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Study on Hypolipidemic Activity of *Cassia fistula*. Legume in Rats

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Abstract : The effect of 50% ethanolic extract of *Cassia fistula* Linn. (Family: fabaceae) legume was assessed on serum lipid metabolism in cholesterol fed rats. Oral feeding of cholesterol (500 mg/kg b.wt./day) dissolved in coconut oil (0.5 ml/rat/day) for 90 days caused a significant ($P<0.001$) elevation in total and LDL-cholesterol, triglycerides and phospholipid in serum of rats. Administration of *C. fistula* legume extract at the doses 100, 250 and 500 mg/kg b.wt./day along with cholesterol significantly prevented the rise in the serum total and LDL-cholesterol, triglycerides and phospholipid in a dose dependent manner. The ratio of HDL-cholesterol / total cholesterol ratio was elevated in serum of *C. fistula* extract treated groups as compared to cholesterol alone fed control rats.

Key words : *Cassia fistula*, Hypolipidemic effect, Cholesterol, Rats.

Cardiovascular disease is a major cause of morbidity and mortality all over the world (WHO, 2000) It is well established that elevated levels of plasma cholesterol or more specifically plasma low density lipoprotein cholesterol (LDL-c) is regarded as a crucial risk factor in the prevalence of atherosclerosis (National Cholesterol Education Program, 2001). Lowering of elevated levels of total cholesterol (Tc) and low density lipoprotein cholesterol reduces the risk of cardiovascular disease (Lipid Study Group, 1998). On the other hand a strong inverse relation between HDL-cholesterol (HDL-c) and risk of coronary heart disease has been advocated (Stein and Stein, 1999). The modulation of risk of coronary heart disease by lowering blood lipid profiles by using natural products of plant origin as a possible therapeutic measure has become a subject of active scientific investigation. Medicinal plants are important source of a large number of active novel compounds which offer themselves as promising substances for the development of hypolipidemic and antioxidant agent (Haber 2001; Anilla and Vijaylakshmi, 2002)

Cassia fistula Linn., (Family: Fabaceae) commonly known as Indian laburnum) has been used in the treatment of various ailments dating back to 'Charak Samhita' and 'Sushrut Samhita'. According to Ayurvedic and Unani systems of medicines various parts of *C. fistula* are highly useful in curing various diseases viz. cardiac disorders, diabetes, skin diseases and snake bite (Kirtikar and Basu, 1933). The pulp from the pod is of great therapeutic value, it is a mild, pleasant and safe purgative, even for children and expectant mothers. Experimental studies have shown that extract of *C. fistula* possesses hypolipidemic (el-Saadany *et al.*, 1991), hepatoprotective

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(Bhakta *et al.*, 2001), antibacterial and antifungal (Duraipandivan and Ignacimuthu, 2007) activities.

Extensive studies have been carried out during the past few decades for isolation and characterisation of chemical constituents of various parts of *C. fistula*. Lal and Gupta (1972) isolated rhein, glucose, sucrose and fructose from the fruit pulp and galactomannans from the seeds of the *C. fistula.* Agrawal *et al.*, (1972) isolated fistulic acid from the pods, and kaempferol and a leucopelargonidin tetramer having free glycol unit from the flowers. The pulp is rich source of minerals and energy and contains a large number of essential amino acids in good amount (Barthakur, 1995). Kuo *et al.*, (2002) have isolated and identified oxyanthraquinones, chrysophanol and chrysophanein from the seeds of *C. fistula.*

In the present investigation hypolipidemic effect of 50% ethanolic extract of *C. fistula* legume was evaluated in cholesterol fed rats.

Materials and Methods

***Cassia fistula* :** Ripe legumes of *C. fistula* were collected at the Rajasthan University campus and authenticated from the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India (Voucher specimen no. RUBL 19870). The plant material was dried in shade and ground to coarse powder and extracted with 50% ethanol for 36 hours at 60-80 ^{0}C. The extract was filtered and evaporated to dryness under low temperature and reduced pressure. The crude extract so obtained was suspended in double distilled water and used for experimental study.

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

Animals : Colony bred, adult, healthy, male Wistar albino rats weighing 175-210 g were utilized for these experiments. The rats were housed in groups in polypropylene cages under controlled conditions of temperature (22 ^{0}C $\pm$ 3 ^{0}C) and light (14:10h light and dark cycle) and provided balanced pallet diet (Lipton India Ltd. Bangalore, India) and water *ad libitum.* The rats were randomly divided in to following groups each having 7 rats:

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

Group II : Rats orally administered with cholesterol (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 cholesterol (500 mg/kg b. wt./day) + *C. fistula* extract (100 mg/kg. b.wt/ day) suspended in distilled water (0.5ml/rat).

Group IV : Rats orally administered with cholesterol (500 mg/kg. b.wt/day) + *C. fistula* extract (250 mg/kg. b.wt/day) suspended in distilled water (0.5ml/ rat).

Group V : Rats orally administered with cholesterol (500 mg/kg. b.wt./day) + *C. fistula* extract (500 mg/kg. b.wt./day) suspended in distilled water (0.5ml/rat).

All the rats of various experimental groups received treatment for 90 days.

Autopsy : At the end of experimental period, the rats were deprived of food overnight, sacrificed under mild ether anesthesia. Blood sample was collected directly from the heart and serum was separated and stored at -20^0 C until analysis. Liver, heart, kidney, lung, adrenal gland were removed, cleaned and weighed on electric balance. Half of the liver tissue was fixed in Bouin's fixative for histopathological observations and remaining half was frozen at -60^{0}C for biochemical analysis.

Biochemical analysis : Pooled serum samples were analysed for total cholesterol

(Zlatkis and Boyle, 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 bodyorgan 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

The mean body weight gain observed in cholesterol fed rats was higher (38.14%) in comparison to normal rats (21.93%). However rats receiving *C. fistula* extract treatment at 100, 250 & 500 mg/kg b.wt./day doses along with cholesterol registered slightly lesser body weight gain (29.34%,23.68% and 26.51% respectively) compared to cholesterol alone fed rats. (Table 1)

Administration of cholesterol in rats caused a significant increase in the relative weights of liver ($P < 0.05$) and kidney ($P < 0.05$) and a non significant change in the relative weight of heart, lung and adrenal gland. Treatment with *C. fistula* extract along with cholesterol showed a slight significant reduction ($P < 0.05$) in the relative weight of liver at 250 and 500 mg/kg dose levels and of kidney at 500 mg/kg dose level as compared to the cholesterol alone fed group. The relative weights of other vital organ viz. heart, lung and adrenal gland remained significantly unchanged as compared to cholesterol alone fed rats. (Table 1)

The concentrations of serum total cholesterol (Tc), low density lipoprotein cholestrol (LDL-c), triglycerides (Tg) and phospholipid increased significantly ($P < 0.001$) after feeding cholesterol. Although the serum high density lipoprotein cholesterol (HDL-c) level remained signficantly unchanged but HDL-cholesterol/total cholesterol ratio (HDL-c : Tc) was markedly lowered in chlesterol/ total cholesterol fed rats. Oral administration of *C. fistula* extract (100, 250 and 500 mg/kg. b.wt./day dose levels) along with cholesterol showed a significant dose dependent decrease in the level of serum total cholesterol ($P < 0.05$, $P < 0.001$, $P < 0.001$ respectively), LDL-cholesterol ($P < 0.001$), triglycerides ($P < 0.05$, $P < 0.001$, $P < 0.001$ respectively) phospholipid ($P < 0.01$, at 250 & 500 mg/kg doses) and a non significant change in the level of serum HDL-cholesterol as compared to the cholesterol fed control rats. However the ratio of HDL-c/Tc was increased dose dependently. Maximum protective effect observed on serum total cholesterol and LDL-cholesterol was 39.25%, and 58.78% respectively. (Table 2)

Discussion

The results of the present study demonstrated hypolipidemic effect of 50% alcoholic extract of *C. fistula* legume in cholesterol fed rats.

The increase recorded 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 *C. fistula* legume 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. Histopatholgical study of liver (Photograph not shown) also supports this finding.

Administration of cholesterol dissolved in coconut oil significantly raised the level of serum cholesterol, triglycerides and phospholipid. Serum LDL-cholesterol concentration was also raised significantly without dipicting and significant change in HDL-cholesterol concentration. However, the ratio of HDL-c : Tc was lowered. These results are in agreement with earlier findings (Sethupathy *et al.*, 2002). Serum concentration

Table 1: Effect of *Cassia fistula* legume extract on body & organ weights and feed intake in cholesterol fed rats.

Treatment	Body Weight (g)		Gain in B.Wt. (%)	Feed intake gm/rat/day	Liver	Kidney	Heart	Lung	Adrenal
	Intial	Final	----------mg/100 gm body weight----------						
Normal (Vehicle treated)	196 ± 7.48	239 ± 10.53	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 ± 12.4	38.14	16.63	4130.96a ± 180.71	870.15a ± 33.32	385.72 ± 40.31	745.45 ± 35.3	21.46 ± 0.849
Cholesterol + *Cassia fistula* extract (100 mg/kg b.wt. /day)	184 ± 6.78	238 ± 15.93	29.34	15.81	3845.91 ± 152.52	796.41 ± 36.4	340.4 ± 25.8	725.73 ± 40.21	21.24 ± 1.27
Cholesterol + *Cassia fistula* extract (250 mg/kg b.wt. /day)	190 ± 4	235 ± 7.07	23.68	16.32	3675.81* ± 92.3	785.95 ± 43.31	326.45 ± 16.32	690.41 ± 32.83	20.52 ± 0.675
Cholesterol + *Cassia fistula* extract (500 mg/kg b.wt. /day)	215 ± 8.36	272 ± 18.18	26.51	17.57	3585.01* ± 125.72	689.30* ± 70.41	342.69 ± 28.73	700.23 ± 28.45	19.12 ± 0.81

(Values are mean ± S.E. of 7 rats)

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

**= P<0.05; C. fistula extract + cholesterol treated rats compared with cholesterol fed control rats.*

Table 2: Effect of *Cassia fistula* legume extract on serum lipid profiles in cholesterol fed rats.

Treatment	Total Cholesterol	LDL Cholesterol	HDL Cholesterol	Triglycerides	Phosphlipid	HDL-c/Tc Ratio
	mg/dl					
Normal	103.2	48.26	39.34	78.01	132.12	0.38
(Vehicle treated)	± 3.1	± 1.13	± 1.1	± 1.39	± 3.7	
Cholesterol fed control	198.71[a]	132.51[a]	42.12	120.40[a]	175.36[a]	0.21
(500 mg/kg b.wt. /day)	± 8.78	± 3.49	± 1.73	± 2.1	± 6.7	
Cholesterol + *Cassia fistula*	159.83*	94.34***	43.84	108.31*	156.44	0.27
extract	± 8.4	± 5.18	± 2.6	± 3.3	± 8.5	
Cholesterol + *Cassia fistula*	145.31***	80.84***	45.63	94.23***	148.41**	0.31
extract	± 3.9	± 2.98	± 1.8	± 1.7	± 5.6	
Cholesterol + *Cassia fistula*	120.70***	54.62***	47.82	88.32***	143.32**	0.39
extract	± 6.3	± 2.13	± 2.3	± 1.8	± 6.4	

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 C. fistula extract + cholesterol treated rats compared with cholesterol fed control rats.*

of LDL-cholesterol is largely dependent on the rate of its production and removal from circulation. LDL-cholesterol is produced from very low density lipoprateins (VLDL), which is secreted by the liver and most of the serum LDL-cholesterol is removed via a hepatoreceptor mediated process. Since the expression of LDL receptors is controlled by feed back inhibition of intracellular cholesterol, increase in hepatic cholesterol in turn decrease the production of LDL receptors and thus elevate blood cholesterol levels by virtue of lower clearance rate (Dietschy *et al.*, 1993).

Administration of 50% ethanolic extract of *C. fistula* legume caused a significant dose dependent decrease in serum total cholesterol, triglycerides and phospholipid. The level of serum LDL-cholesterol was also declined without showing much influence on serum HDL-cholesterol level. The maximum protective effect observed on serum total cholesterol, LDL-cholesterol, triglycerides and phospholipied was -39.25%, -58.78%, -26.64% and 18.27% repectively. The ratio of HDL-c : Tc also improved, indicating beneficial effect. These results are parellel with the finding of (el-Saadany *et al.*, 1991) who also observed a significant decrease in total lipid, cholesterol and triglyceride concentrations in blood and liver of cholesterol fed rats after administration of *C. fistula* legume extract. A similar hypolipidemic activity of *Ginkgo biloba* extract (Gupta and Jain, 2006) and methanolic extract of *Cassia viscosa* seeds (Jain and Agarwal, 2006), aerial part of *Leptadenia pyrotechnica* (Jain *et al., 2007*) have been recorded recently. A large number of phytochemical constituents like galactomannan, anthraquinone glycosides, rhein, flavonoids, polyphenols, proanthocyanidin beside other components have been reported in *C. fistula* legume. Further, hypolipidemic activity of galactomannan (Moriceau *et al.*, 2000) and proanthocyanidins (Bobek, 1999; Yama koshi *et al.*, 1999) and flavonoids (Anilla and Vijayalakshmi, 2002).

Cassia fistula legumes are rich in dietary fibers and mineral contents. It also contain many essential amino acids like aspartic acid, glutemic acid and lysine in the fruit pulp. The hypocholesterolemic effect observed in the present investigation might be due to presence of high fiber components.

Further the pod is rich in anthroquinone and is used as purgative this might effect cholesterol absorption and may enhance bile acid excretion through faeces.

In addition the fruit pulp contains a large number of active components like leucopelargonidin, proanthocynidin, (-) epiafzelechin (+) catechin and kaemferal which are know for their well established hypocholesterlemic and antioxidant properties.

However, the hypolipidemic effect of *C. fistula* legume extract (50% ethanolic) in hypercholesterolemic rats might be due to individual or synergistic action of the active components at various target sites. The exact mechanism of the hypolipidemia caused by *C.fistula* is yet to be understood which warrants further study.

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