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Research Article

HYPOLIPIDEMIC AND ANTIATHEROSCLEROTIC EFFECTS OF *PROSOPIS CINERARIA* BARK EXTRACT IN EXPERIMENTALLY INDUCED HYPERLIPIDEMIC RABBITS

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ABSTRACT

Dietary antioxidants and flavonoids like phytochemicals occurred in several herbs have potential to improve cardiovascular health. *Prosopis cineraria* (Fabaceae) is also widely uses on above basis for traditional therapeutic purposes. It is the Thar Desert prominent tree. This study evaluated the hypolipidemic and antiatherosclerotic effects of *Prosopis cineraria* bark extract in hyperlipidemic rabbits. The rabbits were made to induce exogenously hyperlipidemic through orally administration of high fat diet and cholesterol powder (500mg/Kg body weight per day in 5 ml of coconut oil orally for 15 days). The induced hyperlipidemic rabbits were treated comparatively by bark extract of *Prosopis cineraria* and standard drug. The administration of *Prosopis cineraria* bark extract (70% ethanol) significantly ($P \leq 0.001$) reduced serum total cholesterol (88%), LDL-C (95%), triglyceride (59%), VLDL-C (60%) and also ischemic indices (Total cholesterol/LDL-C and LDL-C/HDL-C). The *Prosopis cineraria* bark extract also significantly ($P \leq 0.001$) prevented the atherogenic changes in aorta. Toxicity profile parameters were also examined and remained under normal ranges. Results indicated that *Prosopis cineraria* bark has hypolipidemic and antiatherosclerotic efficacy along with non-toxic nature.

Keywords: Hyperlipidemia, atherosclerosis, *Prosopis cineraria*, cholesterol

INTRODUCTION

Atherosclerosis is a complex disease that characterized by an excessive inflammatory, fibro-fatty, proliferative response to damage of arterial wall through involving several cells types *i.e.* particularly smooth muscle cells, monocyte derived macrophages, T-lymphocyte and platelet[1]. Landmarks clinical studies have been demonstrated that lowering elevated total cholesterol and low-density lipoprotein cholesterol (LDL) significantly reduces the risk of coronary events, stroke and death in both primary and secondary coronary prevention in patients[2, 3]. Data from epidemiological studies support that potential of dietary antioxidants and flavonoids that are presents in several herbs to improve cardiovascular health[4]. *Prosopis cineraria* (Fabaceae) is commonly known as Khejari or Jandi and also known as Kalpvariksha or boon tree for its myriad virtues[6]. *Prosopis cineraria* is mostly occurs in arid and semi-arid regions and also Known as state tree of Rajasthan[7, 8]. Ancient literature has been also reported that the use of *Prosopis cineraria* as folk medicine for various ailments[8, 9]. Available references and informations indicated that *Prosopis cineraria* may have potential use as an antiatherosclerotic agent. In this study, aimed to explore the hypolipidemic and antiatherosclerotic efficacy of *Prosopis cineraria* bark extract based on serum biochemistry, planimetric studies and histopathological observations.

MATERIAL AND METHODS

Animals

Male New Zealand white rabbits were used as atherogenic animal models. Weight and age of animals were 1.25-1.50 Kg. and 10-12 months, respectively. Animal were kept in standard environmental conditions as cyclic darkness and lightness 12 hrs periods in metallic wire gauges with ample space. The food was also supplemented with green leafy and seasonal vegetables and water *ad libitum*. University departmental ethical committee approved the experimental protocol.

Preparation of extract

The selected plant *i.e. Prosopis cineraria* was identified by experts of botany department, J.N.V.University, Jodhpur. For this selected plant part *i.e.* bark was collected from in and around university campus. This herbal part was allowed to shade dry. Dried bark was coarsely powdered and extracted in 70% ethanol at 70-80°C for 18 h through soxhlation process. The extract was filtered and evaporated to dryness under low temperature and reduced pressure. The crude extract was used for experimentation.

Induction of hyperlipidemia

The hyperlipidemic condition was induced by high fat diet and cholesterol power administration orally with coconut oil in proportion to 500 mg / Kg body weight per day for 15 days. The high fat diet comprised wheat flour base with addition of milk power, dried egg yolk, hydrogenated fat, butter, dried yeast, salt, sugar and vitamins mixture to produce standard proportion.

Standard drug and extract dose

Atrovastatin was used as a standard hypolipidemic drug that was orally administered at the dose of .25mg /Kg body weight per day dissolved in 5 ml. distilled water. The dose of *Prosopis cineraria* bark extract was estimated by LD_{50}30 test.

Autopsy schedule

After completion of 60 days experiment period, the overnight fasted animals were sacrificed under protocol conditions. Blood was collected directly from cardiac puncture; serum was separated and stored at -20^0C for serum biochemistry. Liver, heart, kidney and aorta were removed, cleaned and weighed. The removed organs were fixed in 10% formalin solution for histopathological observations.

Experimental design

Adult rabbits were divided in to four groups as following;-

Group (A):- Vehicle control (60days)

Group (B):- Hyperlipidemic control (60days for atherodiet +Cholesterol powder 500 mg / Kg Body Weight per day)

Group (C):- Cholesterol feeding (500 mg/ Kg BW per day) for 15 days + *Prosopis cineraria* bark extract for 45 days (500 mg / Kg BW per day)

Group (D):- Cholesterol feeding (500 mg/ Kg BW per day) for 15 days + Statins (.25 mg / Kg BW per day) for 45 days.

Serum biochemistry

The total cholesterol (TC), triglyceride (TG), LDL-Cholesterol, HDL-Cholesterol, atherogenic index, ischemic indices and toxicity profile were estimated from serum samples.

1. Total Cholesterol: - Determined by an enzymatic method through use of commercial kit (GPO-POD; Centrionic Gmbh -Germany).
2. Triglyceride: - Commercial triglyceride test kit was used (GPO-POD; Centrionic GmbH Germany).
3. HDL-Cholesterol: - Ana mol (Pvt. Ltd, India) commercial kit was used.
4. LDL-Cholesterol and VLDL cholesterol: - LDL- Cholesterol and VLDL cholesterol were calculated by the Friedewald equation[10].

LDL = TC- HDL – VLDL
Where VLDL = TG / 5

TC / HDL and LDL / HDL were also calculated as Ischemic indices.

Statistical analysis

All the biochemical, toxicity profile, body weight and planimetric values were expressed as mean ± SEM and analyzed statistically using unpaired student's test.

RESULTS

Lipid profile

In hyperlipidemic rabbits, there were observed sixteen folds significantly (P≤0.001) increased levels of total cholesterol and LDL-cholesterol. HDL-cholesterol was not significantly influenced. After treatment of *Prosopis cineraria* bark extract for 45 days, there were observed significant (P≤0.001) reductions in total cholesterol level (88%) as well as in LDL –cholesterol level (95%). The triglyceride and VLDL levels were also reduced respectively up to 59% and 60% significantly (P≤0.001).Total cholesterol/LDL-cholesterol and LDL-cholesterol/HDL-cholesterol were also decreased. Comparatively, statins treatment was also showed reductions near up to control in lipid profile values (Table II).

Toxicity Profile

Toxicity profile parameters studies revealed that administration of *Prosopis cineraria* bark produced no adverse effects on general behavior and metabolism. No significant changes were observed in levels of SGPT and SGOT in treated groups. Blood urea and creatinine were also remained in normal ranges (Table III).

Planimetric studies

A well developed sized atherosclerotic plaque was observed in aorta of cholesterol fed rabbits that covered lumen volume up to 36.59 ± 1.01 (P≤0.001). After treatment of *Prosopis cineraria* bark extract, there were observed reductions in plaque area up to 75.76%, wall thickness 27.49%, intima area 16.13% and lumen volume increased up to 37.89 %. Whereas, statin treated groups showed no plaque like entity that was similar to vehicle control group (Table IV).

Body weight

Body weight and organs weights were not significantly fluctuated except liver and aorta (Table I).

Table I: Body and organs weights of *Prosopis cineraria* bark extract treated intact rabbits

Treatment Group	Body weight (Kg)		Liver	Heart	Kidney	Aorta
	Initial	Final	gm / Kg body weight			
1. Vehicle Control (Gr. A)	1.52±0.11	1.54±0.12	26.34±1.35	2.12±0.18	6.74±0.41	0.16±0.05
2.Hyperlipidemic control (Gr. B)	1.62±0.10	1.45±0.17	40.01±1.35	2.85±0.12	7.23±0.48	0.41±0.17
3.*Prosopis cineraria* Bark Extract (Gr. C)	1.58±0.18	1.48±0.25	30.27±1.47	2.38±0.22	7.41±0.62	0.30±0.11
4.Statin (Gr. D)	1.53±0.17	1.45±0.06	25.76±1.32	2.57±0.14	6.67±0.32	0.35±0.12

Gr. B, C and D were compared with Gr. A and Gr. C & D were compared with Gr.B
P ≤ 0.05= a , P ≤ 0.01 = b
P ≤ 0.001 = c , Nonsignificant= d
P ≤ 0.05 = e, P ≤ 0.01 = f
P ≤ 0.001 = g, Nonsignificant= h

Table II: Lipid Profile of *Prosopis cineraria bark* extract treated intact rabbits (Mean of 5 Values ± SEM)

Treatment groups	CHO. (mg/dl.)	TG. (mg/dl.)	HDL-C (mg/dl.)	LDL-C (mg/dl.)	VLDL (mg/dl.)	CHO/HDL	LDL/HDL
Vehicle Control (Gr. A)	89.48±6.25	101.40±8.23	32.31±1.3	37.6±5.1	20.4±1.2	2.98±0.02	1.62±0.18
Hyperlipidemic control (Gr. B)	1523.59±83.66[c]	366.72±15.23[c]	31.25d±1.2[d]	1499.41±6.50[c]	73.37±2.38[c]	52.77±0.99[c]	49.29±0.02[c]
Prosopis cineraria Bark Extract(Gr.C)	177± 10.34[a,g]	147.2± 9.56[a,h]	29.0±2.63[d,h]	85±5.83[b,g]	29.4±2.42[a,g]	6.1±0.44[a,g]	4.41±0.17[b,g]
Statin (Gr. D)	84.49±4.79[d,g]	95.86±07.72[d,g]	29.79±1.58[d,h]	35.67±4.43[d,g]	20.85±2.33[d,g]	2.81±0.14[a,g]	1.51±0.42[d,g]

Gr. B, C and D were compared with Gr. A and Gr. C & D were compared with Gr. B
P ≤ 0.05 = a , P ≤ 0.01 = b
P ≤ 0.001 = c , Nonsignificant= d
P ≤ 0.05 = e , P ≤ 0.01 = f
P ≤ 0.001 = g , Nonsignificant= h

Table III: Toxicity profile parameters of *Prosopis cineraria* bark extract treated intact rabbits (Mean of 5 Values ± SEM)

Treatment Group	B. Urea mg/dL	S. Creatinin mg/dL	SGOT IU/mL	SGPT IU/mL	T. Protein mg/dL
Vehicle Control (Gr.A)	33.46±2.50	1.20±0.06	64.85±6.85	85.16±8.40	7.32±0.04
Hyperlipidemic Control (Gr. B)	31.12±3.08[d]	1.11±0.05[d]	104.00±10.21[c]	109.16±4.01[d]	7.40±0.13[d]
Prosopis cineraria Bark Exract (Gr. C)	36.25±3.56[d]	1.04±0.03[d,h]	63.1±5.89[d,e]	62.55±7.79[d,h]	6.9±1.08[d,h]
Statin (Gr.D)	30.11±3.34[d,h]	1.33±0.17[d,h]	104.78±10.14[b,h]	93.48±1.37[d,h]	6.96±0.02[d,h]

Gr. B, C and D were compared with Gr. A and Gr. C & D were compared with Gr.B

P ≤ 0.05= a , P ≤ 0.01 = b
P ≤ 0.001 = c , Nonsignificant= d
P ≤ 0.05 = e , P ≤ 0.01 = f
P ≤ 0.001 = g , Nonsignificant= h

Table IV: Planimetric dimensions of ascending aorta of *Prosopis cineraria* bark extract treated intact rabbits (Mean of 5 Values ± SEM)

Treatment Groups	Total Wall Area	Lumen	Intima	Plaque	Media	Adventitia
	----------% of Total Area----------					
Vehicle Control (Gr.A)	49.42± 1.99	50.74 ± 1.44	9.62 ± 0.19	Nil	28.0 ± 0.8	11.34 ± 0.02
Hyperlipidemic control (Gr.B)	68.16 ± 4.61[a]	31.27 ± 2.52[a]	10.06 ± 0.49[d]	36.59 ± 1.01[c]	14.23 ± 0.66[c]	10.11 ± 0.13[a]
Prosopis cineraria Bark Extract (Gr.C)	57.35±5.11[a]	43.12 ± 3.23[a,e]	10.89 ± 0.66[a,h]	8.89 ± 1.38[a,g]	24.42 ± 2.07[d,g]	13.39 ± 0.42[a,g]
Statins (Gr.D)	50.69 ± 1.02[d,e]	49.93 ± 2.44[d,e]	11.66 ± 0.39[a,h]	Nil	28.01 ± 1.84[b,g]	10.49 ± 1.34[c,g]

Gr. B, C and D were compared with Gr. A and Gr. C & D were compared with Gr.B
P ≤ 0.05 = a , P ≤ 0.01 = b
P ≤ 0.001 = c , Nonsignificant= d
P ≤ 0.05= e , P ≤ 0.01 = f
P ≤ 0.001 = g , Nonsignificant= h

The histological investigations of aorta of cholesterol fed rabbits showed a well developed sized atherosclerotic plaque (containing foam cells and extra lipid content) and reduction was observed through *Prosopis cineraria* bark extract treatment, as planimetric statements (Table IV) and microphotographic figures (Fig.2 and 3).

AORTA (x100 HE)

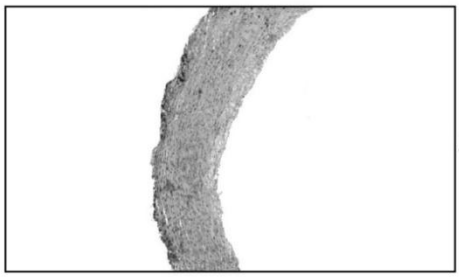

Fig. 3 : Atherodiet + *P. cineraria* Bark Extract (Gr. C)
Microphotograph of P.cineraria bark treated aorta showing reduction in plaque area

AORTA (x100 HE)

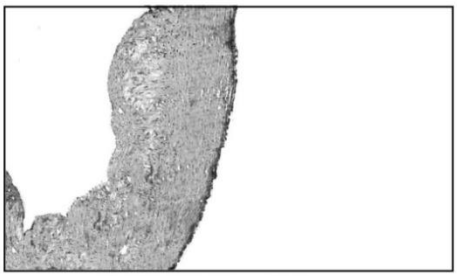

Fig. 2 : Hyperlipidemic Control (Gr. B)
Microphotogragh of aorta exibiting atherosclerotic lesion with thickened intima contained foam cells and extracellular lipid deposition

Statin treatment group and vehicle control were not showed any plaque like entity in aortas (Fig.4 and 1). Treatment of *Prosopis cineraria* bark extract also revealed improvements in histoarchitectures of liver and kidney.

AORTA (x100 HE)

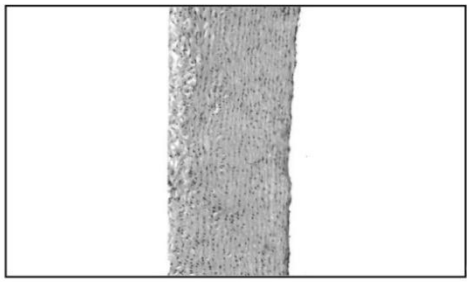

Fig. 1 : Intact Vehicle Control (Gr. A)
Microphotograph of vehicle control aorta exhibiting normal histology with aortal wall consists of intima, media and adventitia

AORTA (x100 HE)

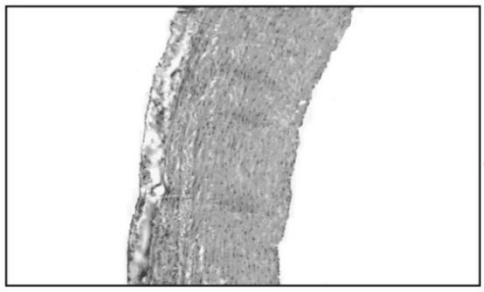

Fig. 4 :Atherodiet + Statins
Microphotograph of statin treated aorta exhibiting normal histology simillar to vehicle control aorta

DISCUSSION

Hypercholesterolemia plays a key role in the development and progression of atherosclerosis and is a prone risk factor for coronary heart disease[16, 17]. Therapeutic interventions aimed to lowering cholesterol level both in primary and secondary prevention show a clear reduction in the incidence of CHD and stroke. The agents that can lower serum cholesterol and scavenger or inhibit free radicals formations have gained wide therapeutic values [20, 19, 12]. Cholesterol administration caused a significant increase in the serum total cholesterol, LDL- cholesterol, VLDL – cholesterol and the ischemic indices (Total cholesterol / HDL- cholesterol and LDL- cholesterol / HDL - cholesterol). Above induced hyperlipidemic status explained that supplementation of cholesterol in diet significantly results in a marked increase in the production of cholesterol and cholesterol esters rich - VLDL cholesterol by the liver and intestine.[13, 14, 25,5] Consequently, serum level of LDL- cholesterol was increased that is independent indication of atherosclerosis.[23,3] A significant increased in ischemic indices (Total cholesterol / HDL- cholesterol and LDL- cholesterol / HDL - cholesterol) indicate atherosclerosis and coronary heart disease.[24] In this study, administration of *Prosopis cineraria* bark extract showed a significant decrease in total cholesterol, LDL-cholesterol, VLDL cholesterol and triglyceride indicating beneficial

modulatory influence on cholesterol metabolism and turnover reduction in ischemic indices (Total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol) in treated group. This may be through increase reverse cholesterol transport from peripheral tissues[25, 11] or inhibition of HMG-reductase[26, 15]or inhibition of cholesterol absorption at intestinal level mechanisms.[21,22,17] Correspondingly, the atherosclerotic plaque area, intima of layer of vascular wall were tended to decreased through treatment of bark extract of *Prosopis cineraria* .These results may be through depletion of deposited lipid content from peripheral tissues by reverse cholesterol transport and inhibit foam cell formation through interactions of containing photochemicals or herbal compounds of *Prosopis cineraria* bark.[20] Cholesterol feeding caused a considerable accumulation of cholesterol content in liver and aorta tissues therefore weight of liver and aorta were increased but other organs not directly influenced.[23] It can be illustrated through results that *Prosopis cineraria* bark extract has efficacy to prevent atherosclerotic plaque formation as well as hypolipidemic potential in cholesterol fed rabbits.

CONCLUSION

Finally, it can be concluded through the results of this study that bark extract of *Prosopis cineraria* has hypolipidemic nature as well as efficacy to prevent atherosclerotic plaque formation in cholesterol fed rabbits along with non-toxic effects. Thus, the study is required further validation through fractionations and compound separation from extract and human subjects to prove its clinical efficacy as a hypolipidemic as well as an antiatherosclerotic agent.

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