

EFFECT OF WATER-EXTRACTED PROPOLIS ON THE ACCUMULATION OF CHOLESTEROL INDUCED BY HIGH-CHOLESTEROL DIET IN THE RAT

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Introduction

Cholesterol has long been known as a risk factor for cardiovascular diseases. The relationship between dietary lipid and atherosclerosis has been a subject of extensive investigation over the last six decades. Epidemiological studies have demonstrated that high cholesterol diets are atherogenic and plasma high-density lipoproteins cholesterol concentrations are inversely correlated with coronary heart disease incidence.

Phenolic acids are widely distributed in all plant. It had been reported that p-coumaric acid exhibited hypocholesterolemic activity in rats.

Propolis is a natural resinous substance gathered by honey bees from the buds and bark of certain trees and plants, and stored inside their hives. It is rich in flavonoid constituent, including p-coumaric acid, ferulic acid, and other phenolic acid. Previous data indicated p-coumaric acid and ferulic acid that water extracted propolis showed a protective effect on CCl₄-induced acute hepatotoxicity in mice. And some clinical reports showed that there was a lowering of hyperlipidemia as well as propolis effect during the treatment of arteriosclerosis or coronary disease.

Objectives

This study was carried out to elucidate the effect of water extracted propolis (WEP) on serum and the blood lipid level and histological change of liver, when rat was fed a high cholesterol diet. Particularly, our study was done to explore propolis as a possible cholesterol-lowering food additive.

Materials and methods

1. Animals

24 male Sprague-Dawley rats weighing 90-120 g each (5 week old), were purchased from Charles River Japan Inc., and used for this study. The rats were divided into 4 groups and subjected to one of the following treatments for 6 weeks.

Group A received basal diets. Group B receives additionally 1% cholesterol and 0.25% sodium cholate; Group C and D received same diets as group B but were additionally administered orally 75 mg/kg and 150 mg/kg of WEP for 6 weeks, respectively.

At the end of the experimental period, rats were fasted 16 h and anesthetized with carbon dioxide for sample collection. Blood was collected and allowed to clot at room temperature. Serum was prepared by centrifuging the blood at 2,000 G for ten min.

Liver were excised, rinsed, blotted dry, weighed and kept on dry ice. A part of liver was excised and microsomes were prepared. Liver microsomes and serum aliquots were stored at -70 °C until analysis.

2. Lipid analysis

Small aliquots of serum from each animal were taken for total – and HDL – cholesterol (Sigma Diagnostics), and triglyceride (Boehringer Mannheim Diagnostics) analysis. Malondialdehyde was determined as previously de-scribed (OHAKAWA et al.).

LDL-cholesterol values were obtained subtracting the HDL-cholesterol from the total cholesterol for each animal.

Hepatic lipids were determined as previously described (JENNINGS et al.). Cholesterol was measured by a colorimetric method, and triglyceride (TG) was measured by using an enzymatic kit (Asan Chemical Co.).

3. Morphologies of hepatic tissues

For microscopic view of hepatic tissues, liver sections were stored in 10% formalin solution, exchanged with fresh solution daily until staining. Specimens stained with hematoxylin-eosin were obtained under 400 times magnification through a light microscope.

4. Data analysis

Data were analyzed by using one-way ANOVA with completely random-ized design and Duncan's new multiple comparison test.

Results

1. Feed intake, body weight gain, and organ weight gain

No significant differences were observed in the average feed intakes and body weight gain among all groups (Table I).

Table I

Body weight gain, feed intake and feed efficiency ratio of rats fed experimental diets for 6 weeks

Group	Body weight gain (g/day)	Feed intake (g/day)	Feed efficiency ratio
A	3.312±0.12	18.95±0.32	0.175±0.08
B	3.383±0.21	18.89±0.40	0.179±0.03
C	3.356±0.18	19.25±0.26	0.174±0.06
D	3.362±0.24	19.14±0.46	0.176±0.03

Group A: basal diets

Group B: basal diets + 1% cholesterol and 0.25% sodium cholate

Group C: group B diets + 75 mg/kg/day WEP

Group D: group B diets + 150 mg/kg/day WEP

Values were means ± S.D. (n=6)

The weights of kidney, heart were not significantly different in each group, but feeding 1% cholesterol and 0.25% sodium cholate diets resulted in significantly higher liver weights compared with basal diets. It may be the result of fatty infiltration in liver (Table II).

Table II

Organ weights of rats experimental diets (g/100 g body weight)

Group	Liver	Kidney	Heart
A	3.53±0.20	0.75±0.05	0.40±0.02
B	3.94±0.12*	0.79±0.06	0.39±0.02
C	3.91±0.23*	0.79±0.04	0.39±0.01
D	3.90±0.18*	0.77±0.04	0.41±0.02

Group A: basal diets

Group B: basal diets + 1% cholesterol and 0.25% sodium cholate

Group C: group B diets + 75 mg/kg/day WEP

Group D: group B diets + 150 mg/kg/day WEP

Values are mean ± S.D. (n=6)

* Significantly different from the basal diets group A (P<0.05)

2. Serum lipid contents

Table 3 illustrates levels of serum total cholesterol, TG- and LDL-cholesterol levels among four groups. There was a significant difference in total serum cholesterol levels among four groups. Serum total cholesterol levels were significantly greater with addition of 1% cholesterol and 0.25% sodium cholate diets compared with basal diets. But feeding 75 mg/kg – and 150 mg/kg – WEP with 1% cholesterol and 0.25% of sodium cholate diets resulted in significant serum total cholesterol reduction compared with basal diets. Feeding 75 mg/kg – and 150 mg/kg – WEP with 1% cholesterol and 0.25% of sodium cholate diets lowered se.rum TG, but the decrease was much larger in the 150 mg/kg WEP group. In the 75 mg/kg – and 150 mg/kg – WEP treated groups on cholesterol added diet, the HDL-cholesterol tends to increase significantly as opposed to the basal diet group. Reduced atherogenic index is observed in two different doses of WEP treated groups; it is also reduced significantly as compared to cholesterol added diet.

Table III

Serum lipid contents of rats fed experimental diets

	Group A	Group B	Group C	Group D
Total-cholesterol (mg/dl)	90.08 ±7.47**	116.71 ±4.07	103.87 ±13.04*	93.57 ±9.58**
HDL-cholesterol (mg/dl)	41.73 ±3.70	45.85 ±5.58	53.79 ±5.86*	51.30 ±5.66**
LDL-cholesterol (mg/dl) ¹⁾	37.15 ±5.11*	49.26 ±3.65	36.10 ±11.04*	29.99 ±8.68*
HDL-cholesterol Total-cholesterol (%)	46.42 ±3.29*	39.20 ±3.61	52.28 ±7.33**	55.52 ±6.47**
Triglyceride (mg/dl)	55.91 ±7.35**	99.73 ±8.91	69.91 ±5.95**	61.40 ±3.96**
Atherogenic index ²⁾	1.16 ±0.15*	1.57 ±0.22	0.94 ±0.27**	0.83 ±0.20**

Group A: basal diets

Group B: basal diets + 1% cholesterol and 0.25% sodium cholate

Group C: group B diets + 75 mg/kg/day WEP

Group D: group B diets + 150 mg/kg/day WEP

¹⁾ LDL-cholesterol: $[LDL-C] = [T-C] - [HDL-C] + [TG/5]$

²⁾ Atherogenic index: $[T-C] - [HDL-C] / [HDL-C]$

Values were mean ± S.D. (n=6)

* and ** were significantly different from the mean for high-cholesterol diets group B (P<0.05 and P<0.01, respectively)

3. Total liver cholesterol and liver TG

Rats fed both 75 mg/kg- and 150 mg/kg-WEP in combination with 1% cholesterol and 0.25% sodium cholate had a significantly lower total liver cholesterol content compared with the group fed basal diets (Fig. 1).

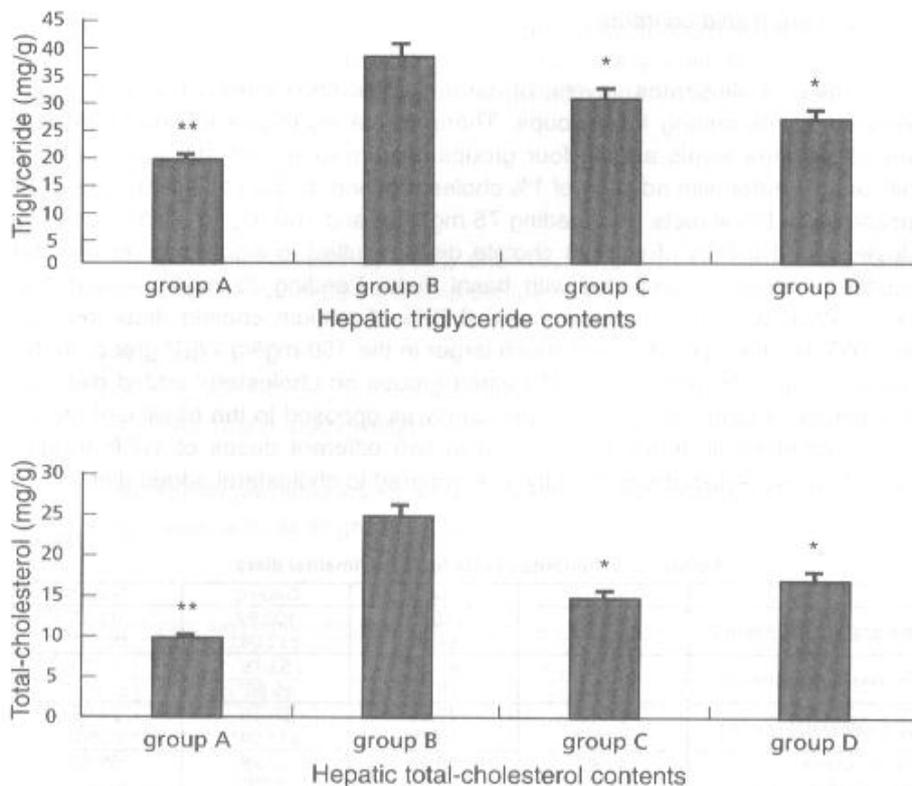


Fig. 1 – Hepatic lipid contents in rats fed the experimental diets

Group A: Basal diets; Group B: basal diets + 1% cholesterol + 0.25% sodium cholate; Group C:

group B diets + WEP 75 mg/kg/day; Group D: group B diets + WEP 150 mg/kg/day;

Values are mean ± S.D. (n=6); * and ** were significantly different from the mean for high-cholesterol diets group B (P<0.05 and P<0.01, respectively)

Total liver TG levels in the groups 75 mg/kg- and 150 mg/kg-WEP supplement were significantly lower compared to 1% cholesterol and 0.25% sodium cholate by 20% and 30%, respectively. (Fig. 1).

4. Serum MDA level

Rats fed both 75 mg/kg- and 150 mg/kg-WEP in combination with 1% cholesterol and 0.25% sodium cholate had a significantly lower serum MDA level compared with the group fed basal diets (Fig. 2).

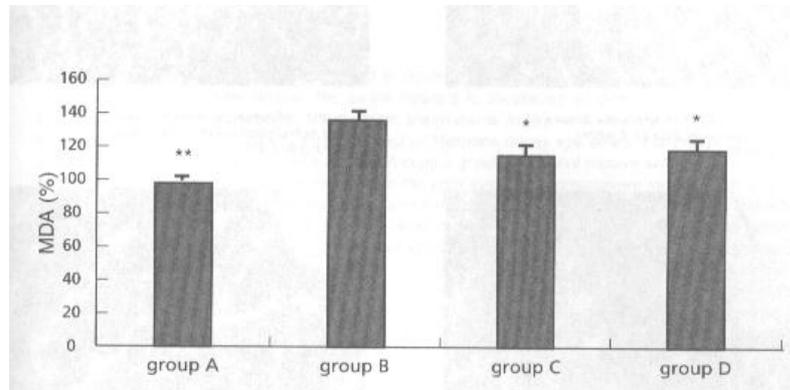


Fig. 2 – Serum MDA concentration in rats fed the experimental diets
 Group A: basal diets; Group B: basal diets + 1% cholesterol + 0.25% sodium cholate; Group C: group B diets + 75 mg/kg/day WEP; Group D: group B diets + 150 mg/kg/day WEP; MDA concentration (%) is compared with group A mean in serum (group A mean = 100%); Values are mean ± S.D., (n=6); * and ** were significantly different from the mean for high-cholesterol diets group B (P<0.05 and P<0.01, respectively)

5. Light microscopic observation of hepatic tissues

As shown in Fig. 3-6, intracellular lipid accumulation and histopathological changes in hepatic tissues were observed in all groups fed with 1% cholesterol and 0.25% sodium cholate diets compared with group fed basal diet. But intracellular lipid dispositions were observed less in the groups WEP supplement.

Conclusion

Adding both 75 mg/kg- and 150 mg/kg-WEP to a high cholesterol diet decreased not only total, LDL cholesterol and MDA, and TG levels in serum but also hepatic concentration of total cholesterol and hepatic TG. Whereas HDL-cholesterol increased by adding both 75 mg/kg- and 150 mg/kg-WEP in rats fed a high cholesterol diet.

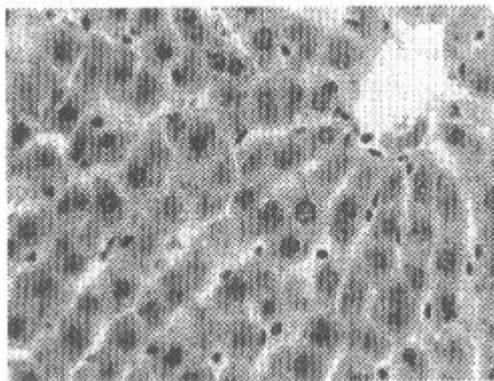


Fig. 3 – Morphology of hepatic tissues in rats fed basal diets for 6 weeks (group A) x 400

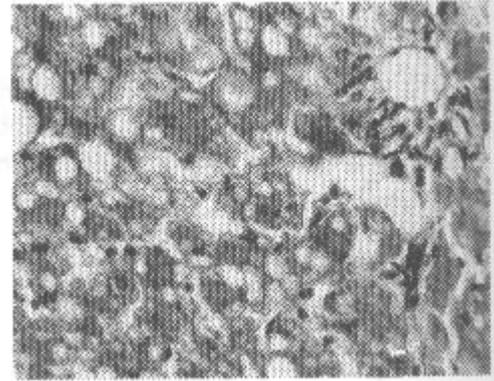


Fig. 4 – Morphology of hepatic tissues in rats fed high-cholesterol diets for 6 weeks (group B) x 400

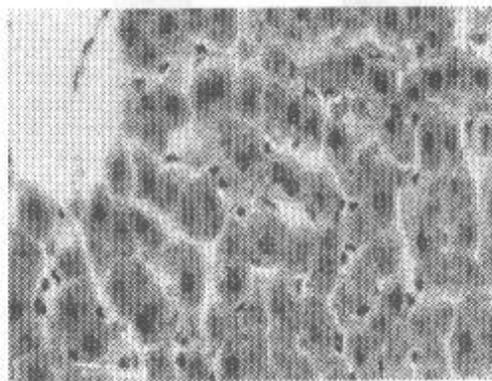


Fig. 5 – Morphology of hepatic tissue in rats fed group B diets + 75 mg/kg/day WEP for 6 weeks (group C) x 400

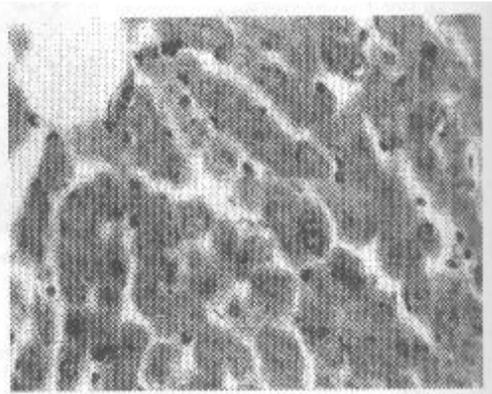


Fig. 6 – Morphology of hepatic tissue in rats fed group B diets + 150 mg/kg/day WEP for 6 weeks (group D) x 400

From these observations, we conclude that WEP addition in diet may show hypocholesterolemic effect.

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