (g) Initial	Body Weight (g) Final	Gain in b.wt (%)	Feed intake g/rat/day	Liver	Kidney	Heart	Lung	Adrenal
					mg/100 g body weight				
Normal (Vehicle treated)	196 ±7.48	239 ±10.39	21.93	17.26	3537..92 ±155.81	764.82 ±30.15	339.55 ±35.31	651.35 ±25.01	19.78 ±1.05
Cholesterol fed control (500 mg/kg b.wt/day)	194 ±7.07	268 ±12.40	38.14	16.63	4130.96[a] ±180.71	870.15[a] ±33.32	385.72 ±40.31	745.45 ±35.30	21.46 ±0.849
Cholesterol + *Ginkgo biloba* extract (10 mg/kg b.wt/day)	208.8 ±7.70	274 ±17.77	31.22	17.84	3915..32 ±100.43	840.37 ±40.31	352.44 ±30.31	727.17 ±28.46	21.43 ±1.13
Cholesterol + *Ginkgo biloba* extract (25 mg/kg b.wt/day)	198 ±10.19	262.4 ±10.64	32.52	17.16	3688.89* ±90.01	792.40 ±38.72	323.73 ±18.5	672.87 ±33.70	22.90 ±0.983
Cholesterol + *Ginkgo biloba* extract (50 mg/kg b.wt/day)	199.6 ±8.15	254 ±7.64	27.25	17.18	3629.37* ±128.99	739.30* ±35.41	318.49 ±25.62	704.37 ±25.45	22.81 ±1.83

(values are mean ±S.E. of 7 rats)

a = P <0.05 Cholesterol fed control rats compared with normal rats.

* = P < 0.05 *Ginkgo biloba* extract + cholesterol treated rats compared with cholesterol fed control rats.

Table 2 : Effact of *Ginkgo biloba* extract on serum lipid profile in cholesterol fed rats

Treatment	Cholesterol	LDL Cholesterol	HDL Cholesterol	Triglycerides	Phospholipid	HDL-c/Tc
	mg/dl					
Normal (Vehicle treated)	103.20 ± 3.1	48.26 ± 1.3	39.34 ± 1.1	78.01 ± 1.39	132.12 ±3.7	0.38
Cholesterol fed control (500 mg/kg b.wt/day)	198.71[a] ±8.78	132.51[a] ±3.49	42.12 ±1.73	120.40[a] ±2.1	175.36[a] ±6.7	0.21
Cholesterol + *Ginkgo biloba* extract (10 mg/kg b.wt/day)	170.32* ±6.2	105.35*** ±3.14	42.42 ±1.9	112.74 ±2.9	166.53 ±6.9	0.24
Cholesterol + *Ginkgo biloba* extract (25 mg/kg b.wt/day)	152.43** ±7.3	87.76*** ±3.21	44.81 ±2.1	99.33** ±3.4	155.40* ±4.8	0.29
Cholesterol + *Ginkgo biloba* extract (50 mg/kg b.wt/day)	135.64*** ±4.9	71.46*** ±2.87	45.62 ±1.3	92.82*** ±3.5	142.21** ±5.2	0.33

(values are mean ±S.E. of 7 rats)

a = P < 0.001 Cholesterol fed control rats compared with normal rats.

* = P < 0.05;**=P<0.01; *** = P <0.001 *Ginkgo biloba* extract + cholesterol treated rats compared with cholesterol fed control rats.

mechanisms including antioxidant and hypolipidemic properties (Gupta *et al.*, 2001; Heber, 2001; Anilla and Vijayalakshmi, 2002).

The results of present study demonstrated hypolipidemic effect of *Ginkgo biloba* in cholesterol fed rats. The increase in the mean relative weights of liver and kidney in cholesterol fed rats might be due to excessive accumulation of lipids in these tissues. Simultaneous administration of *Ginkgo biloba* extract prevented the increase in relative weight of these organs possibly due to less accumulation of lipids by virtue of hypolipidemic effect of the extract.

Administration of cholesterol suspended in coconut oil caused significant increase in total cholesterol, LDL cholesterol, triglycerides, phospholipid, while HDL cholesterol, remained significantly unchanged. Some investigators (Sethupathy *et al*., 2002; Zhang *et al.*, 2002) also observed a significant increase in total and LDL cholesterol after feeding cholesterol and/or high fat diet. The major effect of dietary cholesterol on plasma lipoproteins is to raise LDL cholesterol behind that dietary cholesterol suppresses the synthesis of LDL- receptor (Goldstein and Brown, 1982).

Treatment with *Ginkgo biloba* extract + cholesterol significantly reduced the level of serum cholesterol (-31.73) LDL cholesterol (-46.07%), triglycerides (-22.90%) and phospholipid (-18.90%) concurrently with a non-significant increase (+8.3%) in the level of HDL cholesterol, demonstrating and beneficial moduletory influence on senem lipid profile. The results of present study confirmed the finding of Wojcicki *et al*., (1994) who reported that oral administration of standardized *Ginkgo biloba* extract in high fat diet fed rabbits showed a pronounced improvement in lipid metabolism disturbances by reducing lipid content in serum and increasing serum HDL cholesterol concentration. *Ginkgo biloba* treatment caused an increase in the content of cytochrome P_{450} in the liver microsomes and stimulates synthesis, secretion of bile acid in fecal matter which may be responsible for its hypocholesterolemic effect. Significant increase in fecal cholesterol excretion in *Ginkgo biloba* extract treated rats observed during present study also suggests inhibition of cholesterol absorption and/or increased secretion of cholesterol through bile.

Ginkgo biloba leaves contain large number of metabolites including terpenoids (ginkgolides and bilabolides), flavonoids (catechins, dehydro catechins, flavones and biflavones), flavon glycosides (kempferol-3-rutionside, quercetin-3-rutinoside, isorhamnetin-3-rutinoside), steroids (stilosterol, stigmasterol) and carotenoids. Thus the individual or synergistic activity of these compounds can have diverse action on lipid metabolism and may also influence absorption and excretion of fats, which might be responsible for hypolipidemic effect. Epidemiologic studies have shown dietary flavonoids intake to be inversely associated with mortalty from CHD (Wiesman, 1999).

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