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Antinociceptive Effect of some Amaryllidaceae Plants in Mice

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Abstract

The antinociceptive effects of ethanolic extracts of *Pancratium maritimum* L., *Narcissus tazetta* subspecies *tazetta* and *Leucojum aestivum* L. bulbs have been investigated in mice using the *p*-benzoquinone-induced abdominal constriction and hot-plate tests.

In the *p*-benzoquinone-induced abdominal constriction test the ethanolic extracts of *P. maritimum* (300, 600 or 1200 mg kg^{-1}, s.c.) and *N. tazetta* subsp. *tazetta* (5, 50, 100 or 200 mg kg^{-1}, s.c.) caused dose-dependent inhibition of abdominal constrictions whereas a fluctuating response was obtained from ethanolic extracts of *L. aestivum* (2·5–500 mg kg^{-1}, s.c.). In the hot-plate test *P. maritimum* and *L. aestivum* caused a significant increase of latency only at the highest concentrations used (1200 mg kg^{-1} and 500 mg kg^{-1}, i.p., respectively). However, at these concentrations they also caused significant toxic effects. In contrast with *P. maritimum* and *L. aestivum*, *N. tazetta* subsp. *tazetta* (5–500 mg kg^{-1}, i.p.) extracts had no antinociceptive effect in this test.

These findings indicate that the antinociceptive effect of Amaryllidaceae plants differs depending on the model of nociception investigated.

Alkaloids from some members of the Amaryllidaceae family have attracted significant attention as a consequence of their physiological properties, including antitumour, antiviral, antiplatelet, cholinergic and anticholinesterase activity (Martin & Brossi 1987; Gabrielsen et al 1992; Sener et al 1992; Sener 1994; Harvey 1995; Pettit et al 1995; Weniger et al 1995). The bulbs of *Narcissus tazetta* subsp. *tazetta* L., a species belonging to Amaryllidaceae are used as home remedies for treatment of abscesses in traditional medicine in Turkey because of their antiphlogistic and analgesic properties. Phytochemical research has resulted in the isolation and structure elucidation of new and known alkaloids from *Pancratium maritimum* L., *Narcissus tazetta* subsp. *tazetta* L. and *Leucojum aestivum* L. growing in Turkey (Sener et al 1993a, b, c). Bioassay-guided fractionation of the extracts is in progress.

In this study, the antinociceptive effect of ethanolic extracts of the bulbs of *P. maritimum*, *N. tazetta* subsp. *tazetta* and *L. aestivum* have been investigated using the *p*-benzoquinone-induced abdominal constriction and the hot-plate analgesic tests.

Materials and Methods

Drugs

p-Benzoquinone (Sigma) and morphine sulphate (TMO, Turkey) were dissolved in 0·9% NaCl solution; acetylsalicylic acid (Sigma) was dissolved in 50% ethanol. Solutions were prepared immediately before use.

Preparation of the extracts

Bulbs of *P. maritimum*, *N. tazetta* subsp. *tazetta* and *L. aestivum* were collected during the flowering period from Inkum, Alanya and Samsun (Turkey), respectively. Voucher specimens (GUE 1078, 1079 and 1080) were deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University. Dried and powdered bulbs (10 g) were extracted (10% w/v) with ethanol (50%) by percolation at room temperature, and the combined ethanolic extracts were evaporated to dryness in-vacuo at 50°C. The evaporated extracts were dissolved in saline or ethanol (50%) immediately before the experiments, and completely dissolved portions were administered by an appropriate route.

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Animals

Animals were fed with commercial food (Yem Sanayii Türk A.S.) and had free access to tap-water. They were maintained in the animal house with a 12-h light–dark cycle at room temperature (20–25°C).

p-Benzoquinone-induced abdominal constriction

Experiments were performed on male albino mice, 16–25 g. Plant extracts, vehicles (saline for *P. maritimum* group and ethanol (50%) for *N. tazetta* subsp. *tazetta* and *L. aestivum* groups) and drugs were injected subcutaneously 30 min before intraperitoneal administration of 0·02% *p*-benzoquinone (0·25 mL) (Okun et al 1963). Acetylsalicylic acid (25 mg kg^{-1}, s.c.) was used as a positive control. *P. maritimum* extracts were tested at 300–1200 mg kg^{-1}, *N. tazetta* subsp. *tazetta* at 5–200 mg kg^{-1} and *L. aestivum* at 2·5–500 mg kg^{-1}. Control animals received vehicle.

Immediately after injection of *p*-benzoquinone, each animal was isolated in a glass box for individual observation for 30 min. The number of abdominal constrictions and stretching was recorded. Percentage protection was calculated by:

$$(\text{Control mean} - \text{treated mean}) \times 100/\text{control mean}$$

Table 1. Effect of extracts of *P. maritimum* and *Narcissus tazetta* subsp. *tazetta* against *p*-benzoquinone-induced abdominal constrictions in mice.

Treatment	Dose (mg kg^{-1})	n	Number of abdominal constrictions	% Protection
Control (saline)	–	7	44·3 ± 9·8*	–
P. Maritimum	300	7	23·3 ± 6·8*	47·4
	600	7	11·7 ± 5·1*	73·6
	1200	7	3·0 ± 1·8*	93·2
Control (ethanol 50%)	–	8	39·9 ± 8·2	–
N. tazetta subsp. *tazetta*	5	8	36·9 ± 12·7	3·2
	50	8	34·4 ± 8·4	9·7
	100	8	14·3 ± 3·9*	62·5
	200	8	9·5 ± 3·3*	75·1
Control (ethanol 50%)	–	10	41·2 ± 3·2	–
Acetylsalicylic acid	25	10	20·6 ± 4·2*	50·0

Results are means ± s.e.m. *$P < 0.05$ compared with control.

Hot-plate test

Experiments were performed on male albino mice, 20–30 g. The hot-plate was used to measure response latency according to the method described by Turner (1965). In these experiments, the hot-plate (Micro Hot-Plate Model 792) was maintained at 55 ± 0·5°C. Animals were placed into a 12 cm diameter glass cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as latency. Latency was recorded for control mice and for animals pre-treated with either morphine (positive control) or with extracts from *P. maritimum* (300–1200 mg kg^{-1}, i.p.), *N. tazetta* subsp. *tazetta* (5–500 mg kg^{-1}, i.p.) and *L. aestivum* (5–500 mg kg^{-1}, i.p.). Animals were selected 24 h previously on the basis of their reactivity in the test-animals showing reaction within the range 3·9–6·9 s were selected. On the day of the experiment, the latency of animals was tested twice with a 10 min interval. Ten minutes after these tests, extracts, references or vehicles were injected intraperitoneally and 15, 30 and 45 min after injection the animals were again tested on the hot-plate. If the mice did not react, a cut off time of 20 s was used to prevent damage to the limbs. Control animals received the same volume of vehicle.

Statistics

Results are presented as mean ± s.e.m. and statistical significance between groups was analysed by analysis of variance followed by the Student–Newman–Keuls multiple comparison test. P values less than 0·05 were considered significant.

Results

p-Benzoquinone-induced abdominal constriction test

Ethanol extracts of *P. maritimum* bulbs caused dose-dependent inhibition of *p*-benzoquinone-induced abdominal constrictions in mice (23·3 ± 6·8, 11·7 ± 5·1 and 3·0 ± 1·8 at doses of 300, 600 and 1200 mg kg^{-1}, respectively; Table 1). Inhibition of abdominal constrictions was significant for doses of 600·0 and 1200·0 mg kg^{-1}. The *P. maritimum* extract had no obvious toxic effects up to the maximum dose used. *N. tazetta* subsp. *tazetta* extracts at doses of 100·0 and 200·0 mg kg^{-1} also significantly reduced the number of *p*-benzoquinone-induced abdominal constrictions (Table 1). *N. tazetta* subsp. *tazetta* extracts were, however, extremely toxic at the 500·0 mg kg^{-1} dose, which resulted in 62·5% mortality within 24 h of the experiments (personal observation). *P. maritimum* and *N. tazetta* subsp. *tazetta* extracts resulted in the percentage protection shown in Table 1.

Ethanolic extracts of *L. aestivum* resulted in significant inhibition only when the dose was 500·0 mg kg^{-1}, which also resulted in 57% mortality within 24 h of completing of the experiments. In the dose range 2·5–50·0 mg kg^{-1} the extract had a fluctuating effect which resulted in inhibition or potentiation of the *p*-benzoquinone-induced abdominal constrictions (data not shown).

Acetylsalicylic acid (25 mg kg^{-1}, s.c.), as the reference drug, resulted in a significant antinociceptive effect compared with its own control values (Table 1).

Hot-plate test

In the hot-plate test there was no significant difference between pre-treatment values obtained on the day of the tests and those obtained 24 h previously during animal selection.

Extracts of *P. maritimum* at 1200 mg kg^{-1} and *L. aestivum* at 500 mg kg^{-1} resulted in a significant increase in latency. Morphine (4·0 mg kg^{-1}, s.c.), as the reference drug, resulted

Table 2. Effect of morphine and of extracts of *P. maritimum*, *L. aestivum* and *N. tazetta* subsp. *tazetta* on latency in the hot-plate test in mice.

Treatment	Dose (mg kg^{-1})	n	Latency (s)
Control	–	15	6·8 ± 0·9
P. maritimum	300	7	6·3 ± 0·8
	600	6	3·6 ± 0·6
	1200	6	9·8 ± 1·5*
L. aestivum	5	6	12·4 ± 4·1
	50	6	9·2 ± 1·4
	500	6	10·0 ± 1·4*
N. tazetta subsp. *tazetta*	5	6	7·5 ± 1·1
	50	6	8·6 ± 2·9
	500	6	6·2 ± 1·1
Control	–	4	5·7 ± 2·0
Morphine	4	4	13·7 ± 2·3*

The results are means ± s.e.m. *$P < 0.05$ compared with control.

in a significant antinociceptive effect compared with its own control values (Table 2).

Discussion

The main findings described in this paper concern the antinociceptive effects of extracts of *P. maritimum* and *N. tazetta* subsp. *tazetta* in the *p*-benzoquinone-induced abdominal constriction test. However, in the hot-plate test, *P. maritimum* and *L. aestivum* resulted in significant antinociception, but only at the highest doses used.

The main mechanism of action of the antinociceptive extracts cannot be determined from these data nor with these tests, because they measure different subtypes of pain: chemical (abdominal constriction test) and thermal (hot-plate test). However the dose-response relationship for *P. maritimum* and *N. tazetta* subsp. *tazetta* in the *p*-benzoquinone-induced abdominal constriction test had a common pattern and both extracts gave a similar protection. It is known that some Amaryllidaceae plants contain anticholinesterase, antiviral and analgesic alkaloids (Martin & Brossi 1987; Lewis 1990); we also observed that they have neuropharmacological (especially sedative) and toxic effects. These effects might in some way interfere with the results obtained with these algesiometric tests.

These preliminary findings lend support to the use of these plants in folklore for treatment of rheumatic pain. It should be noted that ethanolic extracts of *L. aestivum* and *N. tazetta* subsp. *tazetta* might contain potentially dangerous toxic substances. Further work is needed for documentation of their anti-inflammatory and analgesic potential.

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References

Gabrielsen, B., Monath, T. P., Huggins, J. W., Kefauver, D. F., Pettit, G. R., Groszek, G., Hollingshead, M., Kirsi, J. J., Shannon, W. M., Schubert, E. M., Dare, J., Ugarkar, B., Ussery, M. A., Phelan, M. J. (1992) Antiviral (RNA) activity of selected Amaryllidaceae isoquinoline constituents and synthesis of related substances. Nat. Products 55: 1569–1581

Harvey, A. L. (1995) The pharmacology of galanthamine and its analogues. Pharmacol. Ther. 68: 113–128

Lewis, J. R. (1990) Amaryllidaceae alkaloids. Nat. Prod. Rep. 1: 549–556

Martin, S. F., Brossi, A. (1987) The Amaryllidaceae alkaloids. In: Brossi, A. (ed.) The Alkaloids, Vol. 30, Academic Press, New York, pp 251–261

Okun, R., Liddon, S. C., Lasagna, L. (1963) The effects of aggregation, electric shock and adrenergic blocking drugs on inhibition of the 'writhing syndrome'. J. Pharmacol. Exp. Ther. 139: 107–109

Pettit, G. R., Pettit, G. R., 3rd, Groszek, G., Backhaus, R. A., Doubek, D. L., Barr, R. J., Meerow, A. W. (1995) Antineoplastic agents, 301. An investigation of the Amaryllidaceae genus Hymenocallis. J. Nat. Prod. 58: 756–759

Sener, B. (1994) Recent results in the search for bioactive compounds from Turkish medicinal plants. Pure Appl. Chem. 66: 2295–2298

Sener, B., Temizer, H., Könükol, S. (1992) Advances in natural product chemistry. In: Rahman, A.-ur. (ed.), Proc. 5th Int. Sym. and Pakistan-US Bi-national Workshop on Natural Product Chemistry, Harwood Academic Publishers, Singapore, p. 401

Sener, B., Könükol, S., Kruk, C., Pandit, U. K. (1993a) Alkaloids from Amaryllidaceae. I. Alkaloids of lycorine and lycorinine class from *Pancratium maritimum* L. Arch. Pharm. 326: 61–62

Sener, B., Könükol, S., Kruk, C., Pandit, U. K. (1993b) Alkaloids from Amaryllidaceae. IV. Alkaloids from the aerial parts of *Pancratium maritimum*. GUEDE-J. Fac. Pharm. Gazi 10: 83–86

Sener, B., Könükol, S., Kruk, C., Pandit, U. K. (1993c) New crinine-type alkaloids from *Pancratium maritimum* L. growing in Turkey. Nat. Prod. Lett. 1: 287–291

Turner, R. A. (1965) Analgesics. In: Turner, R. A. (ed.) Screening Methods in Pharmacology, Academic Press, New York, pp 100–132

Weniger, B., Italiano, L., Beck, J. P., Bastida, J., Bergonon, S., Codina, C., Lobstein, A., Anton, R. (1995) Cytotoxic activity of Amaryllidaceae alkaloids. Planta Med. 61: 77–79