

# Beneficial effects of propolis on experimental hypercholesterolaemia in rabbits

## *Efeito benéfico da propólis sobre a hipercolesterolemia experimental em coelhos*

### ABSTRACT

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*Propolis (bee glue) is one of the major bee hive products and is rich in flavonoids, which are known for their antioxidant effect. To investigate the possible mechanism of propolis therapeutic action, rabbits were distributed into 4 groups (n=6): GI – control; GII – atherogenic diet; GIII – atherogenic diet and ethanol; GIV – atherogenic diet and ethanolic extracts of propolis (EEP=100mg/kg, day). Atherogenic diet (0.14g cholesterol, daily) induced hyperlipidemia in rabbits (male, body weight 2.500±2g) with significant increase of levels of total cholesterol, low density lipoprotein, very low density lipoprotein and triacylglycerol. Treatment with EEP, for 56 days, significantly reduced the levels of these biochemical parameters. The activities of plasma aspartate aminotransferase, alanine aminotransferase and creatine phosphokinase were increased (p<0.05) by atherogenic diet and these increases were largely inhibited by EEP, whereas the administration of EEP produced a marked increase in the levels of high density lipoprotein. Treatment of rabbits with EEP resulted in a decrease of hepatic levels of triglycerides, triacylglycerol and total cholesterol. These results suggest that EEP exerts a hypocholesterolaemic effect in high-cholesterol-fed rabbits, and possesses a protective action against the acute hepatic toxicity caused by administration of atherogenic diet.*

**Keywords: Propolis. *Apis mellifera*. Hypercholesterolaemia. Lipoprotein. Enzymatic activity. Liver. Plasma.**

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## RESUMEN

El propóleo es una sustancia elaborada por las abejas y es rica en flavonoides, los cuales tienen reconocida actividad antioxidante. Para investigar el posible mecanismo de acción terapéutica del propóleo, conejos fueron divididos en 4 grupos ( $n=6$ ) que recibirían dietas diferentes: GI - control; GII - dieta aterogénica; GIII - dieta aterogénica y etanol; GIV - dieta aterogénica y extracto etanólico de propóleos (EEP = 100mg/kg; diariamente). La dieta aterogénica (0.14g colesterol; diariamente) provocó hiperlipidemia en conejos (machos, peso corporal = 2.500g) con aumento significativo en los niveles de colesterol total, lipoproteína de baja densidad (LDL), lipoproteína de muy baja densidad (VLDL) y también de triglicéridos. El tratamiento con EEP durante 56 días, redujo ( $p<0.05$ ) el nivel de todos esos índices bioquímicos. La actividad enzimática plasmática de aspartato transaminasa, alanina transaminasa y creatina fosfoquinasa aumentó ( $p<0.05$ ) con la dieta aterogénica y estos aumentos fueron inhibidos por la EEP. Mientras la administración de EEP mostró elevación del nivel hepático de lípidos totales, triglicéridos y colesterol total. Estos resultados muestran que EEP ejerció efecto hipercolesterolémico en conejos alimentados con dieta rica en colesterol y acción protectora del propóleo frente a la toxicidad hepática causada por la administración de la dieta aterogénica.

**Palabras clave:** Propóleos. *Apis mellifera*. Hipercolesterolemia. Lipoproteínas. Actividad plasmática.

## RESUMO

Própolis, substância elaborada por abelhas, rica em flavonóides, os quais são conhecidos pelas atividades antioxidantes. Para investigar o possível mecanismo de ação terapêutica da própolis, coelhos foram divididos em 4 grupos ( $n=6$ ): GI - controle; GII - receberam dieta aterogênica; GIII - receberam dieta aterogênica e etanol; GIV - receberam dieta aterogênica e extrato etanólico de própolis (EEP=100mg/kg; diariamente). A dieta aterogênica (0.14g colesterol, diariamente) induziu hiperlipidemia em coelhos (machos, peso corporal - 2.500g) com aumento significativo nos níveis de colesterol total, lipoproteína de baixa densidade (LDL), lipoproteína de densidade muito baixa (VLDL) e de triacilglicerol. Tratamento com EEP, por período de 56 dias, reduziu ( $p<0.05$ ) o nível destes parâmetros bioquímicos. As atividades plasmáticas da aspartato aminotransferase, alanina aminotransferase e creatina fosfoquinase aumentaram ( $p<0.05$ ) com a dieta aterogênica e estes aumentos foram inibidos pela EEP. Enquanto que a administração de EEP mostrou elevação no nível hepático de lipídios totais, triacilglicerol e colesterol total. Estes resultados sugeriram que EEP exerceu efeito hipocolesterolêmico em coelhos alimentados com dieta rica em colesterol, e ação protetora da própolis contra a toxicidade hepática causada pela administração da dieta aterogênica.

**Palavras-chave:** Própolis. *Apis mellifera*. Hipercolesterolemia. Lipoproteínas. Atividade enzimática. Fígado. Plasma.

## INTRODUCTION

Propolis, a natural product derived from plant resins collected by honeybees, has been used for thousands of years in folk medicine for several purposes. It contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids, phenolic aldehydes and alcohols), coumarins and steroids. More than 160 constituents have been identified to date, and differ greatly due to variations in their geographical and botanical origins (BANSKOTA et al., 2000; CARDILE et al., 2003; MARTOS; FERRERES; TOMAS-BARBERAN, 2000).

Propolis is a very powerful natural product, which can be used to treat human diseases with great success. Recently, it has been reported to possess versatile biological activities, such as antibacterial, antiviral and anti-inflammatory actions, in addition to prevention of heart diseases (BANKOVA; POPOV; MARENOV, 1983; KUMAZAWA et al., 2004; MARCUCCI, 1995).

It has been suggested that the biological activities of propolis mainly depend on the presence of a large number of flavonoids (BASNET et al., 1997; VENNAT et al., 1995). Propolis contains large amounts of antioxidant compounds and shows high efficiency in the prevention of oxidative processes. This could be explained by the high proportion of polyphenol constituents, especially flavonoids (AHN et al., 2004; KATALINIC et al., 2004; VOLPI, 2004), which are major functional components of many herbal and insect preparations for medical use, among which are propolis (HAVSTEEN, 2003).

Major risk factors for the development of coronary artery disease or atherosclerosis include high plasma LDL-cholesterol concentrations and LDL modifications such as retention, oxidation, and aggregation (AVIRAM; FUHRMAN, 2002). It is important to reduce excessive cholesterol and LDL-cholesterol oxidation to low levels, which represent adequate mechanisms for maintenance of normal body functions (BOK et al., 1999).

Intake of flavonoids may reduce the risk of cardiovascular disease both by reduction in serum lipids, in addition to its antioxidant properties (YOUSEF et al., 2005). Many flavonoids are known to be antioxidants, and several of these, such as quercetin – which has been identified as constituents of propolis – have been shown to be inhibitors of low density lipoprotein oxidation (FRANKEL et al., 1998; NÉGRE-SALVAYRE; SALVAYRE, 1992).

The ethanol extract of propolis leads to decreased levels of total cholesterol, triacylglycerides, low lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL), suggesting that propolis modulates the metabolism of blood lipid (CASTALDO; CAPASSO, 2003; FULIANG et al., 2005). Furthermore, daily intake of flavonoids, increases HDL-cholesterol concentration and decreases the LDL-HDL cholesterol ratio (KUROWSKA; MANTHEY, 2004).

A recent interesting study from Wilcox et al. (2003) examined the molecular mechanisms of flavonoids cholesterol-lowering effects in HepG2 cells. This study showed that flavonoids reduce cellular cholesteryl ester mass, inhibit the activity of acyl-coenzyme A:cholesterol acyltransferase (ACAT) and increase the degradation of LDL. These mechanisms may explain the hypocholesterolemic properties of the flavonoids.

The daily administration of flavonoids, at a dose of 1mg/100g body weight, significantly lowers lipid levels in rats fed cholesterol-containing diets and Hydroxy  $\beta$ -methyl glutaryl coenzyme A reductase (HMG-CoA reductase) is significantly reduced. Highly stimulated activity of the enzyme lipoprotein lipase was observed in flavonoid-treated animals. Hepatic and fecal bile acids and fecal neutral sterols were substantially elevated, indicating a higher rate of cholesterol degradation. Thus, hypolipidemic activity of these flavonoids may be due to a lower rate of lipogenesis and higher rate of LDL degradation (KOSHY; ANILA; VIJAYALAKSHMI, 2003; LEE et al., 2001). These results suggest that the anti-atherogenic effect of flavonoids is involved with decreased hepatic ACAT activity.

Flavonoids effectively reduce lipid levels in serum and tissues of rats in which hyperlipidemia was induced and may increase reverse cholesterol transport and decrease total and LDL cholesterol (REED, 2003). Plasma HDL-cholesterol concentration and HDL to cholesterol ratio were significantly higher with flavonoids. Cholesterol biosynthesis and esterification were concomitantly reduced by flavonoid, as indicated by the decreased HMG-CoA reductase and ACAT activities (LEE et al., 2004).

Flavonoids supplementation results in a significant decrease of hepatic triglycerides and plasma and hepatic total cholesterol increased the HDL-cholesterol and HDL cholesterol/total cholesterol (SEO et al., 2003). ACAT was 27% lower in the flavonoids diet-fed group and flavonoids intake alters hepatic cholesterol metabolism, which may affect VLDL secretion rates and result in less accumulation of cholesterol in the aorta (ZERN et al., 2003). Moreover, diets containing flavonoids significantly reduce serum total and very low density lipoprotein, LDL and either reduced or tended to reduce serum triacylglycerols (KUROWSKA; MANTHEY, 2004).

Flavonoids cause a significant decrease in the levels of plasma total lipids, total cholesterol, triacylglycerols, low density lipoprotein, very low density lipoprotein and LDL:HDL ratio, while the levels of high density lipoprotein increase. Results showed that flavonoids seem to be related to a better plasma lipid and lipoprotein profiles and antioxidant activity (ADARAMOYE et al., 2005; YOUSEF et al., 2005).

The anti-atherogenic effect of flavonoids is involved with a decrease in hepatic ACAT activity in high cholesterol-fed rabbits (LEE et al., 2001).

Thus, the major purpose of the study was to determine the lipid profile in serum and tissues and enzymatic activities in rats fed a high cholesterol diet and treated with propolis.

## MATERIALS AND METHODS

### PROPOLIS ORIGINS

Propolis was collected in the Beekeeping Section of the School of Veterinary Medicine and Animal Husbandry of Botucatu, UNESP, in the year of 2001. Propolis samples were obtained from colonies of African honeybees (*Apis mellifera*) and collected throughout one year from plastic nets.

### Preparation of Ethanolic Extracts of Propolis (EEP)

Propolis obtained was ground and 30% ethanolic extracts of propolis (EEP) were prepared (30g of propolis completing the volume to 100mL of 95% ethyl alcohol), protected from bright light, with moderate shaking, at room temperature (SFORCIN; FUNARI; NOVELLI, 1995). After a week, extracts were filtered.

### Analytical Procedures of EEP

Flavonoid content and total phenolic substances were measured by the method of Woisky and Salatino (1998) (Table 1).

**Table 1 - Concentrations of constituents of EEP (wt/wt)**

Sample, origin	Total phenolic substances	Flavonoids
EEP - Eucalyptus plantation, Botucatu, State of São Paulo	11.7±0.4	3.7±0.1

### ANIMALS AND DIETS

Male rabbits (*Oryctolagus cuniculus*) weighing between 2000 and 2500g were individually housed in stainless steel cages in a room with controlled temperature (25±1°C) and lighting (alternating 12h periods of light and dark).

They were divided into four equal groups of six animals according to the average body weight and received the diets according to a pair-feeding schedule. The experiment was carried out for 56 days; the food consumption and body weight gain were measured every third day.

The animals were randomly divided into four groups:

Group I (untreated control) = received water and basal diet — Purina Rodent Chow (cholesterol-free);

Group II (hypercholesterolaemic) = received water and atherogenic diet;

Group III (hypercholesterolaemic and ethanol control) = received ethyl ethanol (1mL/kg<sup>-1</sup> of body weight) orally, daily and atherogenic diet;

Group IV (hypercholesterolaemic and treated EEP) = received EEP at 100mg/kg<sup>1</sup> of body weight) (MERINO et al., 1996), orally, daily and atherogenic diet.

The animals were given free access to water and food.

The composition of the basal diet and atherogenic diet (cholesterol-rich diet) is described in table 2. The atherogenic diet was formulated from the basal diet, by mixing 2.3g cholesterol/kg diet. Therefore, all diets were offered as pellets and provided sufficient amounts of vitamins, minerals, essential amino acids and fatty acids.

**Table 2 - Composition of experimental diets<sup>a</sup>**

Ingredient	Basal diet	Atherogenic diet
Metabolizable Energy <i>Cal/kg</i>	2500	2500
Protein %	16	16
Carbohydrate %	50	50
Crude Fiber %	15	15
Ether extract %	3	3
Vitamin mix <sup>b</sup> %	1	1
Mineral mix <sup>c</sup> %	11	11
Cholesterol <i>g/kg</i>	0	2.3

<sup>a</sup>Expressed on a dry matter basis.

<sup>b</sup>Composition expressed in international units or mg per kg of vitamin mix: vitamin A, 8000.0 UI/kg; cholecalciferol, 1500.0 UI/kg; vitamin E, 20.0 UI/kg; choline, 900.0mg/kg; nicotinic acid, 40.0mg/kg; pantothenic acid, 12.0mg/kg; riboflavin, 3.0mg/kg; vitamin B<sub>12</sub>, 4.0mg/kg.

<sup>c</sup>Mineral mix consisted of (mg/100g): calcium, 900.0; phosphorous, 750.0; magnesium, 300.0; potassium, 800.0; Iron 3.0; copper 2.5; zinc 26.0; manganese, 10.0; iodine, 0.2; cobalt, 0.15.

At the end of the experimental period, rabbits were fasted overnight (15h), anaesthetized with sodium pentobarbital (Pentobarbital 4%, 60mg/kg body weight) and were sacrificed by decapitation between and 8:00-9:00h.

Blood was taken from the marginal vena ear with heparin-moistened syringes. Plasma samples from each group were prepared by centrifugation and stored at -80°C until analysis. Liver was rapidly removed, rinsed with 150nmol/L ice-cold NaCl, blotted and weighed. Individual aliquots were freeze-clamped and stored at -80°C.

Blood and tissue samples were collected for biochemical estimations.

## PLASMA AND HEPATIC BIOCHEMICAL ANALYSES

Total plasma cholesterol levels were measured using a cholesterol ester/oxidase enzymatic method (LEFFLER; MAC DOYGALD, 1962).

High density lipoprotein (HDL)-fractions were obtained after precipitation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) from the whole plasma with phosphotungstic acid and MgCl<sub>2</sub>, and the cholesterol content, measured (KOSTNER et al., 1979).

VLDL-cholesterol and LDL-cholesterol concentrations were estimated by using the Friedewald equation (FRIEDEWALD; LEVY; FREDRICKSON, 1972). The triacylglycerols levels were measured using a glycerol kinase-based enzymatic procedure (SOLONI, 1971).

Hepatic lipids (total lipid, triacylglycerol and cholesterol) were extracted using the procedure developed by Bligh and Dyer (1959). Part of the sample (approximately 200mg) was homogenized in chloroform-methanol 2:1 (v/v); the chloroform layer contained all the lipids and the methanolic layer contained all the non-lipids. Hepatic cholesterol and triacylglycerol were measured as described above for plasma.

The liver (200mg) was homogenized in 5mL chilled 0.01 M phosphate buffer (pH 7.4) with Potter-Elvehjem homogenizer. The homogenates were centrifuged at 12000 x g for 20 min at 4°C (PEREIRA et al., 1998), and then the supernatant fractions were used for protein and determination of aspartate aminotransferase, alanine aminotransferase and creatine phosphokinase activities.

### **Enzyme assays**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel (1957). Briefly, a decrease of NADH concentration was measured which is proportional to aspartate aminotransferase and alanine aminotransferase.

Plasma creatine phosphokinase (CK) was estimated by the method of Rosalki (1967). This method uses N-acetylcysteine as the thiol activator. AMP and diadenosine pentaphosphate (DAP) were used to reduce adenylate kinase interference to very low levels without loss of creatine kinase activity.

### **STATISTICAL ANALYSIS**

Values reported are expressed as mean  $\pm$  SEM. Statistical significance of the difference between groups was determined by analysis of variance (ANOVA) followed by Tukey's test. The values were considered to be significantly different when *P* value was less than 0.05 (ZAR, 1996).

### **RESULTS**

Table 1 shows the values of the parameters determined in samples of EEP. The values of total phenolic substances stood in the range 11.58g/100g, while those of flavonoids, of about 3.75g/100g.

There were no significant differences in the body weight gain between control and the three experimental groups. However, control rabbits (GI) showed increased ( $p < 0.05$ ) food intake, whereas animals treated with atherogenic diet (GII, GIII and GIV) showed an identical ( $p > 0.05$ ) food intake (Table 3).

**Table 3 - Effects of EEP on weight gain and food intake of atherogenic diet rabbits<sup>1</sup>**

Groups	Control (GI)	Atherogenic diet (GII)	Atherogenic diet + Ethanol (GIII)	Atherogenic diet + EEP (GIV)
Weight gain <i>g/day</i>	14.6±1.2 <sup>a</sup>	17.6±1.6 <sup>a</sup>	15.3±1.4 <sup>a</sup>	18.0±2.1 <sup>a</sup>
Food intake <i>g/day</i>	73.0±5.8 <sup>a</sup>	59.2±4.2 <sup>b</sup>	58.6±5.1 <sup>b</sup>	54.1±3.0 <sup>b</sup>

<sup>1</sup>Mean ±SE, n=6.

<sup>abcd</sup> Means values within a row not sharing a common superscript letter were significantly different, *P*<0.05.

GI – control group; GII – group receiving atherogenic diet; GIII – group receiving atherogenic diet and ethanol; GIV – group receiving atherogenic diet and EEP.

Administration of atherogenic diet caused a marked increase in both plasma and liver levels of lipids biochemical parameters (Table 4). The EEP significantly lowered the levels of plasma total cholesterol, VLDL-cholesterol, LDL-cholesterol and triacylglycerol compared with rabbits treated with atherogenic diet. Similarly, total lipid, triacylglycerol and total cholesterol in liver were significantly elevated (*p*<0.05) in the group treated with atherogenic diet (GII) and treated with atherogenic diet + ethanol (GIII), when compared to control group (GI). The levels were, however, significantly lowered in animals treated concomitantly either with EEP and atherogenic diet (Table 4). However, analysis of HDL-cholesterol concentration showed a marked (*p*<0.05) increase after treatment with EEP.

**Table 4 - Effects of EEP on plasma and hepatic lipids in rabbits fed on atherogenic diet**

Groups	Control (GI)	Atherogenic diet (GII)	Atherogenic diet + Ethanol (GIII)	Atherogenic diet + EEP (GIV)
Plasma				
Total cholesterol <i>mg/dL</i>	39.4±3.5 <sup>a</sup>	360.0±26.0 <sup>b</sup>	382.6±24.3 <sup>b</sup>	123.8±12.1 <sup>c</sup>
LDL-cholesterol <i>mg/dL</i>	19.0±2.6 <sup>a</sup>	337.2±24.2 <sup>b</sup>	355.4±30.9 <sup>b</sup>	94.0±12.2 <sup>b</sup>
HDL-cholesterol <i>mg/dL</i>	13.5±1.1 <sup>ab</sup>	13.2±0.2 <sup>a</sup>	15.6±1.1 <sup>b</sup>	23.0±1.6 <sup>c</sup>
VLDL-cholesterol <i>mg/dL</i>	6.9±1.0 <sup>a</sup>	9.4±1.5 <sup>b</sup>	11.7±1.7 <sup>b</sup>	6.9±0.7 <sup>a</sup>
Triacylglycerol <i>mg/dL</i>	34.5±4.8 <sup>a</sup>	46.9±7.4 <sup>b</sup>	58.4±8.7 <sup>b</sup>	35.6±3.8 <sup>a</sup>
Liver				
Total Lipids <i>md/g tissue</i>	49.8±4.7 <sup>a</sup>	79.3±3.3 <sup>b</sup>	71.8±2.4 <sup>c</sup>	61.3±2.9 <sup>d</sup>
Triacylglycerol <i>mg/g tissue</i>	10.7±0.9 <sup>a</sup>	20.6±1.6 <sup>b</sup>	21.1±2.0 <sup>b</sup>	11.4±2.2 <sup>a</sup>
Total cholesterol <i>mg/g tissue</i>	4.2±0.6 <sup>a</sup>	7.0±1.2 <sup>b</sup>	7.2±0.8 <sup>b</sup>	4.1±0.8 <sup>a</sup>

<sup>1</sup>Mean ±SE, n=6.

<sup>abcd</sup> Means values within a row not sharing a common superscript letter were significantly different, *P*<0.05.

GI – control group; GII – group receiving atherogenic diet; GIII – group receiving atherogenic diet and ethanol; GIV – group receiving atherogenic diet and EEP.



Plasma AST, ALT and CK activity was significantly elevated ( $p < 0.05$ ) in animals treated with atherogenic diet compared to controls (Table 5). However, the activity was significantly reduced in animals treated concomitantly with EEP ( $p < 0.05$ ).

The enzymatic activities of ALT and AST in liver homogenates were decreased with atherogenic diet (GII). Treatment with EEP (GIV) significantly increased ( $p < 0.05$ ) the hepatic activities of ALT and AST.

**Table 5 - Effects of EEP on plasma and hepatic aminotransferase (ALT and AST) and creatine phosphatase (CK) activities in rabbits fed on atherogenic diet<sup>1</sup>**

Groups	Control (GI)	Atherogenic diet (GII)	Atherogenic diet + Ethanol (GIII)	Atherogenic diet + EEP (GIV)
Plasma				
ALT U/L	54.3±10.0 <sup>a</sup>	182.8±22.0 <sup>b</sup>	189.1±27.6 <sup>b</sup>	50.2±12.2 <sup>a</sup>
AST U/L	80.0±11.4 <sup>a</sup>	294.5±21.5 <sup>b</sup>	279.7±25.2 <sup>b</sup>	81.3±23.0 <sup>a</sup>
CK U/L	160.6±35.1 <sup>a</sup>	320.5±41.6 <sup>b</sup>	330.2±40.7 <sup>b</sup>	151.2±29.5 <sup>a</sup>
Liver				
ALT U/g	27.2±5.0 <sup>a</sup>	15.4±4.1 <sup>b</sup>	16.2±4.3 <sup>b</sup>	25.3±3.2 <sup>a</sup>
AST U/g	31.7±5.2 <sup>a</sup>	18.6±4.1 <sup>b</sup>	17.4±4.0 <sup>b</sup>	29.5±5.3 <sup>a</sup>

<sup>1</sup>Mean ±SE,  $n=6$ .

<sup>ab</sup>Means values within a row not sharing a common superscript letter were significantly different,  $P < 0.05$ .

GI – control group; GII – group receiving atherogenic diet; GIII – group receiving atherogenic diet and Ethanol; GIV – group receiving atherogenic diet and EEP.

## DISCUSSION

In present study, daily feeding with cholesterol ( $\pm 0.14$  g) for 56 days led to a significant increase in plasma total cholesterol, VLDL-cholesterol, LDL-cholesterol and triacylglycerol in rabbits. EEP (GIV) caused a significant decrease in plasma levels of lipids in rabbits made hypercholesterolaemic.

The present results showed that EEP feeding triggered off an increase in HDL-cholesterol. Flavonoids supplementation significantly increased HDL-cholesterol and HDL-cholesterol/total-cholesterol ratio (SEO et al., 2003; YOUSEF et al., 2005).

Administration of EEP produced a significant reduction of serum triacylglycerol, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels. The favorable lipid profile indicates a possible anti-atherogenic property of the flavonoids (ADARAMOYE et al., 2005).

The mechanisms and consequences of the reverse cholesterol transport, which is the movement of cholesterol from the extra-hepatic tissues to the liver, and the role of plasma

HDL-cholesterol as a physiological acceptor of tissue cholesterol, have been reviewed (MILLNER, 1999). The data reported recently by Packard, Caslake and Shepherd (2000) support the protective effect of HDL-cholesterol while LDL-cholesterol is a risk factor. Thus, the data suggest that EEP may be protective against atherosclerosis and cardiovascular disease, particularly because they also decreased plasma LDL-cholesterol level.

The ethanol extract of propolis resulted in decreased serum levels of total cholesterol, triacylglycerol, LDL-cholesterol, VLDL-cholesterol of fasting rats; and to increased serum levels of HDL-cholesterol. This suggests that propolis can modulate the metabolism of blood lipid (FULIANG et al., 2005).

Rabbits fed atherogenic diet (GII) had significantly higher liver total lipids, triacylglycerol and total cholesterol concentration than the GI and GIV groups. The cholesterol-fed group (atherogenic diet) and EEP (GIV) had lower hepatic total lipids, triacylglycerol and total cholesterol concentrations than cholesterol-fed groups (GII and GIII). In rabbits fed cholesterol diet, lipid metabolism was altered and there was an evident decrease in hepatic lipids concentration due to EEP.

The supplementation with flavonoids resulted in a significant decrease in hepatic triacylglycerol and cholesterol. The cholesterol biosynthesis and esterification were concomitantly reduced by flavonoids, by decreased HMG-CoA reductase and acyl CoA-cholesterol acyltransferase (ACAT) activities (LEE et al., 2004).

Bok et al. (1999) suggest that flavonoids reduce cholesterol biosynthesis by means of inhibition of hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and acyl CoA: cholesterol o-acyltransferase (ACAT), resulting in lower hepatic cholesterol level. Reduced ACAT activity may lead to lower availability of cholesterol ester for VLDL-cholesterol packing, thereby resulting in a reduction of VLDL-cholesterol secretion from the liver, as suggested by Carr, Parks and Rudel (1992). In addition, concentrations of cholesterol in the aorta were 33% lower in guinea pigs fed flavonoids diet. These results suggest that flavonoids intake alters hepatic cholesterol metabolism, which may affect VLDL secretion rates and result in less accumulation of cholesterol in the aorta (ZERN; WEST; FERNANDEZ, 2004). In addition, the area covered by fatty streaks in the thoracic aorta was substantially decreased by flavonoids (CHOE et al., 2002). Diets containing flavonoids reduced the VLDL (KUROWSKA; MANTHEY, 2004).

The results revealed the protective effects of EEP on atherogenic diet-induced disorders of AST and ALT activities in the liver and blood of rabbits (Table 5). Atherogenic diet administration significantly decreased AST and ALT activities in the liver and greatly increased these enzymatic activities in plasma. These results suggest that AST and ALT activities in the liver are directly inhibited by atherogenic diet. AST and ALT activities in plasma markedly increased after atherogenic diet administration, indicating a release of these enzymes from the liver injured by the administration of atherogenic diet. EEP treatment prevented both the decrease in AST and ALT activities in the liver and the

increase in these enzymatic activities in plasma that was caused by atherogenic diet-treated.

The changes in these enzymatic activities by administration atherogenic diet were recovered to the control values after EEP treatment. Flavonoids effectively decreased serum alanine aminotransferase levels (CHOE et al., 2002). In this study, EEP significantly prevented lipid accumulation in liver cells and normalized ALT and AST level in blood.

A certain reduction of steatosis degree as well as decreased concentration of liver triacylglycerol and ALT activity was found on liver damage in rats treated with propolis (MERINO et al., 1996). The exact mechanism of the hepatoprotective effect of propolis is unknown, although we believe that propolis has either a membrane-stabilizing effect or an inhibitory effect on the lipid uptake by hepatocytes. This result implies that propolis may be an excellent alternative to the statins for hypercholesterolemic patients with fatty liver.

Twenty-seven different constituents have also been isolated and reported from Brazilian propolis, of which one was new and 15 were isolated for the first time from propolis with hepatoprotective activity (BANSKOTA et al., 2000).

The CCl<sub>4</sub>-induced liver injury model in rats, in which the water extract of Brazilian propolis exhibited stronger hepatoprotective activity (BASNET; MATSUMO; NEIDLEIN, 1997). Sugimoto et al. (1999) found that the 95% ethanolic extract Brazilian propolis possessed strong hepatoprotective activity on D-Galactosamine (D-GalN).

The protective effect of propolis was also demonstrated by looking at the creatine phosphokinase activity in plasma. The creatine phosphatase activity, which were significantly elevated following myocardial injury by atherogenic diet, was significantly reduced to physiological activity in plasma in animals treated concomitantly either with EEP and atherogenic diet.

## CONCLUSIONS

In conclusion, the present study suggests that dietary propolis may be an anti-atherogenic agent. This benefit was observed by the decrease in total cholesterol, VLDL-cholesterol, LDL-cholesterol and triacylglycerol levels, and by the increase of HDL-cholesterol level. The results here indicate that hepatic total lipids, triacylglycerol and cholesterol concentration were lower in rabbits fed EEP. EEP treatment prevented atherogenic diet-induced changes in the activities of AST and ALT in the liver and plasma, indicating the protective effect of EEP against the acute hepatic toxicity caused by atherogenic diet administration.

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