

## EFFECT OF INDIAN PROPOLIS ON HAEMATOLOGICAL PARAMETERS IN EXPERIMENTALLY INDUCED HYPERLIPIDEMIC MALE ALBINO RABBITS

ASHOK PUROHIT, KUMUD JOSHI, BHARAT KOTRU AND SUSHIL KOTRU

Department of zoology, Jai Narain Vyas University, Jodhpur-342005 (India), Department of Biochemistry, Sera Care Lab and Research Centre, Jodhpur-342001 (India), Email: drdiafoot@gmail.com

Received: 23 August 2012, Revised and Accepted: 18 September 2012

### ABSTRACT

This study was conducted to evaluate the safety potential of the propolis as drug, which is used in order to control the elevated level of cholesterol in white albino male New Zealand rabbits using hematological indices of toxicity. Four groups of 6 male albino rabbits in each, were used for study. To Intact control group no drug was given, to hyperlipidemic control group atherogenic diet with cholesterol powder (500 mg/kg body weight) mixed in 5ml coconut oil was given. To group 3 & 4 propolis and statin was given as drug by oral administration, the drug treatment was carried out for complete 60 days. Animals were sacrificed by prolonged ether anesthesia after the 24 hours of last dose of drug and blood sample was collected for the hematological assays. The hematological parameters like erythrocyte count, leucocyte count, hemoglobin count etc. were evaluated and the platelet count showed variation from its normal range suggesting dose dependent anti platelet activity in propolis.

**Keywords:** Hyperlipidemia, atherosclerosis, propolis, cholesterol

### INTRODUCTION

Atherosclerosis disease causes over 19 million deaths annually, yet our understanding of the fundamental aspects of atherogenesis of the disease remains incomplete<sup>1</sup>. Several studies have shown that an increased dietary intake of cholesterol result in hypercholesterolemia, which is known to eventually generate atherosclerosis and enhance the risk of coronary heart disease (CHD), fatty liver disease & cancer associated with hydroxyl free radical formation<sup>2</sup>. The endothelial cells are thought to become dysfunctional or denuded allowing blood products and macrophages to adhere and penetrate the vascular wall. The denudation or endothelial cell dysfunction also leads to platelet clumping. Platelets are anucleate cells having a limited life span. Platelets are a rich source of mediators and lead to prominent release of mediators diffusing into the wall, these mediators cause continuous recruitment and activation of monocytes mainly through activation of the monocyte chemoattractant protein-1 (MCP-1) pathway which have a central role in atherogenesis<sup>3</sup>. Thus the platelet, once thought to be solely involved in clot formation, is now known to be a key mediator in various other processes such as inflammation, thrombosis and atherosclerosis. Supported by the wealth of evidence from clinical trials demonstrating their benefits in patient outcomes, antiplatelet agents have become paramount in the prevention and management of various diseases involving the cardiovascular, cerebrovascular and peripheral arterial systems. Despite being among the most widely used and studied classes of medical therapies, new discoveries regarding important clinical aspects and properties of these agents continue to be made<sup>4</sup>.

Propolis contains at least 200 compounds that have been identified in different samples with more than 100 being present in any given sample. These include fatty and phenolic acids and esters, substituted phenolic esters, flavonoids, steroids, aromatic aldehydes and alcohols etc.<sup>5</sup> The main types of flavonoids are rutins, quercetin, galangin<sup>6</sup> & caffeic acid phenethyl ester<sup>7</sup>. The protective activity of propolis on atherosclerotic lesion may be attributed to its antioxidant action<sup>8</sup>. Some studies concluded that consumption of flavonoids like antioxidants are inversely related to the risk of developing coronary heart diseases<sup>9</sup>. Past research reports showed link between flavonoids and atherosclerosis because of the antioxidant activities of phenolic adhesion of platelets in the blood<sup>10</sup>.

Hence assessment of hematological profile becomes a prerequisite to understand the normal functioning of the system and to confirm the toxic nature of the administered crude drug propolis and compare it with standard drug statin (Synthetic drug). The present study was designed to investigate the antiplatelet activity 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. It was dissolved in distilled water and administered orally at 9 AM in a dose of 25mg/kg body weight<sup>11</sup> for 45 days.

#### 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<sub>50</sub> 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 in E.D.T.A vial for blood parameters determinations.

#### Hematological observation

Hemoglobin concentration, WBC, RBC, Platelet Counts, MCV, MCH, MCHC, Lymphocytes, Monocytes, Granulocytes, RDW, PCT, MPV, PDW and Hematocrit were all determined on a Celltac-  $\alpha$  Hematology analyzer (NIHON KOHDEN JAPAN).

#### Results (Table-I)

The results of all the haematological parameters of vehicle treated control group (Gr. 1) and all other experimental groups (Gr.2-4) were found to be within the normal range except platelet count .

**Table – I : Hematology Of Drug Treated Intact Rabbits (Mean Of 5 Values  $\pm$  Sem)**

Hemato Logy Para-meters.	Control (gr.1)	Hyperlipi- Demia (gr.2)	Propolis (gr.3)	Statin (gr.4)
TLC/cu.mm.	7090.0 $\pm$ 378.0	7033.3 $\pm$ 388.0 <sup>d</sup>	7750.00 $\pm$ 250.00 <sup>d,h</sup>	7400.00 $\pm$ 208.16 <sup>d,h</sup>
RBC.ml/dl	6.34 $\pm$ 0.30	5.32 $\pm$ 0.37 <sup>d</sup>	5.20 $\pm$ 0.24 <sup>d,h</sup>	5.00 $\pm$ 0.24 <sup>d,h</sup>
HGB.gm/dl	11.46 $\pm$ 0.42	10.46 $\pm$ 0.24 <sup>d</sup>	10.25 $\pm$ 0.26 <sup>d,h</sup>	10.53 $\pm$ 0.29 <sup>d,h</sup>
HCT.%	35.33 $\pm$ 0.598	36.00 $\pm$ 3.732 <sup>d</sup>	31.65 $\pm$ 2.26 <sup>d,h</sup>	30.00 $\pm$ 3.08 <sup>d,h</sup>
MCV.fl.	64.19 $\pm$ 2.70	64.466 $\pm$ 2.90 <sup>d</sup>	62.75 $\pm$ 3.00 <sup>d,h</sup>	66.26 $\pm$ 2.74 <sup>d,h</sup>
MCH.pg.	17.620 $\pm$ 0.434	18.666 $\pm$ 0.66 <sup>d</sup>	18.150 $\pm$ 0.60 <sup>d,h</sup>	17.76 $\pm$ 0.47 <sup>d,h</sup>
MCHC.gm/dl	28.610 $\pm$ 2.309	28.00 $\pm$ 2.08 <sup>d</sup>	28.95 $\pm$ 2.00 <sup>d,h</sup>	27.43 $\pm$ 2.41 <sup>d,h</sup>
PLT.lacs/cu.mm.	2.909 $\pm$ 0.900	3.75 $\pm$ 0.19 <sup>c</sup>	2.0 $\pm$ 0.25 <sup>c,g</sup>	1.0 $\pm$ 0.52 <sup>c,g</sup>
LYM.%	39.290 $\pm$ 3.057	39.680 $\pm$ 3.48 <sup>d</sup>	43.40 $\pm$ 4.40 <sup>d,h</sup>	44.23 $\pm$ 2.98 <sup>d,h</sup>
MO.%	10.700 $\pm$ 0.651	11.666 $\pm$ 0.88 <sup>d</sup>	10.20 $\pm$ 0.65 <sup>d,h</sup>	9.99 $\pm$ 1.10 <sup>d,h</sup>
GRN.%	50.710 $\pm$ 3.315	48.666 $\pm$ 3.05 <sup>d</sup>	46.40 $\pm$ 3.75 <sup>d,h</sup>	45.78 $\pm$ 4.761 <sup>d,h</sup>
RDW.%	14.84 $\pm$ 0.59	14.26 $\pm$ 0.40 <sup>d</sup>	12.00 $\pm$ 1.00 <sup>d,h</sup>	12.66 $\pm$ 0.24 <sup>d,h</sup>
PCT.	0.02 $\pm$ 0.002	0.02 $\pm$ 0.003 <sup>d</sup>	0.02 $\pm$ 0.003 <sup>d,h</sup>	0.02 $\pm$ 0.003 <sup>d,h</sup>
MPV.fl.	7.12 $\pm$ 0.23	7.76 $\pm$ 0.05 <sup>d</sup>	7.00 $\pm$ 1.0 <sup>d,h</sup>	7.00 $\pm$ 0.5 <sup>d,h</sup>
PDW.%	18.22 $\pm$ 0.47	17.66 $\pm$ 0.88 <sup>d</sup>	18.50 $\pm$ 0.50 <sup>d,h</sup>	18.93 $\pm$ 0.29 <sup>d,h</sup>

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

P  $\leq$  0.05 = a  
 P  $\leq$  0.01 = b  
 P  $\leq$  0.001 = c  
 Nonsignificant = d

#### Gr. 3 and 4 were compared with Gr.2

P  $\leq$  0.05 = e  
 P  $\leq$  0.01 = f  
 P  $\leq$  0.001 = g  
 Nonsignificant = h

#### Platelet Count

Slightly significant reduction was observed in propolis and statin treated groups when compared with group 1 and group 2. All other hematological parameters showed nonsignificant changes when there comparative study was done.

#### DISCUSSION

Platelets and coagulation factors are involved in thrombotic process that can lead to Myocardial infarction. Aspirin and antiplatelet therapy reduce risk and show that hyper aggregability can predispose to CHD <sup>12</sup> also fibrinogen has been associated with increased risk independent of cholesterol level because low fibrinogen indicates risk even with high total cholesterol levels. Normal unperturbed endothelial cells exhibit anticoagulant properties that include the release of the inhibition of platelet aggregation prostacyclin, however, exposure to inflammation and atherogenic factors induces procoagulant activity <sup>13</sup>. More over apoptosis of endothelial cells increases the expression of phosphatidylserine and the loss of anticoagulant components of the endothelial cell membrane, as phosphatidylserine exposure enhances tissue factor activity, which is highly thrombogenic. Certainly, extra-cellular tissue factor expression is increased in and around apoptotic monocyte/lymphocyte cells in necrotic basis for the generation of microparticles within the circulation, which act as potent pro-coagulant substrates both locally and systemically <sup>14</sup>. These particles are increased in patients with unstable coronary disease and account for the vast proportion of the procoagulant activity of the plaque. Thus prostacyclin reduces atherogenic cholesteryl ester accumulation in macrophages and vessel cell, inhibits platelet activation and mitogen release. A laboratory study examined the inhibitory mechanisms of caffeic acid phenethyl ester (CAPE), derived from propolis, in platelet activation<sup>15</sup>. CAPE (15 and 25mcM) was found to markedly inhibit platelet aggregation stimulated by collagen (2 mcg/mL). Since CAPE is involved in

various inhibitory pathways of platelet aggregation, propolis may exhibit potent antiplatelet activity.

#### CONCLUSION

In the present investigation the hematological parameters in all experimental groups remain unaltered except platelet count. The normal range of hematological parameters suggests non-toxic nature of the drug and indicates no drug related side effects on the animal models and the reduction in plateletcount show antiplatelet activity of the crude propolis similar to that of statin.

#### ACKNOWLEDGEMENT

We would like to thanks to the Professor O.P. Agarwal, Department of Zoology, Jiwaji University, Gwalior for their suggestions and crude propolis collection. None of the author has any conflicts of interest. No funding was provided for this work.

#### REFERENCES

- Tunstall, P. H., Smith, W.C., Cholesterol as a risk factor for coronary heart disease. *Br Med Bull*, 1990; 46, 1075-1087.
- Festi, D., Colecchia, A., Sacco, T., Sondi, M., Roda, E., Marchesini, G., Hepatic Steatosis in obese patients. Clinical aspects and prognostic significance. *Obes Rev* 2004; 5, 27-42.
- Egashira, K., Molecular mechanisms mediating inflammation in vascular disease : special reference to monocyte chemoattractant protein-1. *Hypertension*, 2003; 41, 834-41.
- Chen,H., Shashkin,P., Gleissner, C., Dunson,D., Jain, N., Lee,J., Miller, Y. and Klaus Ley K., Induction of dendritic cell-like phenotype in macrophages during foam cell formation. *Physiological Genomics* , 2007; 29, 149-160.
- Marcucci, M.C., DeCamargo, F.A. and Lopes, C.M.A., Identification of amino acids in Brazilian propolis. *Z Naturforsch*, 1996 ; 51[C], 11-14.

6. Isla, M.I., Moreno, M.I., Sampietro, A.R. and Vattuone, M.A., Antioxidant activity of Argentine propolis extracts. *J Ethnopharmacol*, 2001; 76, 165-170.
7. Natarajan, K., Singh, S., Burkerm, T.R. Jr., Grunberger, D. and Aggarwal, B.B., Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc Natl Acad Sci USA*, 1996; 93(17), 9090-9095.
8. Zhang, Z., Chang, Q., Zhu, M., Muang, Y., Ho, W.K.K., Chen, Z.Y., Characterization of antioxidants present in hawthorn hurts. *J Nutr Biochem*, 2001; 12: 144-152.
9. Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Kotan, M.B., Kromhout, O., Dietary antioxidant Flavonoids and risk of coronary heart disease : The Zutphen Elderly Study. *Lancet*, 1993; 342, 1007-1011.
10. Seong, C.C., Hyo, S.K., Tae, S.J., Song, H.B., Yong, B.P. Naringin Has an Antiatherogenic Effect with the inhibition of Intercellular Adhesion Molecule - I in Hyperchoesterolemic Rabbits. *J Cardiovas Pharmacol*, 2001; 38, 947-955.
11. Bhaduria, M., Nirala, S.K. and Shukla, S., Propolis protects CYP2E1 enzymatic activity and oxidative stress induced by carbon tetrachloride. *Mol Cell Biochem*, 2007; 302(1-2), 215-224.
12. Creager, M.A., Results of the CAPRIE trial : efficacy & safety of clopidogrel. *Vasc Med*, 1998; 3, 257-260.
13. Bombeli, T., Karsan, A., Tait, J.F. and Harian, J.M., Apoptotic vaxulan endothelial cells become procoagulant. *Blood*, 1997; 89, 2429-2442.
14. Mallat, Z. and Tedgui, A. Apoptosis in the vasculature : Mechanisms and functional importance. *Br J Pharmacol*, 2000; 130, 947-962.
15. Chen, T. G., Lee, J. J., Lin, K. H., Shen, C. H., Chou, D. S. and Sheu, J. R., Antiplatelet activity of caffeic acid phenethyl ester is mediated through a cyclic GMP-dependent pathway in human platelets. *Chin J Physiol*, 2007; 50(3), 121-126.