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# The antiinflammatory and liver protective effects of *Boehmeria nivea* and *B. nivea* subsp. *nippononivea* in rats

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## Summary

The pharmacological effects of *Boehmeria nivea* and *B. nivea* subsp. *nippononivea* were studied against carrageenan-induced paw edema, acetaminophen (APAP) and D-galactosamine (D-GalN) induced hepatotoxicity in rats, respectively. Water extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* roots were found to maintain significant antiinflammatory activity against carrageenan-induced edema. Moreover, the water extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* significantly decreased the acute increase in serum GOT and GPT levels caused by APAP and D-GalN-intoxication. The histopathological changes of hepatic lesions caused by these hepatotoxicants were improved by treatment with *B. nivea* and *B. nivea* subsp. *nippononivea,* which were compared with silymarin as a standard.

**Key words:** *Boehmeria nivea, B. nivea* subsp. *nippononivea,* carrageenan, hepatoprotective, acetaminophen, D-galactosamine.

## Introduction

*Boehmeria nivea* (L.) Gaud. and *B. nivea* (L.) Gaud. subsp. *nippononivea* (Koidz.) Kitam. (Urticaceae) are widely distributed in China and Taiwan. *Boehmeria nivea* has been used traditionally for diuretic and antipyretic purposes and *B. nivea* subsp. *nippononivea* has been used to eliminate inflammation, neutralize poison and to dissipate heat. Recently, *B. nivea* subsp. *nippononivea* has also been used locally as a remedy for hepatitis in Taiwan. However, the pharmacological effects of these species are obscure. (Kan, 1986)

The components of *B. nivea* subsp. *nippononivea* have been studied. Behenic acid, palmitic acid, stearic acid, ursolic acid, 19α-hydroxyursolic acid, β-sitosterol, β-sitosteryl-β-D-glucoside, a hydroxyaliphatic acid ester and two unsaturated aliphatic alcohols were isolated from the roots of *B. nivea* subsp. *nippononivea* (Matsuura and Lee, 1974). In Taiwan, people use the aqueous extracts from the roots of *B. nivea* and *B. nivea* subsp. *nippononivea* as a remedy for disease. So we selected the roots of these plant in this study.

The aim of the present study is to clarify the antiinflammatory and hepatoprotective effects of these two crude drugs. The pharmacological studies were evaluated in carrageenan-induced paw edema; acetaminophen (APAP) and D-galactosamine (D-GalN) induced hepatotoxicity in rats, respectively. Liver damage was assessed by means of biochemical studies (sGOT and sGPT) and by histopathological examination.

## Materials and Methods

### *Test animals*

Male Wistar albino rats were purchased from the National Laboratory Animal Breeding and Research Center, National Science Council, and were maintained for one week on a commercial diet under environmentally controlled conditions (room temperature 22 ± 3 °C, relative humidity 55 ± 5%) with free access to food and water. A

controlled 12 h light/dark cycle was maintained. Rats weighing 150–180 g were used for carrageenan-induced edema and D-GalN-induced hepatotoxicity, respectively; rats weighing 120–150 g were used for APAP-induced hepatotoxicity.

*Preparation of crude extracts*

Dried roots of *B. nivea* (200 g) or *B. nivea* subsp. *nippononivea* (200 g) were decocted for 1 hr with 1 L of boiling water three times. The decoction was mixed, filtered, concentrated and lyophilized. The crude drug powder was dissolved in normal saline to make a solution (100, 300, 500 mg/ml/kg in rats) prior to intragastric administration to the experimental animals. These plant materials were identified by C. C. Lin, School of Pharmacy, Kaohsiung Medical College, and their voucher specimens were deposited at the Herbarium of the School of Pharmacy, Kaohsiung Medical College.

*Carrageenan induced rat paw edema*

Edema in the left hind paw of each rat was induced by injection of 0.05 mL of 1% (w/v) carrageenan (Sigma Chemical Co., USA) in saline according to the method described by Winter et al. (1962). The animals received the crude drugs or 10 mg/kg of indomethacin (Sigma Chemical Co., USA) suspended in 1% CMC s.c., respectively 1 hr before carrageenan injection. The paw volume was measured with a Plethysmometer 7150, UGO Basil (Italy) before and at 1, 2, 3, 4, and 5 hr after injection of the irritant. The edema and inhibition rates of each group were calculated as follows:

$$\text{Edema rate (E)}\ \% = \frac{Vt - Vo}{Vo} \times 100$$

$$\text{Inhibition rate (I)}\ \% = \frac{Ec - Et}{Ec} \times 100$$

Vo = The volume before carrageen injection. (ml)
Vt = The volume t-hour after carrageen injection. (ml)
Ec = Edema rate of control group.
Et = Edema rate of treated group.

*APAP-induced hepatotoxicity in rats*

A method of acute hepatotoxicity induction was used based on our previous report (Lin et al., 1995). Animals were fasten for 16 hr, then divided into nine groups of six rats each. Group 1 was given normal saline (10 ml/kg, i.p.) as a normal control group. Group 2 was injected with APAP alone (500 mg/kg, in 25% P.E.G. 400) (Sigma Chemical Co., USA). Groups 3–9 were intragastricly (i.g.) administered tested drugs and silymarin (25 mg/10 ml suspended in 1% CMC) (G & F Hanse Biopharma), once 2 h before the injection of APAP and twice at 2 and 6 h after injection, respectively.

*D-GalN induced hepatotoxicity in rats*

Animals were divided into nine groups of six rats each. Group 1 was administered i.g. with saline (10 ml/kg) as normal controls. Group 2 was injected i.p. with D-GalN alone (Sigma Chemical Co., USA; 400 mg/ml/kg in saline) (Lin et al., 1993). Groups 3–9 were administered with different doses of test drugs (i.g.) or silymarin 2 h prior to the injection of D-GalN. Furthermore, two additional treatments of test drugs were given i.g. at 2 h and 6 hr after D-GalN administration.

**Table 1.** Antiinflammatory activity of the aqueous extracts from the roots of *B. nivea* (BN) and indomethacin on carrageenan-induced paw edema in rats.

| Groups | Dose (mg/kg) | Edema rate (E%) after carageenan administration (hours) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Control | – | 18.0 ± 1.15 | 39.85 ± 0.73 | 58.37 ± 2.04 | 51.52 ± 0.42 | 39.27 ± 1.96 |
| Indomethacin | 10 | 13.38 ± 0.40[a] (25.6) | 22.42 ± 1.13[a] (43.8) | 27.33 ± 1.45[a] (53.3) | 20.98 ± 0.85[a] (59.3) | 19.27 ± 1.96[a] (50.9) |
| BN | 100 | 14.95 ± 0.58[a] (16.9) | 26.68 ± 1.55[a] (33.0) | 31.3 ± 0.83[a] (46.4) | 28.77 ± 1.09[a] (44.1) | 25.72 ± 1.69[a] (34.5) |
| | 300 | 13.88 ± 0.75[a] (22.8) | 27.77 ± 1.87[a] (30.3) | 30.92 ± 2.06[a] (47.1) | 21.17 ± 2.02[a] (59.0) | 17.2 ± 2.6[a] (56.2) |
| | 500 | 13.7 ± 0.8[a] (23.9) | 29.62 ± 1.94[a] (25.7) | 32.17 ± 2.98[b] (45.0) | 25.38 ± 1.56[a] (50.7) | 18.7 ± 2.2[a] (52.4) |

Values represent the mean ± S.E. of 6 animals each group.
[a] $p < 0.01$; [b] $p < 0.05$ (Student's *t*-test), significantly different from control group.
Values within parentheses indicate the inhibition percentage (I%).

**Table 2.** Antiinflammatory activity of the aqueous extracts from the roots of *B. nivea* subsp. *nippononivea* (BF) and indomethacin on carrageenan-induced paw edema in rats.

| Groups | Dose (mg/kg) | Edema rate (E%) after carageenan administration (hours) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Control | – | 20.54 ± 1.78 | 40.00 ± 1.86 | 58.31 ± 1.72 | 59.47 ± 1.23 | 44.40 ± 1.28 |
| Indomethacin | 10 | 16.02 ± 1.24 | 20.50 ± 2.20[a] (48.8) | 28.76 ± 2.88[a] (50.7) | 29.21 ± 2.41[a] (50.9) | 26.09 ± 2.14[a] (41.2) |
| BF | 100 | 23.58 ± 2.42 | 32.07 ± 2.71[b] (19.8) | 36.70 ± 1.80[a] (37.1) | 32.05 ± 2.27[a] (46.1) | 26.30 ± 0.82[a] (40.8) |
| | 300 | 18.81 ± 1.61 | 30.93 ± 1.93[a] (22.7) | 30.93 ± 1.93[a] (47.0) | 33.58 ± 0.97[a] (43.5) | 23.37 ± 1.00[a] (47.4) |
| | 500 | 17.84 ± 2.85 | 22.90 ± 0.73[a] (42.8) | 25.93 ± 0.99[a] (55.5) | 29.46 ± 1.8[a] (50.5) | 22.61 ± 1.46[a] (49.1) |

Values represent the mean ± S.E. of 6 animals each group.
[a] $p < 0.01$; [b] $p < 0.05$ (Student's *t*-test), significantly different from control group.
Values within parentheses indicate the inhibition percentage (I%).

**Table 3.** The hepatoprotective effect of the aqueous extracts of *B. nivea* (BN), *B. nivea* subsp. *nippononivea* (BF) roots and silymarin on acetaminophen (APAP)-induced hepatitis in rats.

| Groups | Dose (mg/kg) | sGOT (IU/L) | sGPT (IU/L) |
|---|---|---|---|
| Control | – | 116.8 ± 6.8 | 26.8 ± 0.7 |
| APAP | 500 | 7761.3 ± 518.1 | 1989.8 ± 212.0 |
| BN | 100 | 2098.5 ± 209.7[a] | 499.8 ± 45.8[a] |
| | 300 | 195.0 ± 8.3[a] | 100.6 ± 3.6[a] |
| | 500 | 197.2 ± 20.2[a] | 90.3 ± 9.7[a] |
| BF | 100 | 236.7 ± 6.9[a] | 156.5 ± 2.8[a] |
| | 300 | 255.1 ± 47.3[a] | 196.6 ± 31.3[a] |
| | 500 | 739.8 ± 134.0[a] | 229.3 ± 20.9[a] |
| Silymarin | 25 | 402.3 ± 74.0[a] | 138.4 ± 16.1[a] |

Values represent the mean ± S.E. of 6 animals each group.
[a] $p < 0.01$ (Student's *t*-test), significantly different from APAP-treated group.

**Table 4.** The hepatoprotective effect of the aqueous extracts of *B. nivea* (BN), *B. nivea* subsp. *nippononivea* (BF) and silymarin on D-galactosamine (D-GalN)-induced hepatitis in rats.

| Groups | Dose (mg/kg) | sGOT (IU/L) | sGPT (IU/L) |
|---|---|---|---|
| Control | – | 64.8 ± 7.1 | 27.9 ± 2.4 |
| D-GalN | 400 | 413.9 ± 27.8 | 184.4 ± 7.0 |
| BN | 100 | 119.9 ± 13.0[a] | 41.3 ± 4.4[a] |
| | 300 | 104.9 ± 7.9[a] | 36.7 ± 3.1[a] |
| | 500 | 80.6 ± 13.9[a] | 25.4 ± 3.5[a] |
| BF | 100 | 101.8 ± 4.3[a] | 25.9 ± 2.5[a] |
| | 300 | 146.3 ± 17.5[a] | 54.1 ± 6.6[a] |
| | 500 | 381.9 ± 36.8 | 187.1 ± 23.4 |
| Silymarin | 25 | 153.4 ± 20.2[a] | 47.8 ± 2.4[a] |

Values represent the mean ± S.E. of 6 animals each group.
[a] $p < 0.01$ (Student's *t*-test), significantly different from D-GalN-treated group.

*Assay of serum GOT and GPT activities*

All animals were anaesthetized with ether and then blood was withdrawn from the carotid artery 24 h after APAP or D-GalN intoxication. The blood was centrifuged with 3000 × rpm at 4 °C for 10 min to separate the serum. Glutamic oxaloacetate transaminase (sGOT) and glutamic pyruvic transaminase (sGPT) activities were measured according to the method previously described (Reitman and Frankel, 1957).

*Histopathological observation*

After blood was collected, sections were taken from each lobe of the liver immediately. The tissues were fixed in 10% neutral formalin for at least 24 hrs, dehydrated in gradients of alcohol (50%–100%) and embedded in paraffin, cut into 4–5 μm thick sections, and stained with haematoxylin – eosin for photomicroscopic assessment.

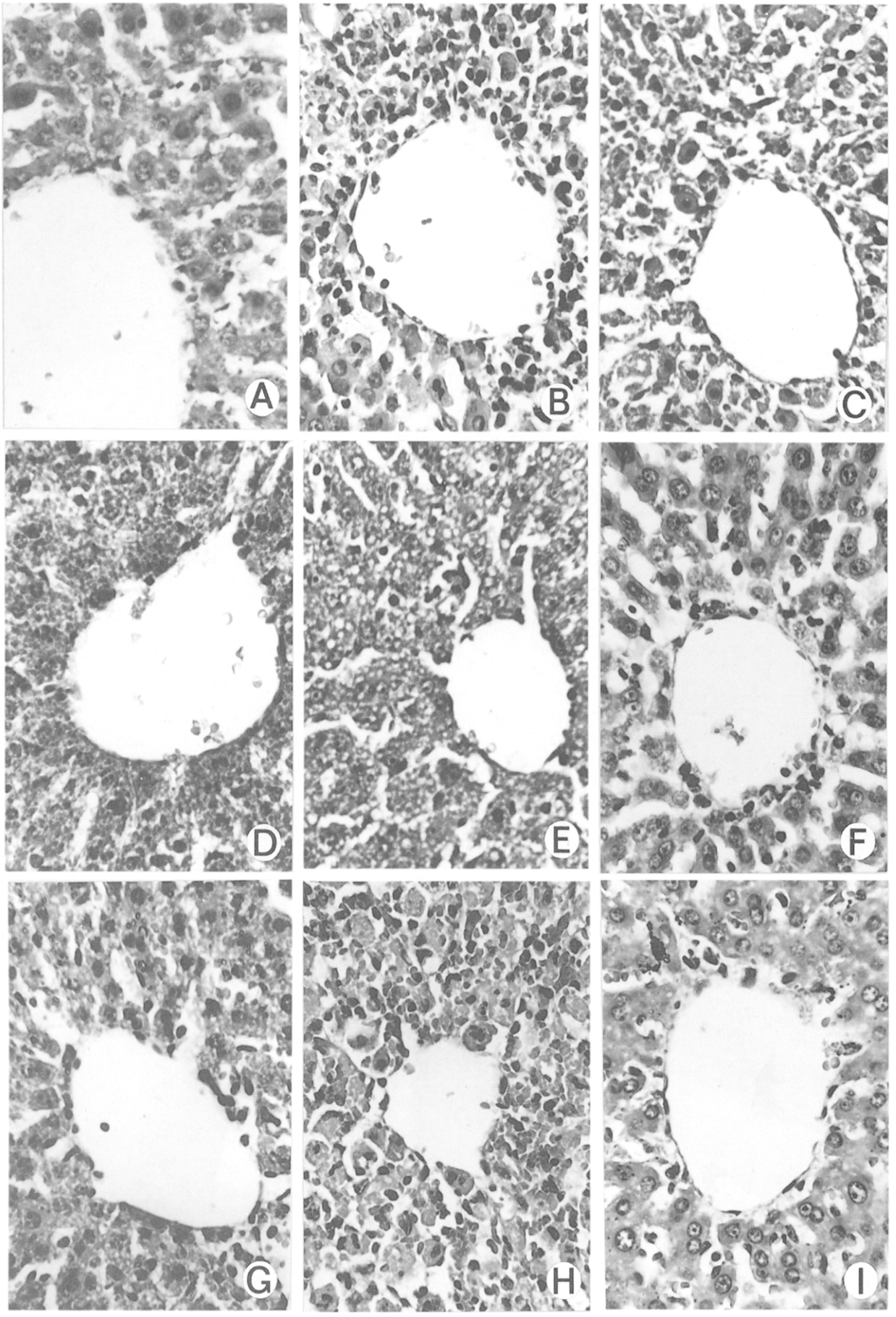
*Statistical analysis*

All serological data were expressed as means ± S.E. The student's *t*-test was applied for detecting the significance of difference between different groups. $p < 0.05$ was regarded as significant.

## Results

*Carrageenan edema test*

Table 1 and Table 2 showed the time course of edema and inhibition rate after subplantar injection of carrageenan. After the injection of carrageenan the paw volume increased rapidly and rose to a maximum at 3 h. Treatment with *B. nivea, B. nivea* subsp. *nippononivea* and indomethacin showed significant antiinflammatory effects against carrageenan-induced paw edema at all time intervals, but it was not significant at 1 hr. ($P < 0.01$, $P < 0.05$). Both the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water extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* displayed a maximum inhibition rate at 3 h or 4 h.

*Effect on APAP-induced hepatotoxicity*

The activities of sGOT and sGPT after administration of APAP are summerized in Table 3; the protective effect was compared with silymarin. The sGOT and sGPT levels were elevated significantly after APAP injection. In contrast, treatment with *B. nivea, B. nivea* subsp. *nippononivea* and silymarin markedly reduced enzyme activities caused by APAP administration ($P < 0.01$). According to these results, the 300 mg/kg and 500 mg/kg doses of *B. nivea* showed the best hepatoprotective effect (Newman-keuls test). The histopathological changes of rat liver tissues under microscopic examination in the APAP induced group are shown in Fig 1. The focal vacuolization of centrilobular hepatocytes, sinusoidal dilation and congestion, broad infiltration of the lymphocytes and Kupffer cells, loss of cell boundaries and ballooning degeneration around the central vein were markedly observed in the APAP-induced group (Fig. 1-B). In contrast, drug-treated groups showed the moderate changes of focal vacuolization of centrilobular hepatocytes, broad infiltration of the lymphocytes and Kupffer cells, and ballooning degeneration around the central vein.

*Effect on D-GalN-induced hepatotoxicity*

The histopathological changes of rat liver tissues under microscopic examination in the D-GalN induced group are shown in Fig 2. Liver damage produced by D-GalN was assessed by measuring the sGOT and sGPT levels. It can be observed from Table 4 that sGOT and sGPT levels induced by D-GalN intoxication were elevated significantly in comparison with the control groups. The enzyme activities were significantly decreased in all drug-treated groups ($P< 0.01$), except the 500 mg/kg of *B. nivea* subsp. *nippononivea*. The 100 and 300 mg/kg of *B. nivea* and the 100 and 300 mg/kg of *B. nivea* subsp. *nippononivea* extracts exhibited moderate effects based on declining sGOT and sGPT levels (Newman-Keuls test). On histopathological examination, inflammatory infiltration of the increase of lymphocytes and Kupper cells, hyaline degeneration, necrosis, after D-GalN administration were observed more remarkably around the portal vein than in the central vein area (Fig 2-B). In contrast, extract-treated groups showed moderate changes of hyaline degeneration and slight increases of fatty changes, Kupffer cells and lymphocytes (Fig. 2 C-I).

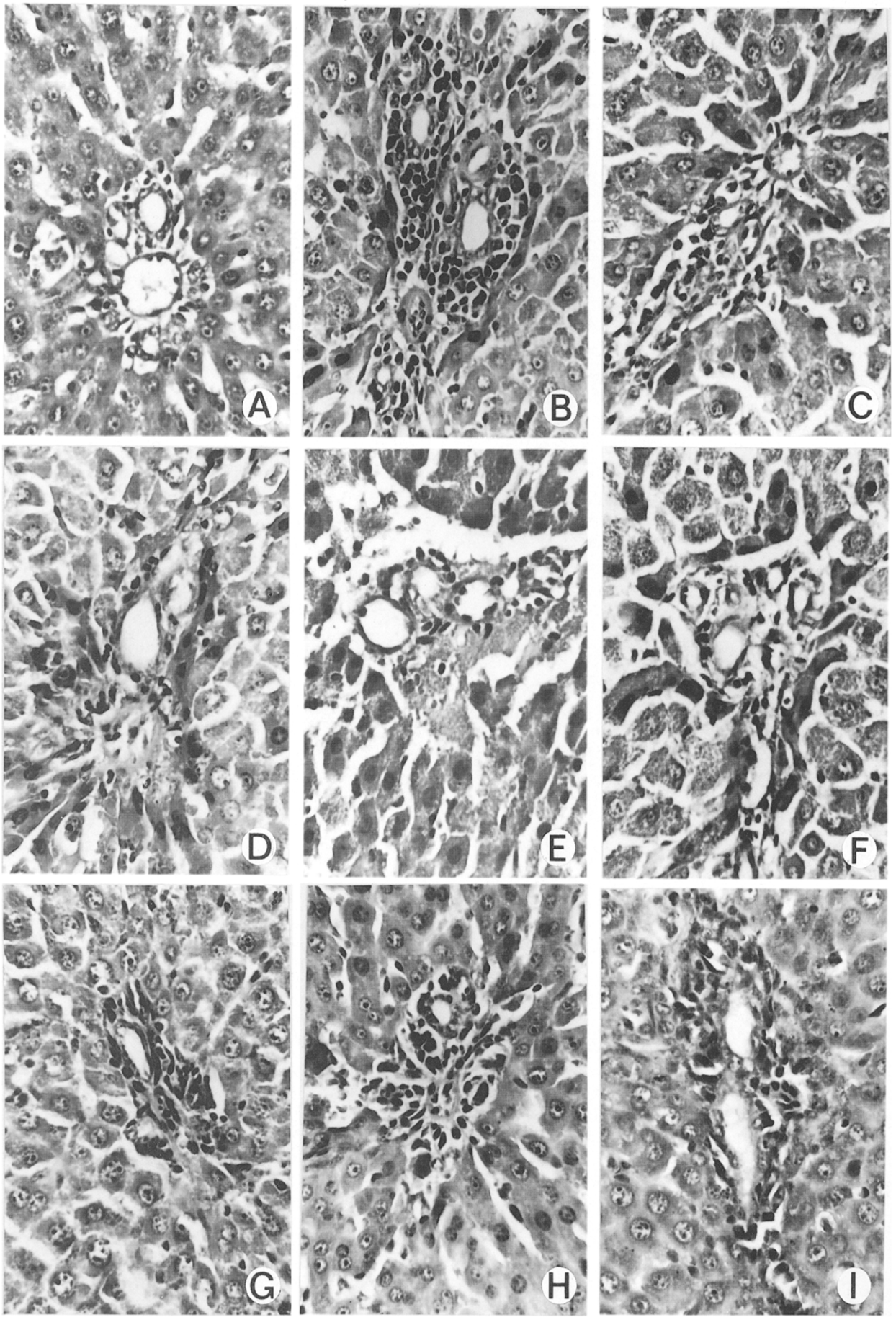
**Fig. 1.** The photomicrographs of liver section taken from rats. A: control group (oral); B: APAP + P.E.G. 400 (500 mg/kg, i.p.); C: APAP + BN (100 mg/kg, oral); D: APAP + BN (300 mg/kg, oral); E: APAP + BN (500 mg/kg, oral); F: APAP + BF (100 mg/kg, oral); G: APAP + BF (300 mg/kg, oral); H: APAP + BF (500 mg/kg, oral); I: APAP + Silymarin (25 mg/kg, oral).

## Discussion

The present study clearly showed that the aqueous extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* demonstrated significant effects against carrageenan-induced inflammation in rats.

The edema after carrageenan injection is believed to be biphasic in nature, the first phase, beginning 1 hr after administration of the irritant, is due to the release of histamine and serotonin; the second phase, occurring 3 to 5 h after the initial injection, is induced by the release of bradykinin, protease, prostaglandin and lysosome (Di Rosa et al., 1968; Vinegar et al., 1969; Crunkhon et al., 1971; Flower et al., 1972; Kubota et al., 1979; Vinegar et al., 1987). It has been reported that the second phase of edema is sensitive to most clinically effective antiinflammatory agents such as indomethacin (Vinegar et al., 1969; Di Rosa et al., 1971). According to our results, the inflammatory phenomenon peaked at 3 hr and 4 hr. The drug-tested groups showed the best inhibition at 3 hr or 4 hr. Our results indicate that all drug-treated rats showed significant suppression of edema formation as compared with controls at 2, 3, 4 and 5 hr. This indicates that the test drugs were effective against the release of bradykinin, protease, prostaglandin and lysosome. Statistical analysis suggested that the water extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* reduced the rat paw edema response.

Acetaminophen (APAP) is widely used as a nonprescription analgesic. However, high doses of APAP can cause centrilobular hepatic necrosis and death in experimental animals and humans (Boyd and Bereczky, 1966; Prescott et al., 1971; Mitchell et al., 1973a, 1973b; Black, 1980). APAP is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P-450 system to produce liver injury (Moldeus, 1978). The toxicity of APAP has been related to the production of *N*-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI is initially detoxified by conjugation to reduced glutathione (GSH) to form mercapturic acid (Moore et al., 1985). However, when the rate of NAPQI formation exceeds the rate of detoxication by GSH, NAPQI covalently binds to macromolecules, cellular proteins and also oxidizes lipids (Mitchell et al., 1973a, 1973b) and alters homeostasis of calcium (Nelson, 1990) after depleting of glutathione. Our studies indicate that treatment with APAP produced severe centrizonal hemorrhagic hepatic necrosis, ballooning degeneration and fatty changes. It also remarkably increased the levels of sGOT and sGPT after APAP administration. Compared with APAP group, we found that *B. nivea* 300 and 500 mg/kg represented the best hepatoprotective effect in serum enzyme examinations (Newman-Keuls test). A dose of 100 mg/kg of *B. nivea* excepted, all drug-treatment groups showed moderate inhibition. The phenomenon was also confirmed by liver biopsy. It was found that the histopathological damages induced by APAP were moderately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improved in rat liver treated with all test drugs, but not including 100 mg/kg of *B. nivea*.

D-Galactosamine has been shown to produce liver damage similar to human viral hepatitis (Keppler and Decker, 1969). It was assumed that D-GalN induced UDP-galactosamine formation must trap many uridine phosphates (Keepler, 1976; Rasenack et al., 1980), which often leads to a marked decrease of several uracil nucleotides, including UTP, UMP, UDP-galactose, and UDP-glucose concentration (Decker and Keppler, 1974), and finally impairs m-RNA and glycoprotein synthesis (Anukarahanonta et al., 1973), and alters the composition of cellular membranes (Hendrina et al., 1983). In addition, intense galactosamination of hepatocellular membrane structures may be responsible for the impairment of the calcium pump, leading to a disruption of calcium ion homeostasis, with a consequent increase in intracellular calcium. This leads to calcium accumulation in the hepatocytes (De Ferreyra et al., 1985) and their eventual destruction, considered to be responsible for cell death (Stinozuka et al., 1973). Our studies demonstrate that the levels of sGOT and sGPT, increased 24 h after D-GalN intoxication and declined significantly in animals treated with *B. nivea* (100, 300, 500 mg/kg), *B. nivea* subsp. *nippononivea* (100, 300 mg/kg) and silymarin, respectively. This observation was also confirmed by histological examination, including periportal inflammation, hyaline degeneration, mitoses, necrosis, as well as increased number of lymphocytes and Kupffer cells, caused by D-GalN intoxication.

In conclusion, the water extracts of *B. nivea* and *B. nivea* subsp. *nippononivea* possessed marked antiinflammatory activity against carrageenan-induced edema and exhibited a liver protective effect against $CCl_4$ and D-GalN induced hepatotoxicity. However, the relationship of the mechanisms and activity of pure compounds from *B. nivea* and *B. nivea* subsp. *nippononivea* require further study.

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**Fig. 2.** The photomicrographs of liver section taken from rats. A: control group (oral); B: D-GalN group (400 mg/kg, i.p.); C: D-GalN + BN (100 mg/kg, oral); D: D-GalN + BN (300 mg/kg, oral); E: D-GalN + BN (500 mg/kg, oral); F: D-GalN + BF (100 mg/kg, oral); G: D-GalN + BF (300 mg/kg, oral); H: D-GalN + BF (500 mg/kg, oral); I: D-GalN + Silymarin (25 mg/kg, oral).

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