

Research Article

Anti Hyperlipidemic Activity of Flavonoid of *Cassia tora*

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ABSTRACT

Since the advent of modern drug treatments, traditional medicine has greatly receded in occidental societies. Moreover, only a limited number of medicinal plants have received detailed scientific scrutiny thereby prompting the World Health Organisation to recommend that this area be comprehensively investigated. *Cassia tora* Linn is used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite production being most probably responsible for the plant's beneficial effects. This study was focused to know antihyperlipidemic activity of leaves of *Cassia tora* on dietetical model. Thirty male rats are divided into five groups, each group contain six animals. First group served as a normal control, which receive the normal diet. The second group receive the high cholesterol diet, third one high cholesteric diet and standard drug atorvastatin (10mg/kg), fourth group receive high cholesteric diet and 100mg/kg extract of cassia tora, last one receive the rich fat food with 200mg/kg extract of cassia tora for 26 days. Serum levels of total proteins, albumin, (AST) and piruvic (ALT) transaminases. Total cholesterol, HDL-, LDL- VLDL- and triglycerides. Extract of leaves of *Cassia tora* treated group showed significant decrease in LDL-cholesterol, total cholesterol, triglycerides, AST, ALT,ALP, an increase in HDL-cholesterol, Albumin, Total protein, and further was conformed by histopathological studies.

Key words: *Antihyperlipidemic, atorvastatin, cassia tora. Cholesterol, diet.*

INTRODUCTION

Hypercholesterolemia is a major obligation faced by the many countries in the world. Clinical studies indicated that hypercholesterolemia is an essential risk factor for coronary artery disease (CAD), where low-density lipoprotein (LDL) cholesterol plays a major role in the atherosclerosis and pathogenesis of CAD. Several studies noted that hyperlipidemia induces oxidative stress and the oxidative modification of lipoproteins in vessel walls might play a key role in atherogenesis (Wittenstein et al., 2002). Cardiovascular problems are most common reasons for the deaths in world wide. Changes in the levels of lipoprotein and abnormalities in lipid metabolism stands for best understood risk factor for atherosclerosis and cardiovascular diseases. Roughly 10% of global populations are affected by dyslipidemia¹. Therapeutic collections for diagnosis the atherosclerosis are mostly depend on the use of statins, which inhibits the rate limiting enzyme for cholesterol biosynthesis. Presently inhibiting the intestinal cholesterol absorption by interfering with sterol transporting system repoted as novel mechanism for lowering the serum cholesterol². Statins were found in less beneficial persons having LDL receptors are low in hypercholesterolemia³. Myopathy (muscle pain), liver problems, contraindicated in pregnancy, is the most common adwers effect of statins⁴. Importance of natural products in modern medicine is increased recently. *Cassia tora* Linn.(Caesalpinaceae) is a small annual herbs or under shrub growing as common weed in Asia countries. It is found as a weed throughout

India, universally in wild state in Himachal pradesh, Bihar, and Orissa. Constituent an ayurvedic preparation "dahughnavati" which is one of the successful antifungal formulations.⁵ It is a well known ayurvedic medicinal plant as a laxative, antiperodic and is useful for leprosy, ringworm, bronchitis, and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic, haemorrhoids. It was reported that leaves of *Cassia tora* has antioxidant activity and contain many active substances including chrysofenol, emodin, rhein etc. Many medicinal properties such as antimicrobial, antihepatotoxic and antimutagenic activities have been attributed to this plant.⁶

MATERIALS AND METHODS

Acute Toxicity Test: Acute toxicity test of leaves of *Cassia tora* was done by following OPPTS guidelines by up and down method⁷.

Plant Material: Plant material – *Cassia tora* was collected from Tirupathi located in Chittoor district of Andhrapradesh, India.

Preparation of Extracts: Powdered leaves were subjected to successive extraction in a soxhlet extractor with methanol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get a constant weight.

Experimental Animals: In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220g were included for the study. Animals were housed in polypropylene cages on clean paddy husk bedding.

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Effect of flavonoid of leaves of *Cassia tora* on plasma total cholesterol, TG, Total protein, Albumin, LDL, VLDL, HDL, AST, ALT levels in cholesterol diet (CD) induced hypercholesterolemia in rats.

Treatment group	Triglycerides	Total protein	Albumin	Total cholesterol	LDL	VLDL	HDL	AST	ALP
Normal control	72.98 ± 0.344***	6.053 ± 0.190*	4.162 ± 0.159*	205.1 ± 1.628**	180.2 ± 1.565**	14.59 ± 0.06***	51.57 ± 0.64**	17.60 ± 1.720***	13.94 ± 2.023***
HCC	122.1 ± 1.081***	3.190 ± 0.276***	2.057 ± 0.152***	265.5 ± 1.120***	235.6 ± 1.133***	24.42 ± 0.216***	28.07 ± 1.742***	49.01 ± 2.347***	54.94 ± 1.387***
Atorvastatin 10mg/kg	79.55 ± 1.135	6.960 ± 0.259***	3.518 ± 0.119	217.5 ± 4.23	191.2 ± 4.076	15.90 ± 0.22	46.79 ± 0.894	29.90 ± 1.446	31.16 ± 0.641
Cassia tora 100mg/kg +HCD	94.70 ± 1.293***	5.695 ± 0.223**	2.938 ± 0.031*	247.0 ± 1.163***	220.5 ± 1.160***	18.94 ± 0.26***	37.73 ± 0.490***	41.92 ± 1.286***	51.30 ± 0.724***
Cassia tora 200mg/kg + HCD	86.20 ± 0.942***	5.397 ± 0.124***	4.157 ± 0.211*	207.5 ± 1.719*	179.3 ± 1.539**	17.24 ± 0.189***	54.88 ± 0.627***	18.37 ± 1.832***	23.16 ± 1.002***

Data was analysed using one way ANOVA followed by Dunnett's test. ***P 0.001, **P 0.01, *P<0.05, (n = 6)

Total Protein

Total protein effect in flavonoid of leaves of *cassia tora* in hypercholesteremic rats

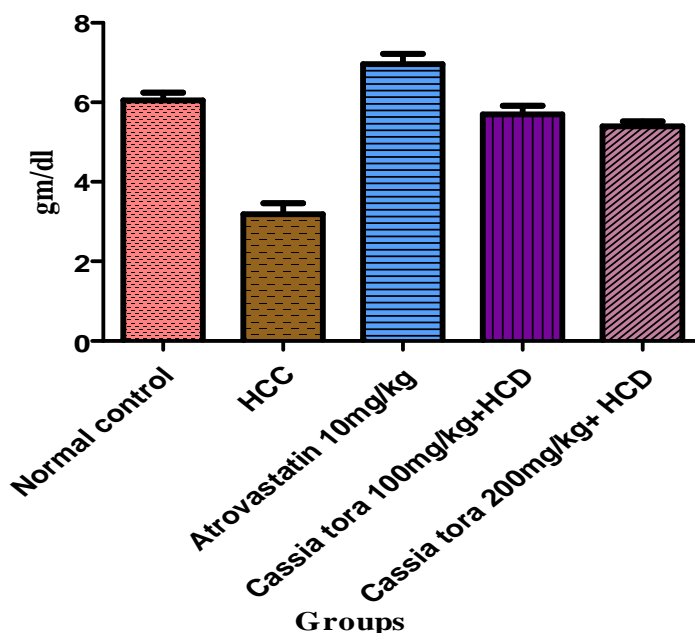


Fig 1. Rats treated with cholesterol along with flavonoid of leaves of *cassia tora* (200mg/kg) shown significant increase in total protein (P 0.001) when compared to standard group.

Animals were maintained under controlled temperature at 25°C ± 2°C with 12hr light/dark cycle. All animals will have a free access to food and water *ad libitum*.

Administration of Drug: The extract was suspended in 1% w/v CMC and the suspension was orally administered to the animals once daily for seven days. Two doses were selected depending on the toxicity profile of the extract upon the oral treatment

METHODOLOGY

Cholesterol – diet induced hypercholesterolemia

Male Wistar rats are divided into 5 groups of six animals each.

Group1: Normal control

Group2: Hyperlipidemic control

Group3: Animals are treated with atorvastatin (10mg/kg)

Group4: Animal are treated with test drug (100mg/kg)

Group5: Animal are rats treated with test drug (200mg/kg)

Atherogenic diet was suspended in coconut oil 25%, cholesterol 1%, and cholic acid 0.5%. The atherogenic

Triglycerides

TG in effect of flavonoid of leaves of *cassia tora* in hypercholesteremic rats

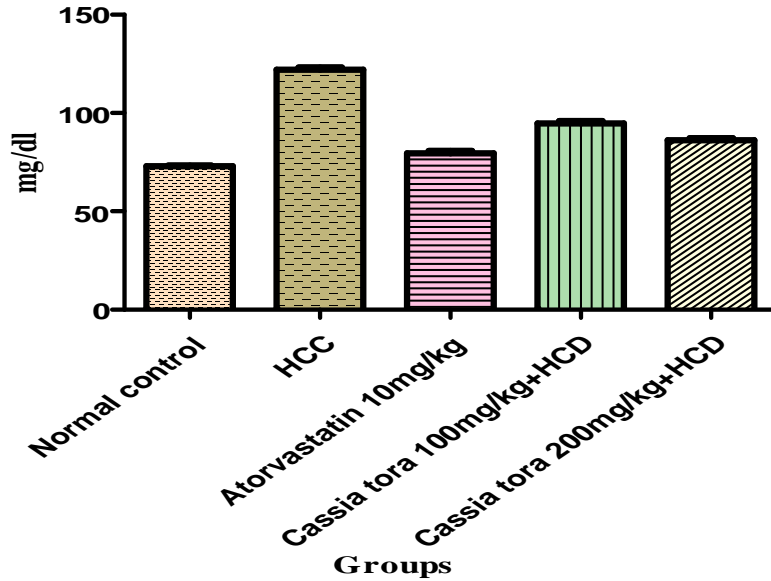


Fig 2.All rats treated with high cholesterol diet and flavonoid of leaves of *cassia* (200mg/kg, b.w. p.o) respectively showed significant decrease ($P<0.001$) in triglycerides when compared with standard group.
HDL- Cholesterol

HDL effect in flavonoid of leaves of *cassia tora* in hypercholesteremic rats

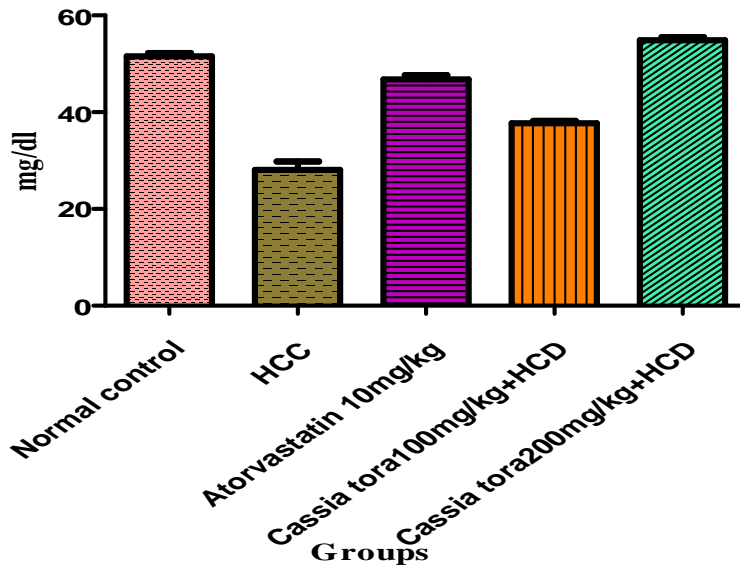


Fig 3.All rats treated with high cholesterol diet and flavonoid of leaves of *cassia tora* (200mg/kg, b.w. p.o) respectively showed significant increase ($P<0.001$) in HDL-cholesterol when compared with standard diet group

diet and the treatment were given simultaneously for 26 days. On the 27th day, blood was collected by retro orbital puncture for the analysis of serum triglycerides, cholesterol, HDL, VLDL and glucose ⁸. A portion of the aorta was fixed in formalin (10%) and subjected to histopathology studies⁹. Parameters Evaluated: Serum levels of total proteins, albumin, creatinin, glucose, urea

and oxalacetic (AST) and piruvic (ALT) transaminases. Total cholesterol, HDL-, LDL- and VLDL-c and triglycerides. Body weight, food efficiency, liver weight and abdominal and liver fat of the rats⁹. Statistical analysis was carried out by one way ANOVA method.

LDL- Cholesterol

LDL effect in flavonoid of leaves of cassia tora in hypercholesteremic rats

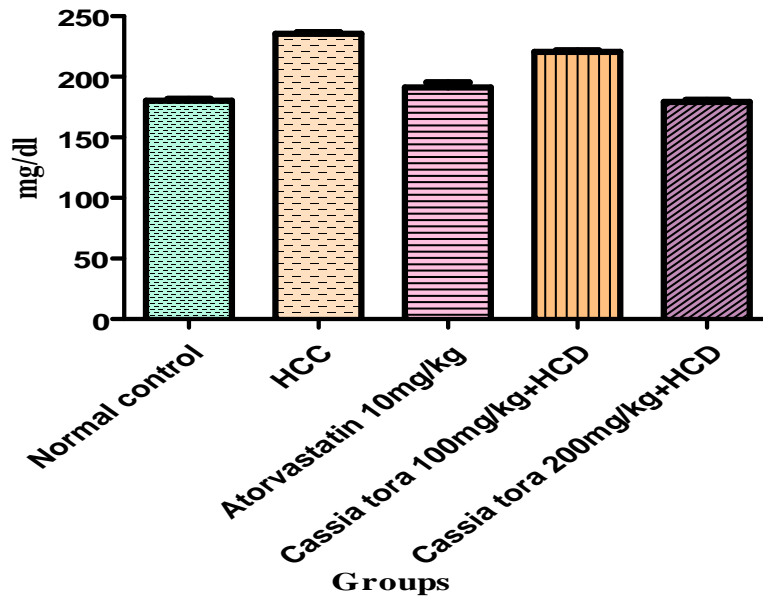


Fig 4 All rats treated with high cholesterol diet and flavonoid of leaves of cassia tora(200mg/kg, b.w. p.o) respectively showed significant decrease ($P<0.05$) in LDL-cholesterol when compared with cholesterol diet group.
VLDL- Cholesterol

VLDL effect in flavonoid of cassia tora in hypercholesteremic rats

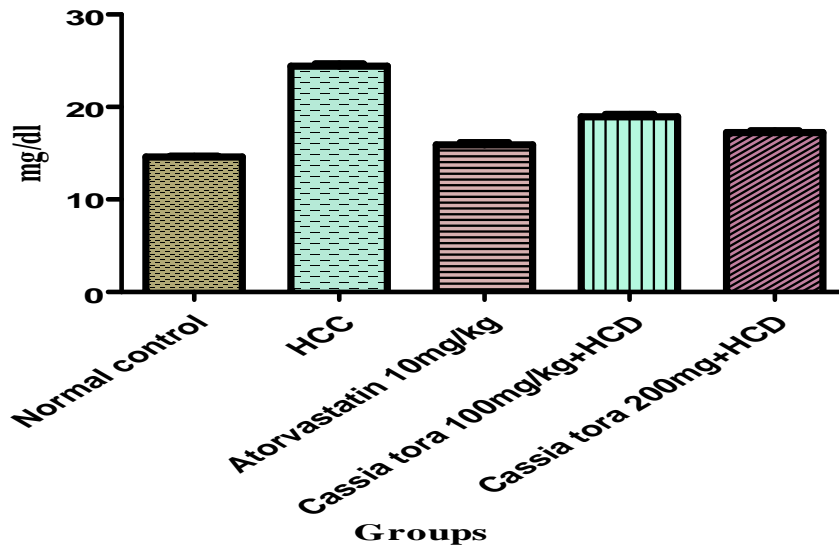


Fig 5 All rats treated with high cholesterol diet and flavonoid of leaves of cassia tora(200mg/kg, b.w. p.o) respectively showed significant decrease ($P<0.001$) in VLDL-cholesterol when compared with cholesterol diet group.

RESULTS AND DISCUSSIONS

In this study, rats fed with High Cholesterol Diet (cocktail) containing 700ml peanut oil and 300ml lard oil, 100g cholesterol, 30g propyl-thio-uracil and 100g cholic acid was given orally 1ml/100g body weight Cholic acid acts by increasing cholesterol absorption by its emulsifying property and concomitant suppression of cholesterol 7 -hydroxlyase activity that results in

decreased cholesterol excretion. The use of propyl-thio-uracil to create hypothyroidism. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the hypercholesterolemia- related metabolic disturbances in animals. Cholesterol feeding alone however does not affect the serum triglyceride (TG) level. It is assumed that a high level of saturated fat in addition to cholesterol is required to significantly elevate

Albumin

Albumin effect in flavonoid of leaves of cassia tora in hypercholesterimic rats

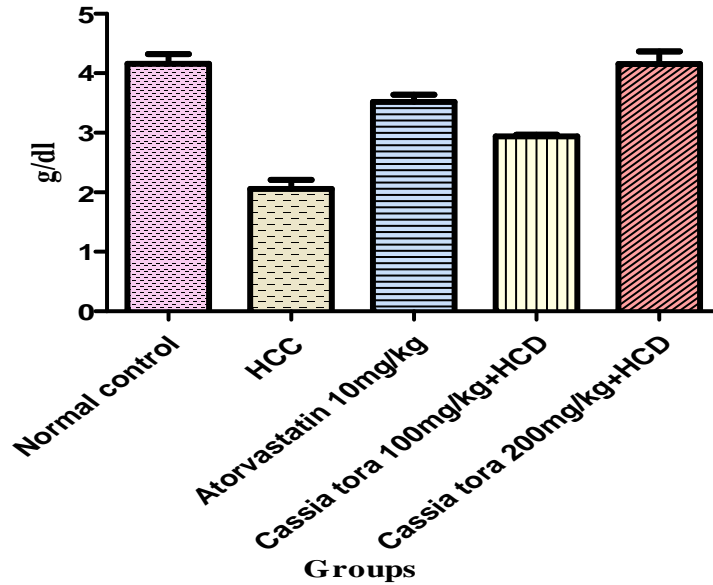
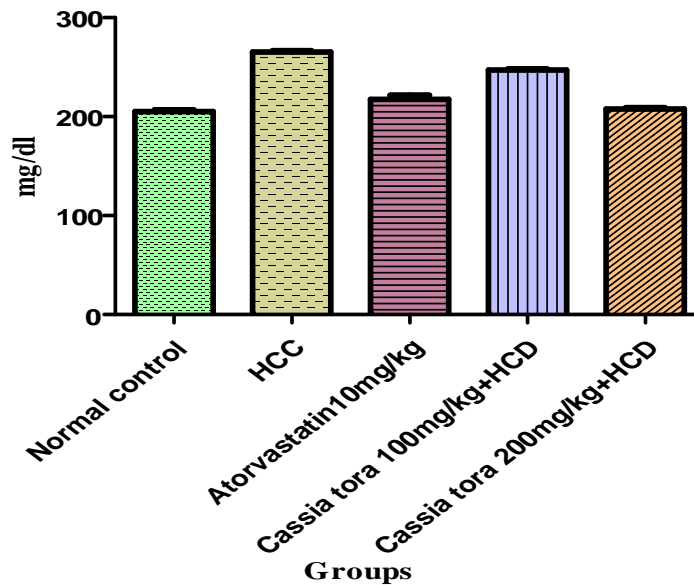


Fig 6 All rats treated with high cholesterol diet and flavonoid of leaves of cassia tora (200mg/kg, b.w. p.o) respectively showed significant increase ($P < 0.05$) in albumin when compared with standard diet group. Total Cholesterol

Cholesterol effect in flavonoid of leaves of cassia tora in hypercholesteremic rats



All rats treated with high cholesterol diet and flavonoid of leaves of cassia tora (200mg/kg, b.w. p.o) respectively showed significant decrease ($P < 0.01$) in total cholesterol when compared with cholesterol diet group.

serum TG level in rat model. Rats are generally considered to be resistant to atherogenesis, although lesions have been produced by heroic measures. The high cholesterol diet (CD) was given for 26 days along with methanolic extract of leaves of *cassia tora*. Cholesterol diet (CD) treated group showed significant increase in total cholesterol triglycerides, LDL-c (265.5

± 1.120 , 122.1 ± 1.081 , 235.6 ± 1.133) levels respectively whereas HDL-c (28.07 ± 1.742) level showed significant decrease when compared to the control TG, TC, LDL-c (72.98 ± 0.344 , 205.1 ± 1.628 , 180.2 ± 1.565) group. On 27th day, atorvastatin treated group showed significant decrease in total cholesterol, triglycerides, LDL-c (217 ± 4.24 , 79.55 ± 1.13 , 191.2 ± 4.07) levels where as HDL-c

AST

AST effect in flavonoid of leaves of *cassia tora* in hypercholesteremic rats

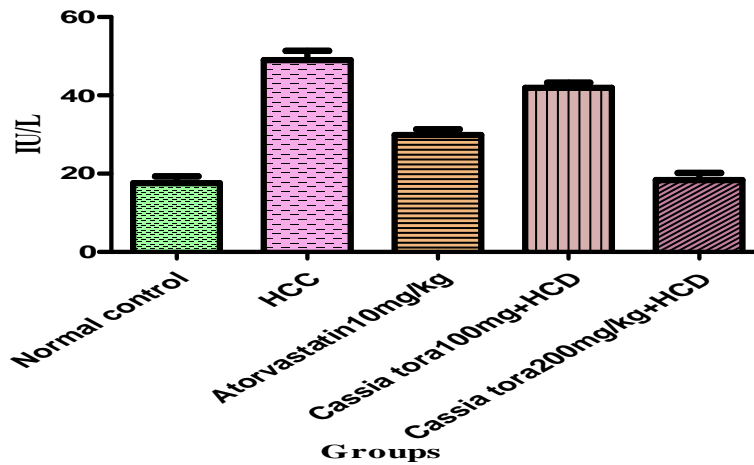


Fig 7 All rats treated with high cholesterol diet and flavanoid of leaves of *cassia tora*(200mg/kg, b.w. p.o) respectively showed significant decrease ($P<0.001$) in AST when compared with cholesterol diet group
ALT

ALT effect in flavonoid of leaves of *cassia tora* in hypercholesteremic rats

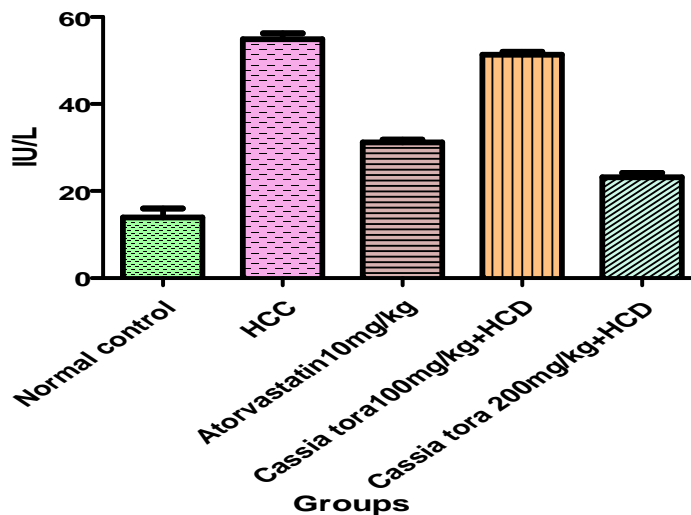


Fig 8 All rats treated with high cholesterol diet and flavanoid of leaves of *cassia tora*(200mg/kg, b.w. p.o) respectively showed significant decrease ($P<0.01$) in ALT when compared with cholesterol diet group.

Histopathology of hcc Normal control

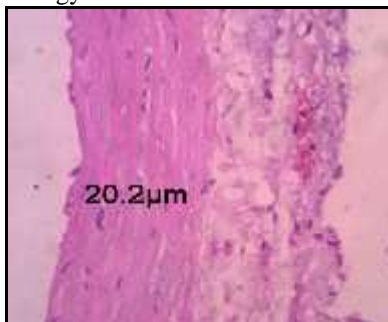


Fig 9 The layers of artery appear intact. The tunica intima, tunica media and tunica adventitia appear within normal limits. The Tunica intima - media thickness – 20.2µm

(46.79 ± 0.894), level was significantly increased when compared to the CD treated group.

On 27th day, rats treated with pure compound of leaves of *cassia tora* (100mg/kg, b.w. p.o) treated group showed significant decrease in total cholesterol, triglycerides, LDL-c (247.0 ± 1.163 , 94.70 ± 1.29 , 220.5 ± 1.160) levels respectively whereas HDL-c (37.73 ± 0.490) level showed significant increase when compared to the CD (28.07 ± 1.742) treated group. And the results were comparable with that of the standard drug Atorvastatin.

On 27th day, rats treated with high CD and flavonoids of leaves of *cassia tora* (200mg/kg, b.w. p.o) treated group showed significant decrease in total cholesterol, triglycerides, LDL-c (207.5 ± 1.79 , 86.20 ± 0.942 , 179.3 ± 1.539) levels respectively whereas HDL-c (54.88 ± 0.627) level was significantly increased when compared

Hyperlipidemic control

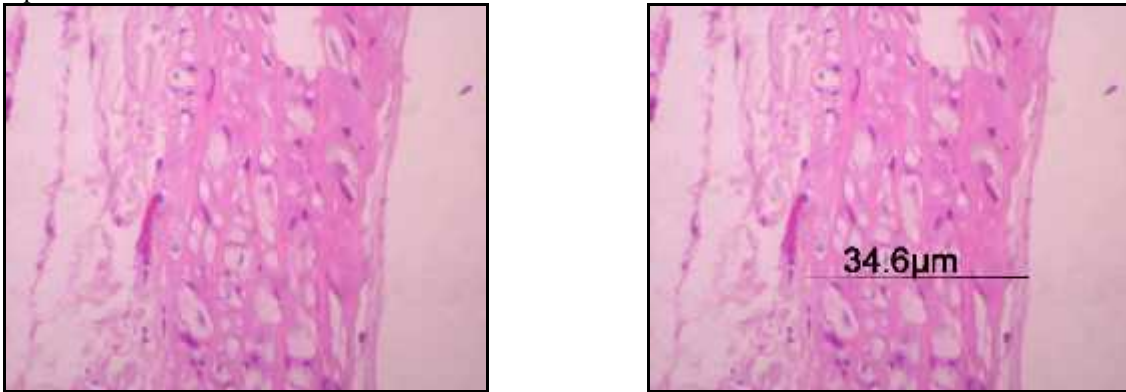


Fig 10 The Tunica intima and endothelium appears disrupted. Within the tunica media and beneath the intima are seen aggregates of lipid containing macrophages (Fig.2, arrow), cholesterol deposits and lipid containing smooth muscle cells. The Tunica intima - media thickness – 34.6μm.

Atorvastatin + high cholesterol diet

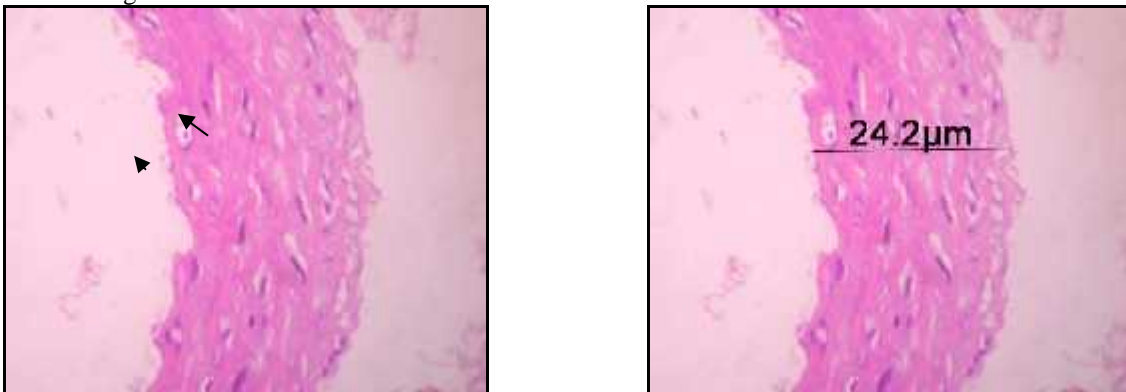


Fig 11 The layers of artery appear intact. The tunica intima, tunica media and tunica adventitia appear within normal limits. Within the tunica intima are seen few scattered foamy macrophages (Fig.2, Arrow). The Tunica intima - media thickness – 24.2μm.

Cassia tora 100 mg/kg

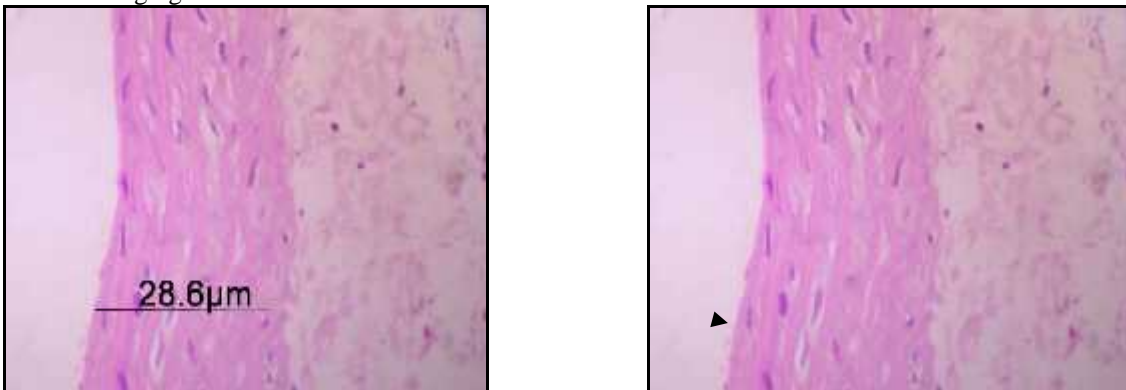


Fig 12 The layers of artery appear intact. The tunica intima, tunica media and tunica adventitia appear within normal limits. Within the tunica intima are seen few scattered foamy macrophages (Fig.2, Arrow). The tunica intima - media thickness – 28.6μm.

to the cholesterol diet treated group. And the results were comparable with that of the standard drug Atorvastatin. Histopathological study was done there was increase in the size of the tunica intima in all CD treated groups in 27th day aorta of all rats. There was reduction in the thickness of the wall of aorta in flavonoids of leaves of *cassistora* (100gm/kg, 200mg/kg).

CONCLUSION

From the experimental studies carried out, flavonoid of leaves of cassia tora at two different administered doses (100mg/kg and 200mg/kg) showed dose dependent antihyperlipidemic activity. The higher dose 200mg/kg showed significant protection compared to lower dose 100 mg/kg. These results were further substantiated with histopathological results. The anti- hyperlipidemic

Cassia tora 200 mg/kg

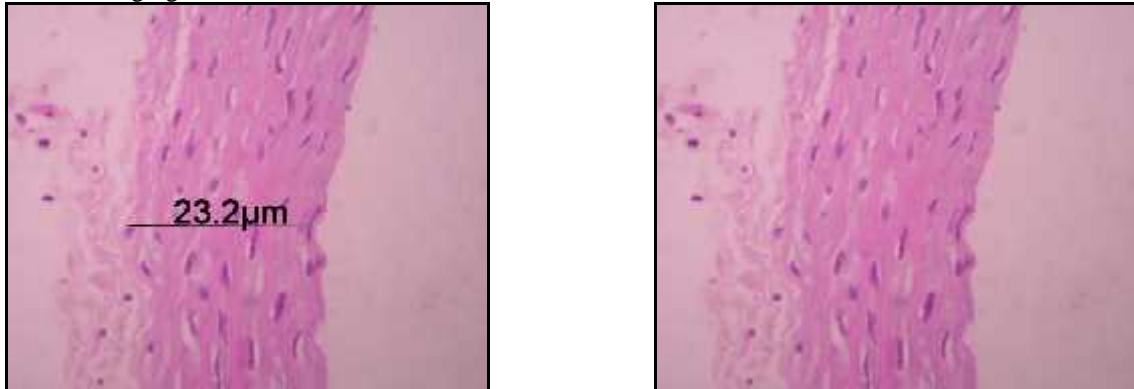


Fig 13 The layers of artery appear intact. The tunica intima, tunica media and tunica adventitia appear within normal limits. The tunica intima - media thickness – 23.2µm.

activity of *cassia tora* may be due to presence of flavonoid, and this requires further investigation. Thus from above results it can be concluded that flavonoid of *cassia tora* has significant anti hyperlipidemic activity.

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