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Anti-nociceptive activity of alcoholic and acetone extracts of *Stephania japonica* (Thunb.) Miers

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Abstract

The present study was aimed to investigate the analgesic effect of alcoholic and acetone extracts of *Stephania japonica* in mice. These extracts (400 mg/kg taken orally) were evaluated for analgesic activity using the acetic-acid-induced writhing test and tail immersion in hot water, and induced significant reduction in writhing and pain in hot water. Acute toxicity studies showed they have a good margin of safety with no lethal effects up to doses of 4 g/kg.

Keywords:

Introduction

Stephania japonica (Thunb.) Miers (Menispermaceae) is used by ethnic tribal societies as a female contraceptive (Mukhopadhyay *et al*. 1950). It is also recorded in the Indian Ayurvedic system of medicine as having significant effect on the uterus (Sharma *et al.* 2003). The plant is mainly distributed in India, Penang-Siam, the Malay archipelago and Australia (Chatterjee *et al*. 1994). The following effects have been demonstrated: a petroleum ether extract of rhizomes promoted fertility while an alcoholic extract was contraceptive (Bhaduri *et al*. 1968); alcoholic and aqueous extracts of the rhizomes had anti-implantation effects (Kamboj *et al*. 1982); an alkaloid aknadine isolated from aerial parts is reported as a uterosedative (Duke *et al*. 1992); aqueous extracts of the leaves caused reduction in the activities of testicular androgenic key enzymes and plasma level of testosterone along with suppression of spermatogenesis in male rats without any hepatic and renal toxicity (Ghose *et al.* 2002); epistephanine, potentially cytotoxic, from aerial parts produced significant adrenergic neuron blocking activity, its activity estimated to be $1/10^{th}$ of guanethidine (Ray *et al*. 1979); freeze-dried juice of the bulbs had a hypoglycemic effect in insulin-dependent diabetes mellitus (IDDM) but marked hyperglycemic effects in non-IDDM and non-diabetic rats (Mosihuzzaman *et al*. 1994; an alkaloidal extract of the vines has been evaluated for its ability to reverse multidrug resistance (Hall *et al*. 1997); the alkaloid isotrilobine was as active as verapamil in reversing doxorubicin resistance in human breast-cancer cells, and possessed anti-platelet aggregant and anti-inflammatory properties (Duke 1992). Apart from alkaloids (Kupchan *et al*. 1968), the reproductive effects of vine leaves in female rats have also been reported (Subhendu Mukherjee *et al* 2006).

The tribal people of the Nilgiri hills use this plant for headache by keeping the paste of the leaves on the affected part, but there is currently no scientific evidence of any analgesic activity from this plant. We therefore tested the analgesic activity.

Materials & Methods

The leaves of *Stephania japonica* were collected from Ootacamund and identified by Dr. Rajan, Department of Botany, J.S.S College of Pharmacy, Ooty.

Swiss albino mice weighing between 20-25 g of either sex were obtained from colonies maintained at the Central Animal Facility, Government Veterinary College, Bangalore. The mice were housed in polypropylene cages with paddy husk as bedding, and with a stainless-steel top grill with facilities for providing food and drinking water in polypropylene bottles with stainless-steel sipper tubes. The animals were housed at temperatures of 25 ± 2 ^{o}C and relative humidity of 30-60%, in a 12L:12D cycle. The experimental protocol was approved by

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Animal Ethical Committee of the Institute, as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (licensed to the Institute under the number 117/99/kvcp) (see CPCSEA 2010).

The acute toxicity study was carried out according to the guidelines of OECD (2001): mortality in each group within 24 hr was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The alcoholic and acetone extracts of *Stephania japonica* has good margin of safety and did not show the lethal effects on the animals up to doses of 4 g/kg. Hence the LD50 of *S. japonica* was considered to be 4000 mg/kg. Our studies were carried out with 1/10 of the LD50 dose, 400 mg/kg.

Two methods were used to evaluate analgesia: tail immersion in hot water, and the acetic-acid writhing test. For the tail immersion test, Swiss albino mice were screened by exposure to the thermal stimulus. Mice showing a positive response were divided into four groups of six animals each. The animals of first and second groups were treated orally with refined ground-nut oil (1 ml/kg) and indomethacin (10 mg/kg), respectively. The animals of third and fourth groups were administered orally with *S. japonica* alcoholic and acetone extracts (400 mg/kg) respectively. About 2 cm of the tail of each mouse was dipped in warm water kept constant at 50 ± 0.7 ^{o}C. The time taken to withdraw the tail clearly out of water was considered as the reaction time, assessed up to 60 sec. Observations were made on each mouse at 0, 30, 60, 120, and 180 mins (Jibon *et al*. 2005)

Extracts were also evaluated for analgesic activity by the acetic-acid-induced writhing test. Swiss albino mice were divided into four groups of six animals each. The first group was a negative control, receiving refined ground-nut oil (1 ml/kg); the second was a positive control, receiving indomethacin (10 mg/kg); the third and fourth groups were administered orally with alcoholic (400 mg/kg) and acetone extract (400 mg/kg) of *S. japonica* respectively. One hour after treatment, 0.6% v/v acetic acid (10 ml/kg) was given via intraperitoneal injection, and all animals observed for 15 min, and the number of abdominal constrictions (writhings) and stretchings with a jerk of the hind limb were counted (Siegmund *et al.* 1957).
The protection was calculated a % Protection = (1-(Experimental/control)) x 100

One-way analysis of variance (Anova) followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. A probability value of <0.01 was considered statistically significant. Values in the text and tables are represented as mean ± SEM.

Results

For the tail immersion data, treatments were compared with the negative control (Group I). The activity of the acetone extract was significant and equipotent to that of the positive control at 60 mins post-treatment. Our results showed that acetone extract possessed better analgesic activity than alcoholic extract.

			Post treatment reaction times in seconds				
Groups	Extracts	Dose (mg/kg)	0 min	30 min	60 min	120 min	180 min
I	Control (groundnut oil)	1 ml	2.5 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.5± 0.1	2.7 ± 0.1
II	Indomethacin	10	2.7 ± 1.1	7.1 ± 1.1 **	7.7 ± 1.1 **	8.2 ± 1.1 **	8.7 ± 1.1 ***
III	*S. japonica* alc extr	400	2.5 ± 1.0	6.1 ± 1.0 *	6.6 ± 1.0 **	7.2 ± 1.0 **	8.0 ± 1.0 **
IV	*S. japonica* acetone extr	400	2.5 ± 1.0	6.3 ± 1.0 **	6.7 ± 1.0 **	7.8 ± 1.0 **	8.2 ± 1.0 **

Values are expressed as mean ± SEM (N=6 in each group), *=p<0.01, **=p<0.001, ***=p<0.0001 with respect to the control group (Dunnett's multiple comparisons). Because of non-independence we quote the Anova only for the final group ($F_{3,20}$ = 2770.3, p<<0.0001).

Table 1: The analgesic activity of *Stephonia japonica* by the tail immersion method

For the acetic-acid-induced writhing test, the acetone and alcoholic extracts showed significant reductions in the number of writhings compared to the negative control (Table 2).

Groups	Treatment	Dose (mg/kg)	Mean number of writhings	Percentage protection
I	Control (Groundnut oil)	1 ml	47.0 ± 1.2	
II	Indomethacin	10	9.1 ± 0.5 ***	80.5
III	*S. japonica* alc extract	400	17.0 ± 0.6 ***	63.8
IV	*S. japonica* acetone extract	400	13.2 ± 0.9 ***	71.9

Values are expressed as mean ± SEM (n=6 per group); ***= $p<0.0001$ with respect to the control group using Dunnett's multiple range test. The Anova was highly significant ($F_{3,20} = 3107.9$, $p<<0.001$).

Table 2: The analgesic activity of *Stephania japonica* by the acetic-acid-induced writhing response in mice

Discussion

The acetic-acid-induced writhing and tail immersion tests are used to study the action of substances on the peripheral nervous system. Our results showed that the acetone extract possessed slightly better analgesic activity than the alcoholic extract, more or less equipotent to the positive control after about 60 min post treatment. Increases in the immersion time of the tail in hot water suggests that the extracts probably inhibit the production of substance p and bradykinin (WheelerAceto *et al.* 1991). Acetic acid causes nociception by liberating endogenous substances including histamine, serotonin, bradykinin and prostaglandin, which may stimulate pain (Costa *et al*. 2003). Therefore the acetone and alcoholic extracts of *S. japonica* might inhibit the synthesis and release of these endogenous substances.

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الملخص العربى

النشاط المضاد للألم لمستخلصات نبات *Stephania japonica* المذابة فى الكحول والأستيون

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تهدف هذه الدراسة لمعرفة مدى تأثير مستخلصات نبات *Stephania japonica* المذابة فى الكحول والأسيتون كمواد مسكنة للألم على فئران التجارب. ولقد تم تقييم مفعول هذه المستخلصات بإعطاء جرعات بتركيز400 ملجم / كجم عن طريق الفم للفئران التي تتلوى بفعل حمض الخليك أو التي تغمر ذيولها في الماء الساخن، ووجد أن هذه المستخلصات قد قللت من التلوي والألم الناجم عن المياه الساخنة بشكل ملحوظ. وأظهرت دراسات السمية الحادة أن هذا النبات آمن لحد كبير وأن ليس له آثار قاتلة حتى تصل الجرعة إلى 4 جم / كجم.