Effect of Propolis Extract on d-Galactosamine-Induced Hepatic Injury in Rats

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The preventive effect of propolis extract on d-galactosamine-induced hepatic injury was examined in rats. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were significantly increased at 24 h after intraperitoneal injection of d-galactosamine (400 mg/kg) in the animals. Propolis extract was administered orally three times in doses of 3 or 30 mg/kg at 18 h and 1 h before and 8 h after d-galactosamine injection. The extract itself and the vehicle alone (dextran) caused no significant changes in serum AST or ALT activities. Treatment with the extract dose-dependently prevented the increases in serum AST and ALT activities induced by d-galactosamine, and significant inhibition was observed at a dose of 30 mg/kg. These results suggested that propolis extract may have an ameliorating effect on hepatic dysfunction.

Key words propolis extract; d-galactosamine; hepatic injury; hepatoprotective effect

Propolis is the sticky, varnish-like substance that is collected by bees.\textsuperscript{1} Several biological activities of propolis extract including its antibacterial,\textsuperscript{2,3} antiviral,\textsuperscript{4} antitumor\textsuperscript{5} and anti-inflammatory effects\textsuperscript{6,7} have been reported. In the last few years, it has been reported that the extract shows a protective effect against liver injury induced by ethanol, acetaminophen and carbon tetrachloride (CCl\textsubscript{4}) in mice and rats.\textsuperscript{8-13} However, acute liver injury and failure are commonly accompanied by a high rate of bacterial and septic complications,\textsuperscript{14} and these infections are frequently of Gram-negative enteric origin. d-Galactosamine intoxication was reported to mimic several aspects of the pathogenesis of human viral hepatitis.\textsuperscript{15} Kasravi \textit{et al.}\textsuperscript{16} however, reported that bacterial translocation is an early phenomenon in acute liver injury induced by d-galactosamine, which corresponds largely to serum levels of liver enzymes. These findings also suggested that d-galactosamine-induced hepatic injury is an important model for viral hepatitis.

In the present study, we investigated the preventive effect of propolis extract on d-galactosamine-induced hepatic injury in rats.

MATERIALS AND METHODS

Materials and Chemicals The propolis used in this study was brown grade from Minas Gerais, Sao Paulo, Rio de Janeiro Espirito, Santo and Parana, Brazil. It is produced throughout the year. The color is brownish or blackish and the aroma is not as strong as green grade propolis. The chemical characteristics are not as high in flavonoids as the green grades, but it has at least 0.7 mg/g of flavonoids (including phenolic and non-phenolic substances). The propolis was resuspended in 95% (v/v) ethanol in a mortar and the suspension was decanted after 48 h at room temperature in our laboratory. This extract was diluted with 1 volume of dextran powder. The propolis extract thus obtained had a concentration of 50% in dextran and was kept in an auto-desiccator. d-Galactosamine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and was dissolved in saline. This solution was adjusted to pH 7.0 with 1 N NaOH, and was filtered through a 0.45 μm disposable syringe filter unit (DISMISC-25, Advantec Toyo, Tokyo, Japan). All other chemicals were reagent grade and obtained from commercial sources. Test drugs were dissolved in water, and were administered orally in a volume of 5 ml/kg of body weight.

Animals and Treatment Six-week-old male Wistar rats were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). Animals were housed in an air-conditioned room maintained at 22—26°C and humidity of 40—70%. Rats were given standard laboratory rodent chow (Oriental Yeast, Tokyo, Japan) and water ad libitum. Rats used ranged from 6 to 8 weeks old at the start of experiments. For the induction of liver damage, the animals were fed until administration of d-galactosamine and then fasted 24 h before sacrifice. d-Galactosamine hydrochloride was dissolved in saline and administered by intraperitoneal injection of a neutral solution, at a dose of 400 mg/kg. Control animals received an equal volume of saline. Propolis extract in 50% dextran, dissolved in water, was administered orally three times in doses of 3 or 30 mg/kg at 18 h and 1 h before and 8 h after d-galactosamine injection. The control group received the same volume of vehicle (dextran water solution) in the same manner. The animals were anesthetized with ether 24 h after injection of d-galactosamine. Blood from the abdominal vein was collected for measurement of biochemical parameters, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Assay for AST and ALT Serum AST and ALT activities were measured by the colorimetric test reported by Reitman and Frankel\textsuperscript{17} using S. TA-test Wako kits from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Data and Statistical Analysis All data are presented as means±S.E. Statistical analysis was performed using one-way ANOVA and Dunnett's test. A probability value of less than 0.05 was considered significant.
RESULTS

Influences of Serum AST and ALT Levels Treated with Propolis Extract, Dextran and D-Galactosamine in Rats

Propolis extract at a dose of 30 mg/kg, p.o., showed no influence on serum AST or ALT level. The vehicle used for propolis extract, dextran, also showed no significant effect in this experimental model (Fig. 1). Serum AST and ALT activities were markedly increased at 24 h after D-galactosamine injection, and serum enzyme activities of AST and ALT in the D-galactosamine-treated group were 14 and 56-fold greater than in the control group, respectively (Fig. 1).

Hepatoprotective Effects of Propolis Extract on D-Galactosamine-Induced Liver Injury in Rats

Propolis extract inhibited the increases in serum AST and ALT activities induced by D-galactosamine injection at doses of 3 and 30 mg/kg in a dose-dependent manner (Fig. 2). A significant effect was observed on these biochemical signs of hepatic necrosis with 30 mg/kg of propolis extract.

DISCUSSION

Oral administration of propolis extract showed no effect on serum AST or ALT levels in rats treated with the extract three times at a dose of 30 mg/kg. Burdock reported recently that propolis is relatively non-toxic, and no remarkable toxic signs were observed following injection of 1400 mg/kg body weight/day for 90 d in mice. Based on these results, the dose of propolis extract used in this experiment was considered appropriate for investigation of pharmacological activity.

The extract was also shown to have a protective effect against hepatocellular necrosis induced by D-galactosamine in rats. Similar results were observed by investigators who reported that rats were protected from CCl₄ or ethanol-induced hepatic injury by propolis extract. The mechanism of action of the extract in D-galactosamine-induced liver injury is still unknown, several investigators have reported that it has free radical scavenging and antioxidant properties. Pascual et al. reported that the scavenging action of propolis extract against oxygen radicals was directed against alkoxy radicals and superoxide anions. They further suggested that its antioxidative properties contribute to the prevention of liver injury induced by acetaminophen in mice and CCl₄ in rats. Mahran et al. also reported that the hepatocyte protective effect of the propolis extract is due to its antioxidant properties, which results from its ability to scavenge free radicals. In association with this, several an-
tioxidants were reported to show hepatoprotective effects against d-galactosamine-induced liver injury, and therefore the pathogenesis of its liver damage is associated with the production of free radicals. It is thus reasonable to presume that the protective effect of propolis extract against d-galactosamine-induced liver injury may be mediated through an antioxidative effect.

On the other hand, it has been shown that the activation of hepatic macrophages by the injection of d-galactosamine, and release of tumor necrosis factor and other cytokines, plays a more important role in the initiation and extension of hepatic necrosis. Hepatic macrophages were found to induce extensive inflammation and necrosis in the liver parenchyma. Shiratori et al. also reported that inhibition of macrophage responses markedly reduced the hepatotoxic effect of d-galactosamine. Khayyal et al. reported that propolis extract has potent anti-inflammatory properties in vivo, and that its activity was correlated well with its effects on the release of various mediators of inflammation. Therefore, the hepatoprotective effect of the extract against d-galactosamine-induced liver injury may be related to its anti-inflammatory activities. These effects of propolis may well be the basis of the often mentioned anti-inflammatory activity of flavonoids. In recent years, a very active propolis ingredient on an inflammation model has become available: caffeic acid phenethyl ester, one of the cinnamic derivatives. There exists a need to identify the key substance of propolis in the inhibition of inflammation. Recently, Miyatake et al. found that the activity of hyaluronidase, known as an inflammatory enzyme, was inhibited by the water extracts from propolis. From these findings, they concluded that the anti-inflammatory action of the water extracts seems attributable to a water-soluble substance contained in the extract, but are not being flavonoids. Furthermore, Miyatake et al. suggested that an unknown substance, which is a poorly water-soluble compound and a non-flavonoid which inhibits histamine release from mast cells, is contained in propolis. It is therefore necessary to isolate the active substance with the hepatoprotective effect.

REFERENCES