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Research Article

CARDIOVASCULAR DISEASE REMAINS THE LEADING CAUSE OF MORBIDITY AND MORTALITY ALL OVER THE WORLD

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ABSTRACT
Hyperlipidemia/hypercholesterolemia and oxidative stress are major risk factors for atherosclerosis and cardiovascular diseases. *Cassia fistula* Linn. is widely used in Ayurvedic and Unani systems of medicines to treat various ailments. The present study was undertaken to investigate antioxidant and antiatherogenic efficacy of 50% ethanolic extract of *C. fistula* legume in cholesterol fed Wistar rats. Results of the study showed that total cholesterol, total phospholipids and triglycerides contents were significantly ($P<0.001$) increased in aorta of cholesterol fed control rats. Cholesterol feeding also resulted in 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. Simultaneous treatment of *C. fistula* legume extract at 100, 250 and 500 mg/kg b.wt./day doses, orally for 90 days along with cholesterol feeding significantly prevented the rise of aortic lipid profile, TBARS level and improved the levels of glutathione and catalase in aorta. The histopathological study of the aorta revealed that *C. fistula* extract treatment significantly prevented the atherogenic lesions induced due to cholesterol feeding. The results of the present study suggest that 50% ethanolic extract of *C. fistula* legume possesses significant hypolipidemic, antiatherogenic and antioxidant activities in Wistar rats.

Keywords: *Antiatherogenic, Antioxidant, Aorta, Cassia Fistula, Lipid Profile, Lipid Peroxidation*

INTRODUCTION
Cardiovascular diseases remain the leading cause of morbidity and mortality all over the world (WHO, 2014). The elevation of serum total cholesterol and low-density lipoprotein (LDL) cholesterol as well as alteration of other lipid parameters has been implicated as a primary risk factor for cardiovascular diseases. Persistent hypercholestromia along with generation of reactive oxygen species (ROS) plays a key role in the development of coronary artery disease (CAD) and atherosclerosis (Parsad and Kalra, 1989). Oxidative stress is currently suggested a mechanism underlying hypercholesteromia. It has been suggested that oxidative modification of low density lipoproteins (LDL) caused by reactive oxygen species results in the formation of foam cells which is the initial lesion of atherosclerosis (Steinberg, 1997; Ross, 1999). The control of cardiovascular risk factors is thus imperative for reducing the morbidity and mortality all over the world. Several modern synthetic drugs such as statins, fibrates nicotinic acid and resins are widely used to as clinical treatment to lower blood cholesterol level via different mechanisms (Davidson *et al.*, 2004). However, their long term consumption causes adverse health effects (Bhatnagar, 1998; Marshall, 2014). 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 as these are easily available, cheap and comparatively safe (Saravanan and Ignacimuthu, 2015). Medicinal plants are important source of a large number of bioactive novel compounds which offer themselves as promising substance for the development of hypolipidemic and antioxidant agents (Haber, 2001; Anilla and Vijaylakshmi, 2002; Joanna *et al.*, 2003; Mahmood *et al.*, 2010) Epidemiological evidences and international studies have also correlated higher level of antioxidant rich food uptake with lower incidence of coronary heart disease (Devasagayam *et al.*, 2004).
Cassia fistula Linn. (Family: Fabaceae commonly known as Indian laburnum) has been used in the treatment of various ailments dating back to 'Charaksamhita' and 'Sushrutsamhita'. According to Ayurvedic and Unani systems of medicines various parts of *C.fistula* are highly useful in curing various

diseases and snake bite (Kirtikar and Basu, 1933). The dried pods and pulp are valued for their laxative properties. The pulp is considered a safe purgative, recommended for pregnant women and children to relive disorders of the liver and biliousness. It is also used to treat leprosy and diabetes and for the removal of abdominal obstructions.

The seeds possess laxative, carminative, cooling and antipyretic properties. They are used to relieve constipation and to treat jaundice, biliousness, skin diseases and swollen throat. In Ayurveda, the bark is used to treat skin diseases, such as eczema and leucoderma, diabetes, dysuria, leprosy, jaundice, syphilis and arthritis. The leaves possess antiperiodic and laxative properties and are used in the treatment of jaundice, piles, rheumatism and externally for skin diseases. The root is useful for treating cardiac disorders, biliousness and rheumatic conditions (Parrotta, 2001).

Experimental studies have shown that extract of *C. fistula* possesses hypolipidemic, hepatoprotective, antidiabetic, anti- inflammatory, antitumor, antimicrobial, antipyretic, antiulcer, antioxidant, antifertility and wound healing properties (Chauhan *et al.*, 2011; Danish *et al.*, 2011; Ashraf Ali, 2014).

Phytochemical studies revealed that the pulp of the *C. fistula* pod is rich in antioxidants and contains anthraquinone glycosides, sennosides A and B, rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid and their ethyl esters, oxalic acid, pectin and tannin (Bahorun *et al.*, 2005; Khare, 2007; Ashraf Ali, 2014).

Proanthocyanidins containing flavon-3-ol units with abnormal 2S configuration have also been observed in the pods of the plant, together with the common flavon-3-ols and proanthocyanidin like catechein, epicatechin, procyanidin B-2 and epiafzelechin (Kashiwada *et al.*, 1990). A new bioactive flavone glycoside 5,3',4'- tri-hydroxy-6-methoxy-7-0-α-L- rhamnopyranosyl-(1→2)-0-β-D-galactopyranoside was also reported by Yadav and Verma (2003). Oxyanthroquinines, chrysophanol and chrysophanein were also isolated from the seeds of *C. fistula* by Kuo *et al.*, (2002).

The pulp is rich source of minerals (Fe and Mn), energy and contains a large number of essential amino acids in good amount (Barthakur *et al.*, 1995). Misra *et al.*, (1997) reported new diterpenes, 3 beta-hydroxy-17-norpimar-8(9)-en-15-one from pods of *C. fistula*.

The present study was designed to investigate anti-hyperlipidemic, antioxidant and antiatherogenic efficacy of 50% ethanolic extract of *C. fistula* legume in dorsal aorta of cholesterol fed Wistar rats.

MATERIALS AND METHODS

***Cassia Fistula*:** Ripe legumes of *C. fistula* were collected from the campus of Rajasthan University, Jaipur 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˚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 (220C + 3 0C) and light (14:10h light and dark cycle) and provided balanced pallet diet (Lipton India Ltd. Bangalore, India) and water *ad libitum.* 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 rats were randomly divided into 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 were collected directly from the heart and serum was separated and stored at-20^{o}c for biochemical analysis.
Dorsal aorta and other vital organs were removed, cleaned and weighted on electric balances. Half of the aorta sample was fixed in 10% formol calcium solution for histological studies and remaining half was refrigerated (-70^{o}c) for biochemical analysis.

Tissue Biochemistry

Quantitative biochemical estimations of total proteins (Lowry *et al.,* 1951), total cholesterol (Zlatkis *et al.*, 1953), phospholipids (Zilversmit and Davis, 1950) and triglycerides (Gottfried and Rosenberg, 1973) were made in the frozen aorta samples.

Lipid Peroxidation and Antioxident Defense System

Lipid peroxidation in aorta was estimated by employing the thiobarbituric acid reactive substance (TBARS) assay (Okhawa *et al.,* 1979). Glutathione (GSH) (Moron *et al*, 1979) and catalase activity (Claiborne, 1985) were also determined in aorta sample.

Histopathological Study

For histopathological study, the aorta were fixed in 10% formol-calcium solution, then dehydrated in graded series of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 µm thickness and stained with hematoxylin and Eosin (H& E) stain for studying histopathological changes.

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.

RESULTS AND DISCUSSION

Results

Aortic Lipid Profile

Cholesterol feeding in rats for 90 days resulted in a significant increase ($P< 0.001$) in aortic cholesterol concentration. Simultaneous treatment of 50% ethanolic extract of *Cassia fistula* legume at 100,250 and 500 mg/kg b.wt. doses significantly countered the increase in aortic cholesterol concentration when compared with cholesterol fed control rats. (Table 1)

Aortic total phospholipid concentration was significantly increased ($P<0.001$) in cholesterol fed control rats when compared with normal control. Treatment of *C.fistula* legume extract at 100,250 and 500 mg/kg b.wt./day dose regimen along with cholesterol feeding showed significant ($P<0.05$; $P<0.01$; $P<0.001$, respectively) dose dependent decrease in aortic phospholipid concentration as compared to cholesterol fed control rats (Table 1).

Concentration of triglycerides in aorta of cholesterol fed rats was also increased significantly ($P<0.001$) as compared to cholesterol fed control rats. Simultaneous treatment of *C.fistula* legume extract at 250 and 500 mg/kg b.wt./day doses along with cholesterol feeding significantly ($P<0.01$, $P<0.001$, respectively) prevented the rise of triglycerides in aorta when compared to cholesterol fed control rats (Table 1)

Lipid Peroxidation and Antioxidant Defense Parameters

Cholesterol feeding in rats for 90 days caused a significant increase ($P<0.01$) in lipid peroxidation (TBARS level) with a concurrent decline in catalase activity ($P<0.05$) and glutathione (GSH) content ($P<0.01$) in aorta as compared to normal control rats. Simultaneous administration of *C.fistula* legume extract at 250 and 500 mg/kg b.wt./day significantly ($P<0.05$) prevented the rise in TBARS level and improved glutathione level ($P<0.05$, $P<0.01$ respectively) and catalase activity ($P<0.05$) when compared to cholesterol fed control rats (Table 1).

Table 1: Effects of *Cassia Fistula* (C.F.) Extract on Aortic Lipid Profile, TBARS and Antioxidant Defense Parameters

Group/ Treatment	Total Cholesterol (mg/g)	Total Phospholipids (mg/g)	Triglycerides (mg/g)	Lipid Peroxide (TBARS) (Mole/mg Tissue)	Glutathione (GSH) (µmole/g tissue)	Catalase (n Mole of H_2O_2 Decomposed/min/mg Protein)
Group I (Normal control rats)	5.60±0.32	7.64±0.62	10.87±0.54	1.17±0.14	2.43±0.14	44.87±2.13
Group II (Cholesterol fed rats)	16.25±0.70^{c}	16.31±0.75^{c}	20.32±0.88^{c}	2.42±0.29^{b}	1.56±0.14^{b}	37.23±2.27^{a}
Group III (Cholesterol + C.F. 100mg/kg)	14.11±0.90*	13.10±0.83*	18.61±1.14^{ns}	1.98±0.14^{ns}	1.77±0.09^{ns}	41.27±3.12^{ns}
Group IV (Cholesterol + C.F.250 mg/kg)	12.32±0.43***	12.40±0.57**	15.36±0.64**	1.83±0.13*	2.12±0.17*	47.26±3.34*
Group V (Cholesterol + C.F.500 mg/kg)	7.85±0.50***	9.62±0.39***	12.15±0.44***	1.40±0.12*	2.28±0.13**	51.37±4.21*

Values are mean ± SEM (n=7)

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

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Histopathology of Aorta

Histopathological examination of cross section of the dorsal aorta of normal control rat showed normal morphology. Tunica intima surface is smooth without any irregularities. While, the histopathological examination of the dorsal aorta in cholesterol fed control rats showed appearance of lipid laden macrophages and foam cells, mild proliferation of smooth muscle cells and thickening of intima layer. Simultaneous administration of *C. fistula* legume extract (100, 250 and 500 mg/kg b.wt./day) doses along with cholesterol feeding showed dose dependent protective effect as indicated by disappearance of foam cells and prevention of smooth muscle cells proliferation and normal appearance of intima layer (Figure I, II, III, IV, V).

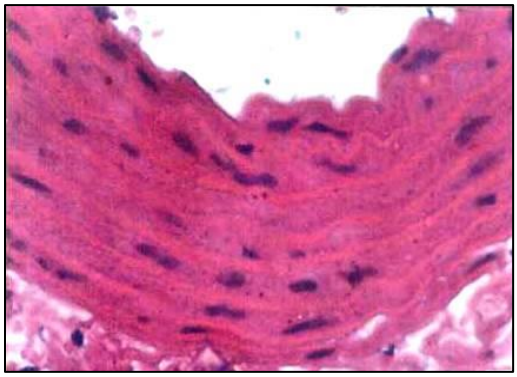

Figure I: C.S. of Dorsal Aorta of Normal Rat

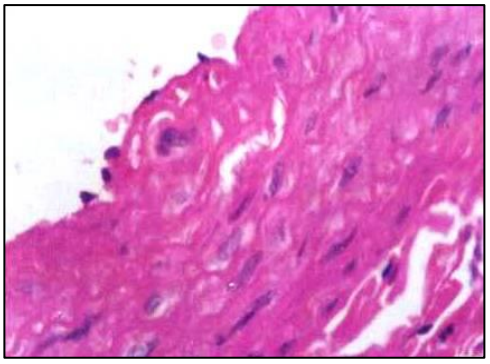

Figure II: C.S. of Dorsal Aorta of Cholesterol Fed Rat

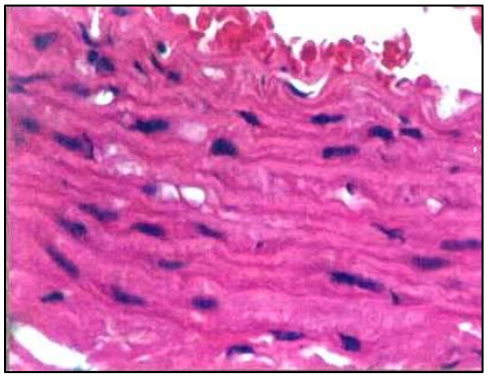

Figure III: C.S. of Dorsal Aorta of Cholesterol + *C. Fistula* Extract 100 mg/kg

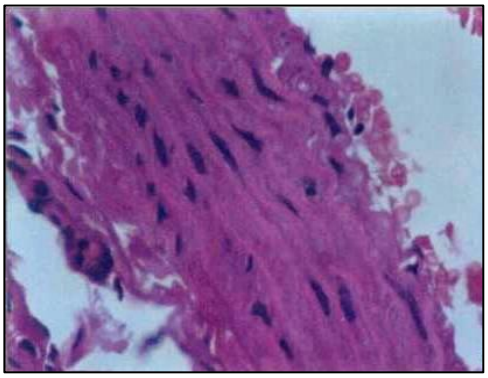

Figure IV: C.S. of Dorsal Aorta of Cholesterol + *C. Fistula* Extract 250 mg/kg

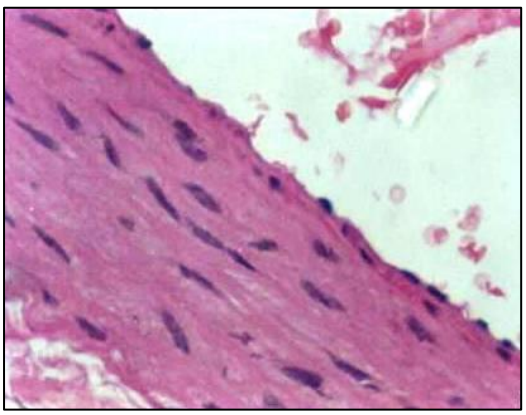

Figure V: C.S. of Dorsal Aorta of Cholesterol + *C. Fistula* Extract 500 mg/kg (H.E. X 400)

Discussion

It is well established that elevated blood lipid levels constitute a major risk factor for atherosclerosis *Cassia fistula Linn.* plant has received increasing attention for their potential role in preventing hepatotoxicity, lipid disorders and diabetic complications. In our earlier communication we have reported significant decrease in the levels of serum total cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides and phospholipids with concomitant improvement of high density lipoprotein (HDL) cholesterol/total cholesterol ratio in cholesterol fed rats receiving 50% ethanolic extract of *C. fistula* legume for 90 days. The decline in serum lipid profile might be due to hypolipidemic action of the phytoconstituent present in the extract and/or increased fecal excretion of bile salts and lipids (Gupta and Jain, 2009).

In the present study, the rats fed with cholesterol diet have shown a significant increase in aortic concentration of total cholesterol, triglycerides and phospholipids. Simultaneous administration of *C.fistula* extract along with cholesterol diet significantly prevented the rise of these lipids in aorta. These results are in agreement with earlier reports which have also shown significant increase in total cholesterol, triglycerides and phospholipids content of aorta in cholesterol/high fat diet fed rats (Mathew and Daniel, 1996; Sethupathy *et al.*, 2002; Deepa and Varalakshmi, 2005; Ntchapda *et al.*, 2015). The decline observed in tissue lipids in *C.fistula* extract treated rats may be correlated with decline of serum lipid profile. Serum lipid lowering efficacy of 50% ethanolic extract of *C.fistula* legume has already been reported in our earlier communication (Gupta and Jain, 2009). Similar to our findings, antihyperlipidemic activities of extracts of *C.fistula* fruit pulp (Tassa *et al.*, 2015), legume (El-Saadany *et al.*, 1991), seeds (Li and Guo, 2002), leaves (Sutar *et al.*, 2015) and bark (Reddy *et al.*, 2015) have also been reported by other workers.

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) in aorta. Lipid peroxidation is regarded as one of the basic mechanism of cellular damage caused by free radicals (Valko *et al.*, 2007). 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 (Ibrahim *et al.*, 1991). Hypercholestremia and the process of lipid peroxidation are believed to be critically involved in the development of atherosclerotic lesions (Loper *et al.*, 1983; Parsad, 2005). Parallel to present finding, other workers have also recorded significant increase in TBARs level in aorta of high cholesterol diet fed animals (De La Cruz, *et al.*, 1999; Ling *et al.*, 2001; Sethupathy *et al*., 2002; Deepa and Varalakshmi; 2005; Ntchapada *et al.*, 2015). The decrease observed in the levels of catalase and glutathione in aorta 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 (Lu and Chaing, 2001; Sethupathy *et al.*, 2002).

Simultaneous administration of *C.fistula* legume extract along with cholesterol diet significantly diminished the TBARs level with concomitant improvement in catalase activity and glutathione concentration in aorta. Phytochemical studies have shown that *C.fistula* legume is rich source of naturally occurring bioactive compounds like galactomanns, anthraquinone glycoside, flavonoids, polyphenols and proanthocyanides (Bahorun *et al.*, 2005; Rizvi *et al.*, 2009; Ashraf Ali, 2014). Parallel to present findings many studies carried out in rats have also suggested that different extracts of *C. fistula* exerts a definite anti-lipidperoxidative effect (Luximon- Ramma *et al.*, 2002; Manonmani *et al*., 2005; Pradeep *et al.*, 2010; Tassa *et al*., 2015). Several studies suggest that naturally occurring flavonoids and polyphenols scavenge free radicals including hydroxyl and superoxide anions, inhibit lipid peroxidation and improve lipid profile (Manach *et al.*, 2005; Salvamani *et al*., 2014).

Aorta is the main site of athermanous changes. Total cholesterol content of the aorta is good indirect measure of atherosclerotic severity in high cholesterol diet fed animals (Nielson *et al.*, 1993). The histological picture of the aorta in cholesterol fed rats showed presence of lipid laden macrophages, foam

cells, thickening of atherosclerotic lesion. These results are in agreement with earlier reports which have also indicated significant increase of total cholesterol and triglyceride in aorta consequently leading to atherosclerotic lesions (Zou *et al.*, 2005; Jain *et al.*, 2007; Ntchapada *et al.*, 2015).

It is well known that persistent hypercholesterolemia results from prolonged circulation of lipid rich lipoproteins that increases oxidative stress leading to oxidative modification of LDL to oxy-LDL. These substances are toxic to endothelial cells, resulting in lesions that stimulate monocytes and macrophages to become foam cells, eventually leading to atheroma (Kakadiya, 2009).

Simultaneous administration of *C.fistula* legume extract in cholesterol fed significantly prevented the atherogenic lesions in aorta.

This might be due to presence of polyphenolic compounds, flavonoids, authroqinone glycosides and proanthocyanidin phytoconstituents present in the extract which lowers lipid profile and improve antioxidant defense system and consequently reduce oxidative stress in aorta.

The present experimental data therefore suggest that 50% ethanolic extract of *C. fistula* legume has antiatherosclerotic and antioxidant potential in cholesterol fed Wistar rats.

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Conflict of Interest Statement

The authors declare that there is no declaration of interest to disclosure.

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