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Research Article

HISTOLOGICAL STUDY OF ANTIATHEROSCLEROTIC EFFECT OF PROPOLIS IN INDUCED HYPERCHOLESTROLEMIC MALE ALBINO RABBITS

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ABSTRACT
Propolis is bee exudates that found at the opening of chambers of bee hives. This study was aimed to investigate the antiatherosclerotic effect of propolis in hypercholesterolemic rabbits. Twenty four male New Zealand white rabbits were divided into four groups *i.e.* control (vehicle and hyperlipidemic) and treated (Propolis and statin). The vehicle control group (Gr.1) was fed with normal diet and hyperlipidemic control group (Gr-2) was fed with high fat diet and cholesterol powder (500 mg/ Kg Body weight per day mixed with coconut oil orally for 15 days). Propolis treated group (Gr.3) was administered with 25 Mg/Kg body weight of propolis as drug and statin treated group (Gr.4) was administered with 0.25 Mg/ Kg body weight per day of statin as standard drug for 45days of supplementation. After completion of experiment, the animals were autopsied under prolonged ether anthesia and the aortic tissues were taken for histological study. In hypercholestrolemic rabbits, the high fat diet and cholesterol powder caused focal atherosclerotic fatty streak lesions. The treatment of propolis (Gr-3) showed significant reduction in fatty streak up to 78.76% and in similar proportion aortic lumen was increased. The observations also showed reductions in foam cells and deposited lipid content through treatments of propolis and statin. Thus the conclusion can be drawn that administration of Propolis as a crude drug has protective effect against atherosclerosis.

Key Words: *Hyperlipidemia, Atherosclerosis, Propolis, Plaque.*

INTRODUCTION
Atherosclerosis is the major cause of morbidity and mortality in the developing and developed countries (Stocken and Keaney, 2004). The magnitude of this problem is profound as atherosclerosis claims more lives than all types of cancer combined and the economic costs are considerable. Today herbal medicines and nutraceuticals obtained by nature directly has grown in popularity all over the world, especially in developing countries, because of absence of adverse effects and cost effectiveness (Ernest, 1998). Propolis is a complex resinous mixture collected by honeybees from buds and exudates of certain plant sources neighboring its hives. Propolis consisting of sap, bark and bee excreta accumulates in bee hives. The chemical consistency of propolis is highly dependent on the flora of the region from where it is collected (Marcucci, 1995). Propolis contains at least 200 compounds that have been identified in different samples of propolis, with more than 100 being present in any given sample. These include fatty and phenolic acids and esters, substituted phenolicesters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, and chalcones), terpenes, β-steroids, aromatic aldehydes and alcohols and derivatives of sesquiterpenes, naphthalene and stilbenes (Marcucci *et al*., 1996). The main types of flavonoids are rutin, quercetin, galangin (Isla *et al*., 2001) and caffeic acid phenethyl ester (Natarajan *et al*., 1996). Medicinal plants and their derivatives have been accumulating wide therapeutic value since ages. Propolis is a plant derived resinous mixture and has been proved as potent hypocholesterolemic drug with lesser side effects. Besides this the studies has indicated that propolis possesses a broad spectrum of biological activities and is extensively being used in health, food, pharmaceutical preparations (Orsolic and Basic, 2006) and beverages with the aim of maintaining or improving human health (Marcucci *et al*., 1996). Some medicinal use of propolis are – Enhancement of immune system activities (Orsolic and Basic, 2006), oxygen radical scavenging (Chen *et al*., 2004), antimicrobial, anti-inflammatory and antitumor activities

(Duarte *et al*., 2006). The present study was designed to investigate the probable hypolipidemic effects of crude propolis and its comparative status with that of synthetic drug statin (atorvastatin) currently present in market as drug treatment of hypercholesterolemia.

MATERIALS AND METHODS

Collection of Propolis

Indian brown propolis was obtained from Apiculture centre, Department of Zoology, Jiwaji University, Gwalior, Madhya Pradesh.

Experimental Animals

Healthy adult male New Zealand rabbits were procured from Forest Department, Jodhpur (Rajasthan). Weights and age of animals were 1.25-1.75 kg and 10-12 month respectively. Animals were housed in well-lighted air-conditioned room in metallic wire gauge cages, under controlled environmental conditions with 12 hours illumination and 12 hours darkness cycle. Animals were fed on standard rabbit chow supplied by Hindustan lever ltd., India. The food was supplemented with green leafy and seasonal vegetables and water ad libitum.

Induction of Hyperlipidemia

The hyperlipidemic condition was induced by cholesterol feeding to rabbits. The cholesterol powder (500 mg/kg body weight) was mixed in 5ml of coconut oil mixture and administered to the animals orally. In addition animals were fed with atherogenic diet. The atherogenic diet was comprised of wheat flour base with addition of milk powder, dried egg yolk, hydrogenated fat, butter, dried yeast, salt, sugar and vitamin mixture to produce the following nutrients in the given proportion as recommended by WHO protocol. The average consumption of diet was 200g/rabbit per day.

Standard Drug

Atorvastatin was used as standard hypolipidemic drug and it was given to the animals at the dose of 0.25mg/kg body weight dissolved in 5ml distilled water.

Feeding of Propolis

The crude propolis (25mg/kg body weight) was suspended in 5ml of distilled water and was orally given to hyperlipidemic models. The dose of the drug was determined by LD_{50} test.

Experimental Groups

Twenty four male albino rabbits were divided into four groups the control and experimental groups, usually consisted of six animals each.

Group 1 – Vehicle treated control or intact control (60 days)

Group 2 – Atherodiet + cholesterol feeding (500mg/kg body weight) for 60 days

Group 3 – Cholesterol feeding (500mg/kg body weight) for 15 days + propolis (25mg/kg body weight) for 45 days

Group 4 – Cholesterol feeding (500mg/kg body weight) for 15 days + statin (0.25mg/kg body weight) for 45 days

Criteria of Observation

At the end of experimental period, animals were autopsized under prolonged ether anesthesia. Blood was collected through cardiac puncture and serum was separated by centrifugation for 10 minutes at 3000 RPM and was divided into 4 to 5 portions for different serum and blood parameters determinations. The tissues were taken for histopathological study through microtomy process.

Histological Studies

For histological observation tissues were cut into small pieces. Some pieces of aorta were fixed in 10% formalin fixative and were subjected to various steps of alcohol series processing and wax blocks were prepared. The wax blocks were cut with the help of a microtome and thin sections of 5μm thickness were obtained. The thin sections were stained using Harris haematoxylin and Eosin. The stained sections were examined for histopathological changes.

Planimetric Studies

Planimetric studies of different layers of aorta were also performed. Sections of aorta were traced on paper with camera lucida (32x). Area occupied by each layer of aorta in comparison to total area was calculated.

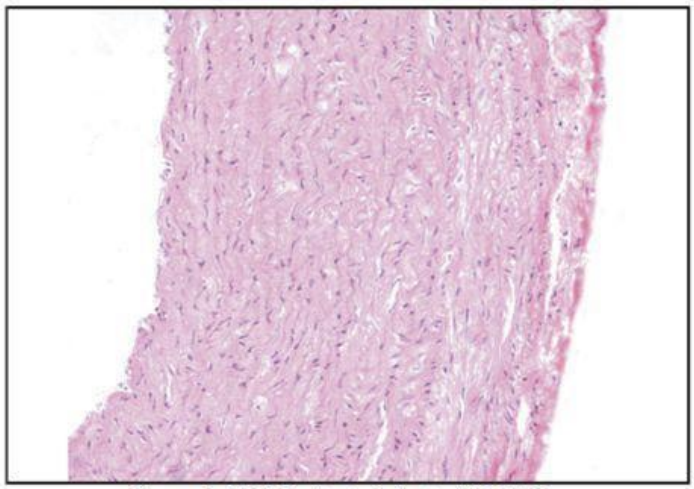

Figure 1: Vehicle Control Aorta (x200HE)

RESULTS

Histological Studies

Intact Control (Gr.1): The wall of aorta is composed of three layers *i.e*., tunica intima, tunica media and tunica adventitia. The tunica intima is composed of collagenous connective tissue with few elastic fibers. The media is particularly broad and extremely elastic and consist of fenestrated sheets of elastin fibers separated by collagenaeous connective tissue and few smooth muscle fibers. The collagenous tunica adventitia contains small vasa vasorum (Figure 1).

Hyperlipidemic Control:

The atherodiet feeding to rabbits (Gr.2) resulted into formation of plaques in aorta. This is characterized by thickened intima, cell proliferation, collagen and lipid deposition. Lumen of aorta became narrow. The tissues lining the aorta consist of large macrophages with abundant foamy cytoplasm accompanied by strands of fibrous tissue. (Figure 2).

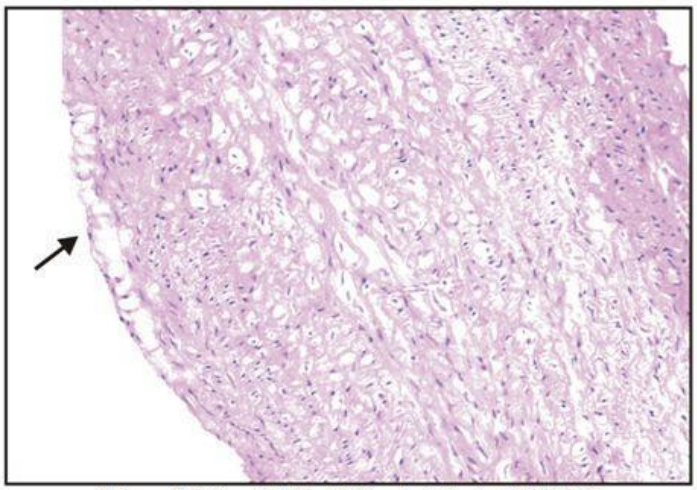

Figure 2: Hyperlipidemic Control Aorta (x200HE)

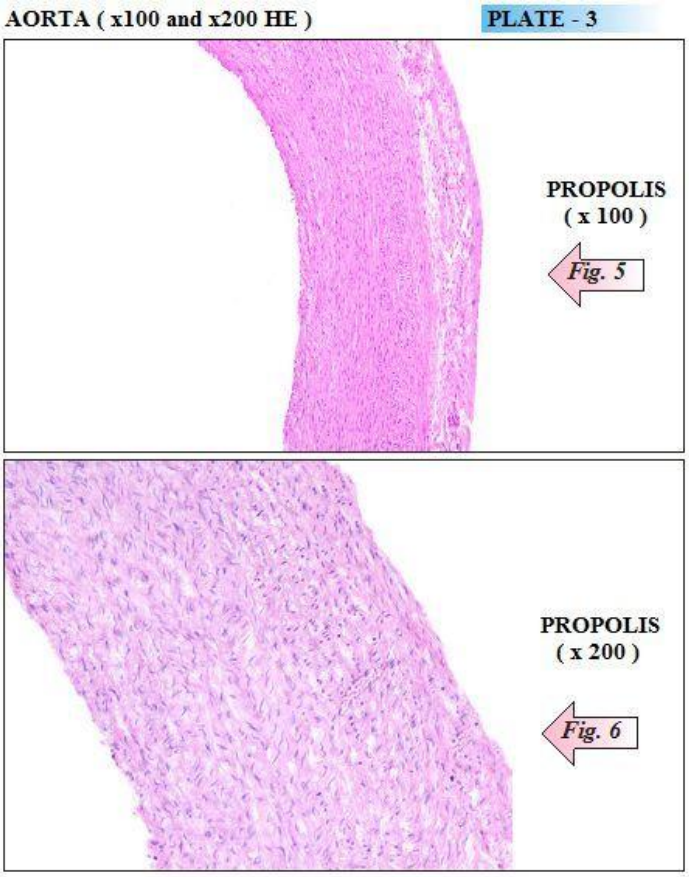

Figure 3: Propolis

The propolis (Plate 3, Figure 5 and 6) treatment reduced the size to greater extent but plaque persists till the end of the experiment, the rabbits treated with statin (Figure 4) showed some plaque traces.

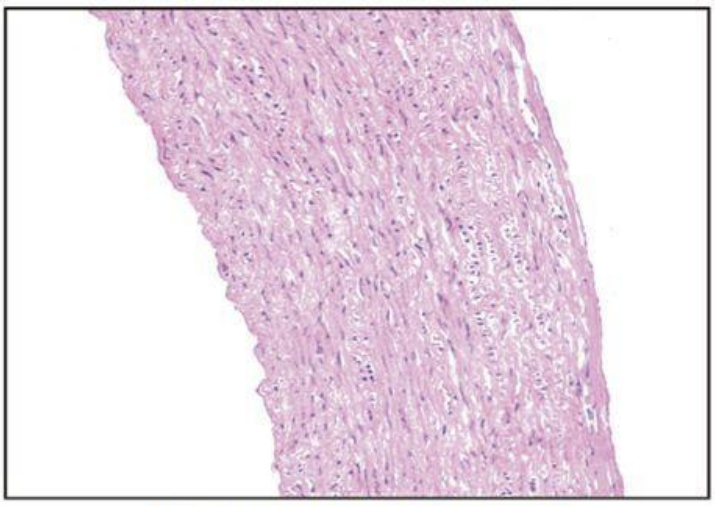

Figure 4: Stain Treated Aorta (x200HE)

Planimetric Studies (Table 1)

Surface area studies of ascending aorta showed highly significant increase in total wall area of aorta in atherodiet fed rabbits (Gr. 2) when compared to intact control group (Gr.1).

Table 1: Planimetric Dimensions Of Ascending Aorta Of Various Drugs (Biowaxes) Treated Intact Rabbits (Mean Of 5 Values ± Sem)

Treatment Groups	Total Wall Area	Lumen	Intima	Plaque	Media	Adventitia
	----------% of Total Area----------					
Control (Gr.1)	48.36 ± 2.03	51.42 ± 1.02	9.13 ± 0.14	Nil	29.0 ± 0.7	10.43 ± 0.06
Hyperlipidemic (Gr.2)	70.12 ± 3.63[a]	29.16 ± 2.31[a]	11.01 ± 0.32[a]	38.43 ± 1.08[c]	13.78 ± 0.63[c]	8.02 ± 0.13[a]
Propolis (Gr.3)	67.96 ± 2.41[a,e]	32.36 ± 4.03[b,e]	11.13 ± 0.63[a,h]	8.16 ± 1.07[a,f]	29.63 ± 3.01[d,g]	19.36 ± 0.76[c,g]
Statin (Gr.4)	40.63 ± 1.06[a,e]	48.93 ± 2.46[d,e]	11.61 ± 0.32[a,h]	Nil	41.01 ± 1.63[b,g]	17.42 ± 1.06[c,g]

Gr. 2, 3 and 4 were compared with Gr.1 Gr. 3 and 4 were compared with Gr.2

$P \leq 0.05$	= a $P \leq 0.05$	= e	
$P \leq 0.01$	= b	$P \leq 0.01$	= f
$P \leq 0.001$	= c	$P \leq 0.001$	= g
Nonsignificant = d	Nonsignificant = h		

The propolis drug treatment (Gr.3) showed slightly significant increase in total wall area and statin drug treatment (Gr.4) showed nonsignificant change when compared with Group 1, while on comparing with Group 2 slightly significant decrease in case of propolis and significant decrease in case of statin was observed. Histological examination showed thickening of tunica intima and tunica media of the abdominal aorta. Three to four cross sections of the aorta were used from each rabbit and the average were taken. In hyperlipidemic control group (Gr.2), there was significant increase in tunica intima and media (90%) when compared with control group (Gr.1). In propolis treated group there was less thickening of tunica intima compared to hyperlipidemic control Group (Gr.2). In statin treated group (Gr.4) there was complete recovery in the intimal thickening. Thus a significant reduction in the tunica intima layer was observed in propolis and statin treated group when compared with hyperlipidemic control group.

The lumen area of aorta was reduced after atherodiet feeding for complete 60 days (Gr.2). The drug treatment (Gr.3-4) lead to significant reduction in lumen area of propolis treated group, while nonsignificant reduction in lumen area of statin treated group was observed when compared with Group 1, while on comparing with group 2 slightly significant decrease in lumen area was observed in case of propolis and significant decrease in case of statin was observed.

DISCUSSION

Hypercholesterolemia is a major risk factor for coronary heart disease, and the Framingham study had reported that a 1% increase of plasma cholesterol level as equivalent to a 2% elevation of coronary heart disease incidence (Kannel and Dawber *et al*., 1961). The present study showed that dietary treatment of

rabbits with high cholesterol diets caused atherosclerotic lesions in an animal model and these findings were in accordance with cartees studies (Stocken and Keaney, 2004). Feeding with excess amount of cholesterol causes rapid hyperlipidemia and atherosclerosis (Stocken and Keaney, 2004). In the present study, we observed that the severity of the atherosclerotic lesions in aorta was associated with hypercholesterolemia which was in accordance with past investigations (Prasad *et al.*, 1994). In microscopy study, we selected randomly the sample from each group for histological analysis. In intact control group no changes were observed in the intima surface layer. In the hyperlipidemic control group (Gr-2), there were foam cells in the intima layer. The tunica intima was thickened in hyperlipidemic group leading to reduction of vascular lumens. In propolis and statin treated groups there were less foam cells and no fat cells were observed. So there was less tunica intima thickening or negligible size of plaque was observed.

The results of the present study also showed that hypercholesterolemia diet produced tunica intimal thickening that contained foam cells which was even reported by past researchers (Stocken and Keaney, 2004). Hypercholesterolemia is also one of the important factors that cause endothelial dysfunction in human arteries (Minor *et al*., 1990). Atheromatous lesions develop in the sub endothelial space due to the accumulation of cholesterol ester in forming foam cells. The mechanism of foam cell formation was unclear because macrophages have few LDL receptors but there is much evidence that oxidized LDL is responsible for cholesterol loading of macrophages foam cell formation in atherosclerosis.

The protective activity of propolis on atherosclerotic lesion may be attributed to its antioxidant action because it reduces the production of free radicals, decreasing the oxidized LDL and alleviating the subsequent damage to the heart tissue (Zhang *et al*., 2001).There are many antioxidant components in Propolis like Flavonoids which have patent action in protecting LDL from oxidation. The antioxidant activity of crude drugs was found to have scavenger free radical activity 90% (Vimala *et al*., 2003). Other studies have also concluded that consumption of flavonoids like antioxidants are inversely related to the risk of developing coronary heart diseases (Hertog *et al.*, 1993).

The present results of experimental rabbits, although not directly applicable to human subjects, suggest that Propolis may be effective as an anti-atherosclerotic agent and will have a great potential of commercialization as medicinal plant in near future.

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