

Assessment of Antihyperlipidemic Properties of Aqueous Extract of Cassia Fistula Leaves

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ABSTRACT

Objective: To study antihyperlipidemic properties of aqueous extract of *Cassia fistula* leaves in mice.

Study Design: Experimental study.

Place and Duration of Study: This study was conducted at Pharmacology Laboratory of Islamic International Medical College, Rawalpindi, and National Institute of Health, Islamabad from February to April 2011.

Materials and Method: 40 male Balb/c mice were used which were randomly divided into 4 groups i.e. Group A, B, C and D, each group containing 10 mice. Duration of study was 8 weeks after acclimatization (1 week). Group A served as control group (received standard mice diet), Group B as disease control (received 2% cholesterol diet) and Group C & D as *Cassia fistula* groups (received 2% cholesterol diet + aqueous extract of *Cassia fistula* leaves 250 and 500 mg/kg respectively).

Results: At the end of 8 weeks, blood samples of all the mice were analyzed. In disease Group B, there was significant rise in the levels of serum total cholesterol, triglycerides and LDL-C and decline in HDL-C. In Group C and D (*Cassia fistula* groups), there was much less rise in cholesterol, triglycerides and LDL-C especially in Group D. Also, there was significant increase in HDL-C level in group D.

Conclusion: The results demonstrate that administration of aqueous extract of *Cassia fistula* leaves, along with cholesterol diet, not only prevented the rise in serum cholesterol, triglycerides and LDL-C levels but also increased the HDL-C level, demonstrating antihyperlipidemic properties of the extract.

Key Words: *Cassia fistula*, total cholesterol, serum triglycerides, HDL-C (high density lipoprotein cholesterol), LDL-C (low density lipoprotein cholesterol).

INTRODUCTION

Hyperlipidemia is the condition in which lipid or lipoprotein levels are elevated in the plasma. There is a related term "dyslipidemia" which also includes any decreased lipid levels.¹ Hyperlipidemia is the major underlying pathology of atherosclerosis, which is the leading cause of death.² Elevated LDL-C and reduced HDL-C are major risk factors for ischemic heart diseases.² HDL-C serve as a good lipid and recruits cholesterol from the vessels and inhibit the lipoproteins oxidation, so has antiatherogenic effects.³

Drugs derived from the natural resources are found to be less harmful to the human body due to biological friendliness, in contrast to the synthetic drugs.⁴ Cassia fistula is one such plant, which is called as 'Amaltas' in Pakistan and in English it is known as 'Golden shower'. Numerous parts of Cassia fistula have been verified to possess several medicine properties such as hypoglycemic, hepatoprotective, antibacterial, antifungal, antifertility, antitumor, wound healing, hypolipidemic, antipyretic, analgesic, immunomodulator, laxative and purgative properties.⁵⁻¹³ This study was conducted to find out antihyperlipidemic properties of aqueous extract of Cassia fistula leaves.

MATERIALS AND METHODS

Chemicals/Instruments: 2g Cholesterol, extra pure (Applichem, Germany), coconut oil, commercially available kits (Randox) for biochemical analysis of serum lipids, pre-coated TLC (Thin Layer Chromatography) plate, silica gel GF254, toluene, ethyl acetate, formic acid and methanol as solvent system. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid. The instruments used were incubator and centrifuge (Germany), TLC scanner III (Camag, Switzerland) with win CATS software.

Preparation of 2% Cholesterol Diet: 2% cholesterol diet was prepared at animal house of NIH. 2 grams cholesterol powder along with coconut oil, was mixed and mashed with 98 grams of standard mice diet and the prepared diet was given pellet form.^{14, 15}

Plant Materials and Preparation of the Extract: The leaves of the Cassia fistula were collected from Amaltas road, Wah Cantt. The plant was identified with the help of a botanist at National Agriculture Research Council (NARC), Islamabad. The leaves were dried in shade for 7 days and then crushed to form coarse powder.^{14, 15} Three beakers (1000ml each) were taken and 500ml distilled water was put in each beaker, with proper labeling. 100 grams of dried Cassia fistula leave powder was soaked in 500 ml of distilled water in each

beaker, with periodic stirring for 76 hours, and filtered using Whatmann filter paper no. 1 and rota sucker. The filtrate was evaporated at 55° C in hot air oven. The extract was stored in air tight bottles, protected from light and kept in refrigerator at 2-8 °C.^{16, 17} The yield of aqueous extract of Cassia fistula with respect to original dry plant material was about 25% (75g was obtained from 300g).

Standardization & Phytochemical testing of the Extract: The Cassia fistula leaves extract was tested for phytochemicals by performing thin layer chromatography and standard biochemical tests as demonstrated by Goodwill et al.¹⁸ It was found that the extract contains alkaloids, flavanols, and glycosides including flavonoids, saponins, anthraquinones and triterpenoids. In addition to these some carbohydrates, proteins and amino acids were also present.

Animals: 40 male Balb/c mice (weighing 20–40g, age 7–8 weeks) were procured for this study. They were kept in the experimental research laboratory of NIH, Islamabad.. Prior to the commencement of the experiments, all animals were kept for one week (week zero) under the same laboratory conditions, at a temperature of 22 ± 2 °C, relative humidity of 70 ± 4% and 12 hour light/day cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines.

Experimental Procedure: At the start of week 1, the mice were randomly divided into 4 groups i.e. Group A, B, C and D, each group containing 10 mice. Group A served as control group (received standard mice diet). Group B as disease control (received 2% cholesterol diet) and Group C & D as Cassia fistula groups (received 2% cholesterol diet + aqueous extract of Cassia fistula leaves, 250 & 500 mg/kg respectively).

Sample collection: At the end of week 8, blood sampling of all the mice was done. 24 hours after

administration of the last dose of extract and after overnight fasting, the animals were weighed and anaesthetized under ether vapours. A sample of 1-2ml blood was drawn through cardiac puncture from all animals. Blood was transferred to the sterile vacuotainers with gel and allowed to clot at room temperature for one hour. It was then centrifuged for 10 mins at a speed of 3000 rpm. Serum was separated and stored in sterile Eppendorf tubes at -20°C for analysis of biochemical parameters.

Biochemical Analysis: The blood drawn was assessed to measure serum total cholesterol, triglycerides, LDL-C and HDL-C, using commercially available kit (Randox, UK).

Statistical Analysis: The data obtained was analyzed using SPSS 20. All data are shown as mean ± S.E.M. One way ANOVA (Analysis of Variance) was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. P-value of <0.05 was considered as statistically significant.

RESULTS

The results indicate that as compared to Group A, in Group B there was significant rise in total cholesterol, triglycerides and LDL-C, but the HDL-C levels fall. In Groups C & D, in contrast to Group B, the Cassia fistula leaves extract not only prevents the rise in serum total cholesterol, LDL-C and triglyceride levels but also causes increase in HDL-C level in dose dependent manner. At the dose of 500mg/kg body weight, there was no significant difference in the serum total cholesterol, triglycerides and LDL-C of Groups D and control Group A, also the HDL-C levels were significantly higher in group D as compared to Group A.

Table 01: Fasting lipid profile of all groups at the end of week 8

Groups		Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Group A (N=10)	Control	91.30 ± 2.43	73.00 ± 2.39	39.00 ± 1.57	32.10 ± 1.47
Group B (N=10)	2 % Cholesterol diet	170.90 ± 2.60*	124.20 ± 2.34*	102.40 ± 1.89*	23.00 ± 1.12*
Group C (N=10)	2 % Cholesterol Diet + 250 mg/kg CF	122.20 ± 2.76**	91.90 ± 2.18**	61.10 ± 2.79**	38.10 ± 1.23**
Group D (N=10)	2 % Cholesterol Diet + 500 mg/kg CF	95.60 ± 2.78**	78.00 ± 2.00**	43.70 ± 1.48**	44.80 ± 2.17**

*p<0.05 when compared with group A (control), **p<0.05 when compared with group B

DISCUSSION

The Cassia fistula leaves extract contains alkaloids, flavanols (tannins & proanthocyanidins) and glycosides including flavonoids, saponins, anthraquinones & triterpenoids. These findings are also consistent with the findings of Panda SK et al.¹⁹ The anthraquinones have

stimulatory effect on gastrointestinal wall increasing peristalsis and decreasing the contact time between food and gut wall, decreasing cholesterol absorption. Similarly, the high fiber contents in Cassia fistula leaves might be involved in decreasing cholesterol absorption from the gut.

The tannins present in the extract might potentially inhibit the activity of lipases found in mice, inhibiting fat absorption. Previous studies using tannins from grape seed extract showed their antihypercholesteromic effects.²⁰ Furthermore, antihyperlipidemic activity of flavonoids and proanthocyanidins has been demonstrated in different studies.^{21, 22}

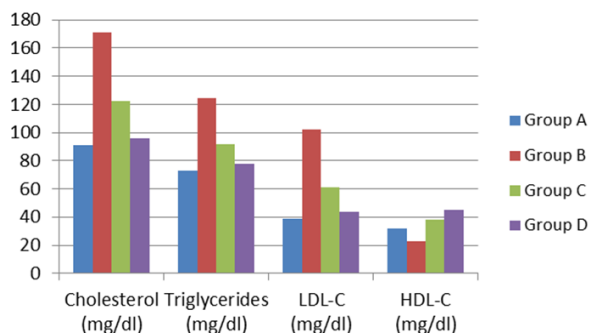


Figure No.1: Comparison of fasting lipid profile of all groups at the end of week 8

The results of this study correlate with a study done in 2009 by Gupta and Jain, in which they have demonstrated the antihyperlipidemic role of ethanolic extract of *Cassia fistula* legumes in diet induced hyperlipidemia.²³ The results of present study are also parallel with the finding of el-Saadany et al. who also observed a decrease in cholesterol and triglyceride concentrations in blood and liver of cholesterol fed rats after administration of *Cassia fistula* legume extract.²⁴ Another study performed by Christine et al. in 2011, has demonstrated weight lowering effect of methanol extract of *Cassia fistula* leaves and its possible role to be used as a hypolipidemic drug.²⁵ Some constituents of *Cassia fistula* leaves, especially flavonoids, anthraquinones and proanthocyanidins, have strong antioxidant activity, which may account for its inhibitory effects on lipid peroxidation.⁷

The dose, of the *Cassia fistula* extract, used was quite high. To reduce the dose, active principles of the extract are needed to be separated and tested for antihyperlipidemic activity, individually & comparatively, and the component with maximum activity should be selected.

CONCLUSION

The results of present study have demonstrated antihyperlipidemic properties of aqueous extract of *Cassia fistula* leaves, in dose dependent manner.

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