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Neuropharmacological Activity of *Humulus lupulus* Extracts

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Abstract—Neuropharmacological profile of *Humulus lupulus* (hop) extract was studied in mice. At doses above 100 mg/kg(i.p.), it decreased spontaneous locomotor activity and raised the nociceptive threshold in the hot-plate test. At doses above 250 mg/kg (i.p.), it increased pentobarbital-induced sleeping time and produced muscle relaxant effect. At the dose of 500 mg/kg, anticonvulsive effect against pentylenetetrazole-induced convulsion and hypothermic effect was observed.

Keywords—*Humulus lupulus* L. (hop) • hot-plate test • rotarod test • locomotor activity • sleep potentiation • hypothermia • anticonvulsant property

Humulus lupulus L. (hop) (Cannabiaceae) has been reported to have hypnotic and mild sedative effects[1~3]. But there has been no recent report concerning the detailed behavorial effects of the hop. The present study was carried out to elucidate the neuropharmacological effects of hop extract on spontaneous locomotor activity, skeletal muscle relaxation, sleep potentiation, body temperature, and thresholds for pain and seizure in mice.

Experimental

Animals—ICR mice of either sexes(30~40 g, 6~8 weeks of ages) were obtained from Hallym University Laboratory Animal Center and housed in cages with 12hr light-dark cycles with free access to food and tap water.

Hop extract—Hop extract was obtained from Flachsmann Pharmaceutical Company (Zűrich, Switzerland). Various doses of hop extract was administered intraperitoneally(i.p.) thirty minutes before each test, except the spontaneous locomotor activity experiment.

Spontaneous locomotor activity — The locomotor activity (4 mice in each cage) was measured with a motor meter(Rhema-Laboratechnic, Karl Kolb Co., Germany). The size of each cage was 42 cm×26 cm and 18 cm in height. Each mouse was placed in the activity cage immediately after i.p. injection of extract, and spontaneous motor activity was recorded for 1 hr. To avoid any variability, the experiments were conducted from 9:00 a.m. to 12:00 a.m.

Rotarod test—A rotarod test was employed for the measurement of motor incoordination as described by Miller *et al.*[4] Mice were trained on a rota-rod(Rhema-Laboratechnic, Type 436~700, Karl Kolb Co., Germany) until they were able to remain on the rotating rod(13 rev min^{-1}, diameter of 5.5 cm) for at least 120 seconds. Animals that fell several times were discarded. Thirty minutes after i.p. injection of extracts, the time spent on the rotating rod was determined. The cutoff time of each trial was

2 min.

Anticonvulsant action—According to Goodman *et al.*[5] mice were observed for 15 min after i.p. injection of pentylenetetrazole (100 mg/kg) and the time to the onset of tonic extensor convulsion and time to the death were measured.

Sleep potentiation test—The methods of Rachid *et al.*[6] were used. Thirty minutes after intraperitoneal administration of extracts, mice were given i.p. injection of pentobarbital (35 mg/kg). The time between loss and reappearance of righting reflex was measured.

Antinociceptive test—Antinociceptive effect was measured using the hot-plate test according to a method described by Nathan *et al.*[7] The temperature of hot-plate was fixed at 53±0.5° C. Latency for licking the fore-paws was measured for each mouse. Various doses (100~500 mg/kg) of hop extract were administered i.p. for 30 min and the hot-plate response was measured.

Body temperature—Rectal temperature was measured at 0, 20, 40, 60 and 120 min after i.p. administration of hop extracts.

Statistics—Data are expressed as the mean ±S.E.M. The statistical significance of differences among the group means was determined with the Student's *t*-test. Discrepancies with $p<0.05$ were considered statistically significant.

Results and Discussion

Hop extract dose-dependently supressed the spontaneous locomotor activity at doses above 100 mg/kg (i.p.) (Fig. 1). The spontaneous locomotor activity in mice treated with hop extract at doses of 100, 250, and 500 mg/kg were reduced to 53, 11, and 3% of saline-treated control mice, respectively. Hop extract dose-dependently increased the sleeping time in mice treated by a hypnotic dose of pentobarbital (Fig. 2). Hop extract at the dose of 100 mg/kg did not affect the pentobarbital-induced sleeping time. However, at the doses of 250 and 500 mg/kg, hop extract increased the pentobarbital-induced sleeping time 1.9- and

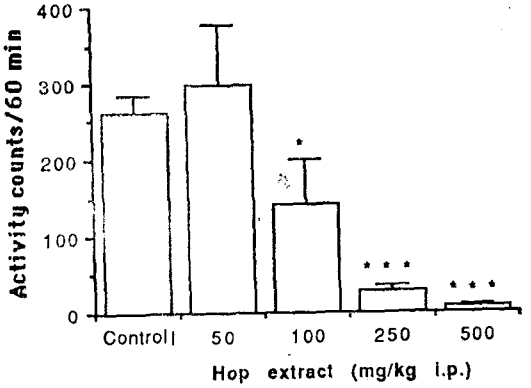

Fig. 1. Effects of *Humulus lupulus* extracts on the spontaneous locomotor activity in mice
Four mice in each cage were placed in the activity cage immediately after i.p. injection of extract, and the spontaneous motor activity was recorded for 1hr. The experiments were conducted from 9 : 00 a.m. to 12 : 00 a.m. The vertical bars indicate the standard error of the mean. *$p<0.05$, ***$p<0.001$

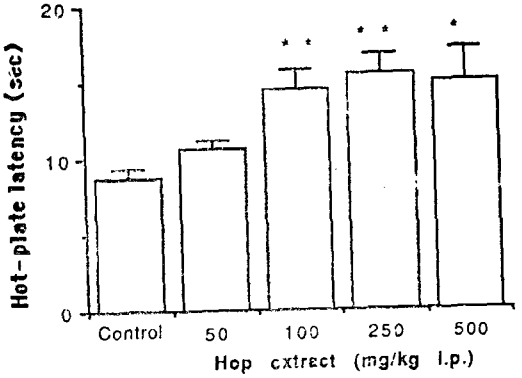

Fig. 2. Effects of *Humulus lupulus* extracts on sleeping time-induced by sodium pentobarbital in mice (n=5~6)
Thirty minutes after i.p. administration of extracts, mice were given i.p. injection of pentobarbital (35 mg/kg). The time between loss and reappearance of righting reflex was measured. The vertical bars indicate the standard error of the mean. *$p<0.05$, **$p<0.01$

2.6-fold, respectively. In the hot-plate test, hop extract at the dose of 50 mg/kg did not inhibit the hot-plate response. However, hop extract significantly increased the the latency for licking the fore-paws at doses above 100 mg/kg(Fig. 3). In the rotarod performance, at doses of 250 and 500 mg/kg(i.p.), it decreased the time that mice were able to remain on the rotarod by 59% and 65%, respectively (Fig. 4). Pentylentetrazole(100 mg/kg, i.p.) induced severe tonic and clonic convulsions followed by 100% mortality in control mice (Table I). At the dose of 500 mg/kg (i.p.), hop extract delayed the onset time of convulsions and significantly prolonged

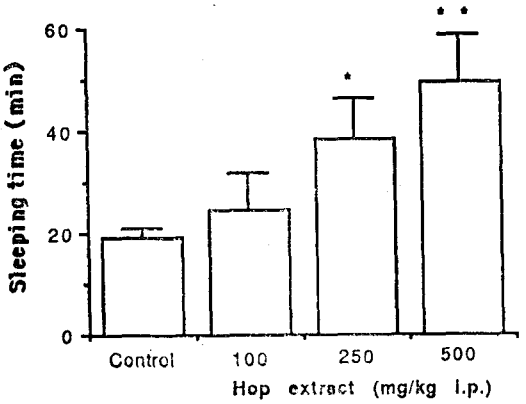

Fig. 3. Analgesic effects of *Humulus lupulus* extracts in the hot-plate test in mice (n=3~6)
The temperature of hot-plate was fixed at 53±0.5°C. Latency for licking the fore-paws was measured for each mouse. Hop extract was administered 30 min before the test at doses of 100, 250 and 500 mg/kg (i.p.). The vertical bars indicate the standard error of the mean. *p<0.05, **p<0.01

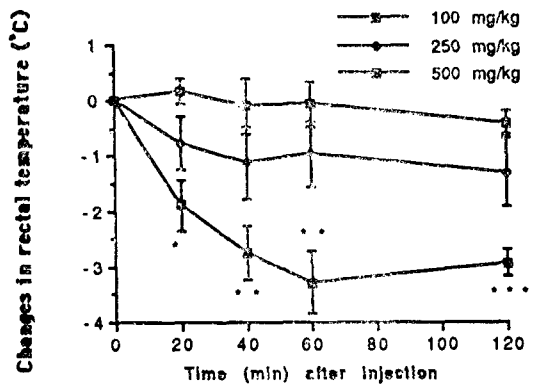

Fig. 4. Effects of *Humulus lupulus* extracts upon the time for which mice were able to stay on a rota-rod (n=4~8)
Mice were trained on the rota-rod until they were able to remain on the rotating rod (13 rev min^{-1}, diameter of 5.5 cm) for at least 120 seconds. Animals that fell several times were discarded. Thirty min after i.p. injection of extracts, the time spent on the rotating rod was determined. The cutoff time of each trial was 2 min. The vertical bars indicate the standard error of the mean. *p<0.05

Table I. Anticonvulsant effects of *Humulus lupulus* extracts on pentylenetetrazole-induced seizure in mice

Dose (mg/kg)	Death[1]	Onset of seizure (min)	Survival time (min)
Control	5/5	1.28±0.19	5.60±0.75
250	6/6	1.02±0.18	6.49±1.75
500	4/6	6.26±2.78	13.38±1.09***

Mice were observed for 15 min after i.p. injection of pentylenetetrazole (100 mg/kg) and the time to the onset of tonic extensor convulsion and time to the death were measured. Mean±S.E.M.
***p<0.001
[1]Number of mice that died/number of mice tested.

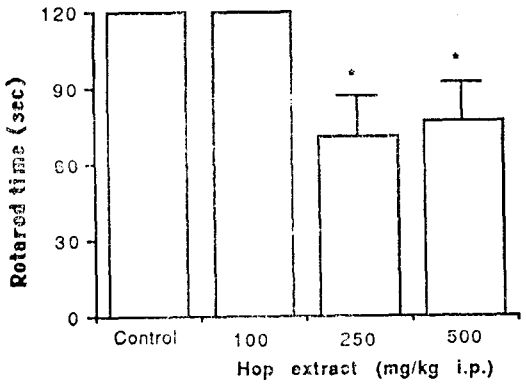

Fig. 5. Rectal temperature changes after administration of *Humulus lupulus* extracts (n=3~6)
Rectal temperature was measured at 0, 20, 40, 60 and 120 min after i.p. administration of various doses of extracts. The vertical bars indicate the standard error of the mean. *p<0.05, **p<0.01, ***p<0.001

survival time in mice. And it protected one third of mice (2 of 6) from death. Finally, at the dose of 500 mg/kg, hop extract caused a significant time-dependent fall in the rectal temperature (Fig. 5).

The above results indicate that hop has following activities: suppression of spontaneous activity, muscle relaxing activity, potentiation of pentobarbital anesthesia, hypothermia, anti-nociceptive and anticonvulsant properties. At lower doses, it decreased spontaneous locomotor activity, raised the nociceptive threshold in the hot-plate test, and increased pentobarbital-induced sleeping time. At higer doses above 250 mg/kg (i.p.), it produced muscle relaxant, anticonvulsive, and hypothermic effect. These results confirm the previous reports on the sedative and hypnotic effect of hop extract[1~3]. Further investigations to find the mechanisms involved in these responses, especially in relation to the possible interaction with neurotransmitter systems, are needed.

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