Pharmacological Trials of Crude Extract of *Passiflora alata*

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Abstract: Dry extract obtained from leaves of *Passiflora alata* yielded 0.217 mg% of harman-like alkaloids and 44.8 mg% of flavonoids. Intraperitoneal administration (i. p.) of 75 and 150 mg/kg of this extract reduced the spontaneous motor activity and prolonged the sleeping time induced by pentobarbital sodium in mice. The extract showed anticonvulsant action on pentylenetetrazole induced seizures, increasing both its onset time and survival period. All animals treated previously with 75 mg/kg of extract died after clonic and tonic convulsions, while 16.7% of the group treated with 150 mg/kg survived. The DL50 value of the extract was estimated as 456 (424–491) mg/kg i. p. in mice.

Introduction

A number of plants of the genus *Passiflora*, known in Brazil as *maracuja*, have widespread use in popular medicine as sedatives and tranquilizers. *Passiflora alata* is one of these plants and it is the only one officially reported by the Brazilian Pharmacopoeia (4). Unlike *Passiflora incarnata* and some other species, which have been extensively described chemically and biologically (3, 5, 7, 9, 14), there is no pharmacological data available on *Passiflora alata* that can justify its medicinal application. The presence of a relatively high amount of 2″-xylosylvitexin in leaves of *Passiflora alata* has been reported by Ulubelen and Oksuz (18), but whether this flavonoid derivative is responsible for any reputed pharmacological effects is not known.

This work was carried out in an effort to determine alkaloid and flavonoid contents in extracts of *Passiflora alata* leaves and to test some pharmacological effects of its crude extract on the central nervous system in mice.

Material and Methods

Plant Material

The plant material used in the present study (adult leaves of *Passiflora alata* Aiton) was obtained from the collection of *Passiflora* species of the department of Phytotechnics at the Faculty of Agrarian and Veterinarian Sciences of São Paulo State University at the Campus of Jaboticabal and found to be identical with samples from the herbarium at the São Paulo Botanical Institute registered under number SP 44606. The material was harvested in March 1983 and dried in the shade.

Animals

Both male and female mice, weighing 35 ± 5 g were used.

Preparation of extract

The fluid extract from leaves of *Passiflora alata* was first prepared according to the French Pharmacopoeia, VIII ed. (12); this extract was evaporated under reduced pressure at a temperature below 50° C to yield a dry extract. The dry extract was dissolved in distilled water for pharmacological trials.

Evaluation of alkaloids and flavonoids

The content of alkaloids was determined according to the method of Bennati (3). The alkaloid fraction was diluted in methanol and chromatographed on silica gel GF-254, by TLC, with a solvent mixture (chloroform, methanol and ammonia 90 : 10 : 0.6) as mobile phase. The evaluation was made by measuring the fluorescence by means of a Photovolt, mod. 520, Densitometer with a 445 nm filter. The total amount of alkaloids was calculated, based on a standard curve of harman, in a concentration range from 0.02 to 0.1 μg/μl. The flavonoid fraction was diluted in methanol and chromatographed on silica gel GF-254, by TLC, with a solvent mixture (ethyl acetate, formic acid and water, 9 : 1 : 1) as mobile phase. After reaction of flavonoid components with a solution of boric acid 3 % and oxalic acid 10 % (3 : 1), a Photovolt mod. 520, densitometer with a 570 nm filter was used to measure the fluorescence at 355 nm wavelength. The total flavonoid amount was calculated, based on a standard curve of orientin, in a concentration range from 0.25 to 1.25 μg/μl.

Acute Toxicity

The extract was administered intraperitoneally (i. p.) in mice, and its toxicity was observed during 48 h. The lethal dose 50 % (LD50) was estimated by the method of Thompson (17).

Pentobarbital Sleeping Time

Sixty minutes after i. p. administration of 75 or 150 mg/kg of extract, a dose of 25 mg/kg of pentobarbital sodium was injected i. p. in mice. A loss of righting reflex was taken as sleeping parameter.

Pentylenetetrazole Convulsion

Sixty minutes after i. p. injection of extract, in doses of 75 and 150 mg/kg, pentylenetetrazole (50 mg/kg, i. p.) was administered and the convulsive effect was observed for 60 successive minutes. The interval from pentylenetetrazole injection to manifest convulsion and the survival period (time from onset of convulsion to death) as well as the percent lethality were recorded in all groups. Diazepam was used as a reference drug.

Analgesic Effect

The analgesic effect of the extract was studied according to the method of Swingle et al. (16) slightly modified, using Ugo Basile Analgesy-Meter. The pain threshold was determined, applying the force to the animal's paw and calculating the analgesia coefficient (i) by the expression:

$$i = \frac{^{a}60 + {}^{a}120 + {}^{a}180}{^{b}60 + {}^{b}120 + {}^{b}180}$$

where a represents the value obtained from the animals treated with extract and b the value obtained from the animals treated with saline. The numbers represent the time in minutes, after drug administration. Two doses of the extract, 75 and 150 mg/kg, were administered i. p. and indoprofen was used as a reference drug.

Spontaneous Motor Activity

Four groups of mice were injected i. p., respectively, with saline, test extract (150 mg/kg), amphetamine (3 mg/kg), and extract plus amphetamine. Amphetamine was used as reference drug. Thirty minutes after each treatment, spontaneous motor activity was recorded for 90

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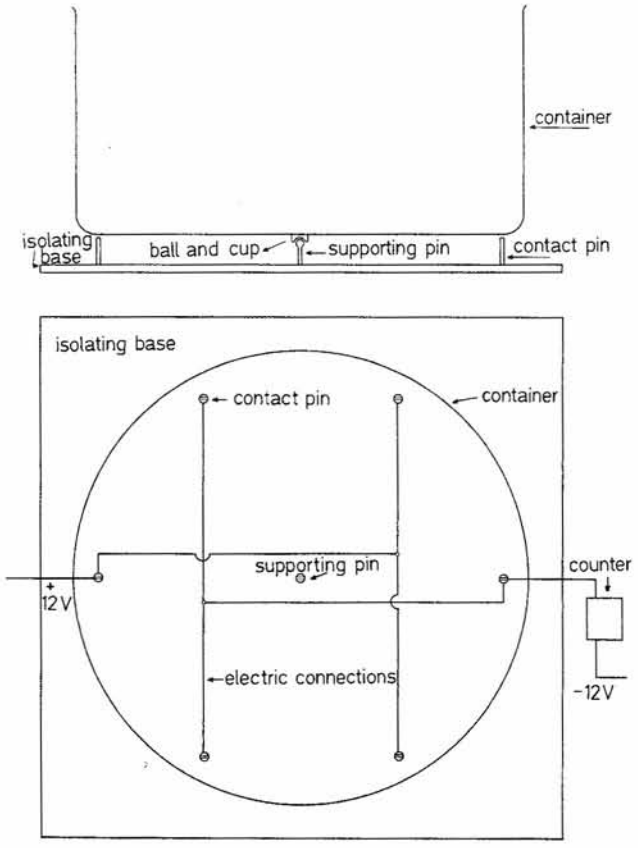

Fig. 1. General layout of apparatus for measuring spontaneous motor activity of mice. Lateral view (upper) of the apparatus and its electrical connections in view from top to bottom of isolating base (lower).

minutes by means of a simple apparatus, idealized and constructed by Wasicky (19), consisting of a cylindrical metal container with a diameter of 30 cm and a height of 15 cm, in which the animal was placed, and an electrically isolating base provided with 6 contact pins and a supporting pin. This pin is placed at the centre of the base and supports the container at a level 1 mm above the contact pins which are placed circumferentially at regular intervalls at a radius somewhat smaller than that of the container. The contact pins are alternately connected in parallel (in groups of three). One group is connected directly to a 12V DC source and the other, in series with an electric impulse counter, to the other pole of the source. The container is placed on the supporting pin by a ball and cup fitting at the centre of its bottom and being in unstable equilibrium, always supported in addition by any two adjacent contact pins. As the animal moves around the container, the contact between pins and container's bottom changes, interrupting momentarily the circuit and producing impulses which are recorded by the counter (Fig. 1).

Statistical Evaluation

The statistical assessment of data was carried out by analysis of variance. Sequential differences among averages were tested according to Tukey contrast analysis (15).

Results

Alkaloid and Flavonoid Contents

Dry extract obtained from leaves of *Passiflora alata* yielded 0.217 mg% of alkaloids expressed in harman. In the flavonoid fraction, a presence of vitexin, isovitexin, and isoorientin was confirmed (Fig. 2). Total amount of flavonoid derivatives was estimated at 44.8 mg% in the dry extract.

Acute Toxicity

The extract submitted for the acute toxicity test in mice revealed that lethal effect is caused in doses over 432 mg/kg. All the

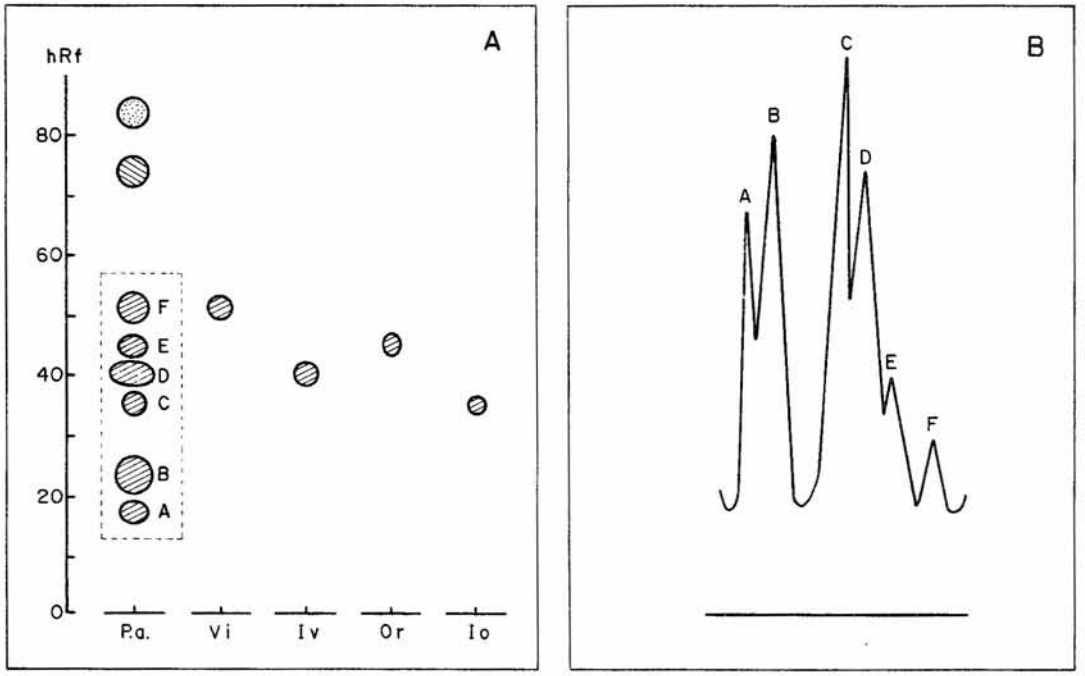

Fig. 2. (*A*) TLC of flavonoid fraction from *Passiflora alata* on silica-gel 60 GF_{254}. Visualization: boroxalic acid, observed in U. V. On the right (*B*) the densitometric recording showing the peaks corresponding to the spots inside the area limited by the dotted line (yellowish green; blue; beige).
P. a. = extract of *P. alata*; Vi = vitexin;
Iv = isovitexin; Or. = orientin; Io. isoorientin.

animals presented depressive symptoms with sleepiness, and in lethal doses death occurred within 24 h. The LD50 value was estimated as 456 (424–491) mg/kg.

Sleeping Time Induced by Pentobarbital

The extract, at doses of 75 and 150 mg/kg, increased significantly the pentobarbital induced time (Table I). The intervals from barbiturate injection to manifest righting reflex loss were slightly reduced by administration of both 75 and 150 mg/kg of extract.

Table I. Effect of dry extract of *P. alata* on pentobarbital induced sleeping time in mice

Group	Sleeping time (min)	Potentiation (%)
Saline (control)	45.1 ± 10.0	100
Extract, 75 mg/kg	107.1 ± 13.0*	138
Extract, 150 mg/kg	139.8 ± 3.4*	210

Sixty minutes after drug administration, 25 mg/kg i.p. of pentobarbital sodium were injected. Each value represents the mean ± standard error of 10 animals.
* Significantly different ($p < 0.05$) from the control.

Pentylenetetrazole Convulsion

As Table II shows, 50 mg/kg of pentylenetetrazole caused clonic and tonic convulsions followed by death in mice. The extract showed anticonvulsant activity, at doses of 75 and 150 mg/kg, prolonging onset time and survival period. All animals treated with 75 mg/kg of extract died, whereas those treated with 150 mg/kg of extract and 5 mg/kg of Diazepam showed, respectively, 83.3 % and 66.6 % lethality.

Table II. Effect of dry extract of *P. alata* on pentylenetetrazole convulsion in mice

Group	Onset Time (s)	Survival Period (s)	Lethality (%)
Saline (control)	26.1±1.7 (12)	74.6± 4.9** (12)	100
Extract, 75 mg/kg	34.0±1.8* (12)	105.6± 8.1* (12)	100
Extract, 150 mg/kg	53.3±6.2** (12)	191.8±22.5* (10)	83.3
Diazepam, 5 mg/kg	54.5±3.4** (12)	292.5±28.5** (8)	66.6

Sixty minutes after drug administration, 50 mg/kg i.p. of pentylenetetrazole were injected. Each value represents the mean ± standard error. Number of animals is shown in the parenthesis.
* Significantly different ($p < 0.05$) from the control
** Significantly different ($p < 0.01$) from the control

Analgesic Effect

The extract did not show analgesic effect at doses of 75 or 150 mg/kg. The analgesic coefficient of indoprofen (25 mg/kg) was 2.01 (Table III).

Spontaneous Motor Activity

As Figure 3 shows, amphetamine (3 mg/kg) increased the spontaneous motor activity during a period of 90 minutes. The extract (150 mg/kg) reduced significantly the spontaneous motor activity of mice, as well as antagonized the amphetamine stimulant effect.

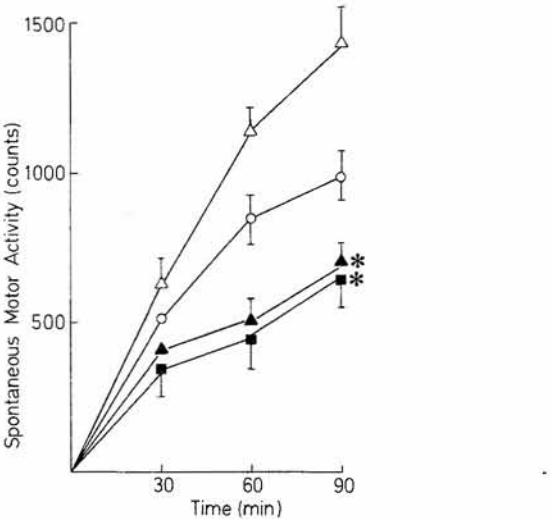

Fig. 3. Effect of dry extract of *P. alata* on spontaneous motor activity in mice.
○- saline; △- 3 mg/kg of amphetamine; ■- 150 mg/kg of extract; ▲- amphetamine plus extract. Each point represents the mean of 6 animals and bars the standard errors.
* Significantly different from the control ($p < 0.01$)

Discussion

Dry extract of *Passiflora alata* caused central nervous system depression in mice, as reported by Lutomski and Wrocinski (10) and Aoyagi et al. (2) for *Passiflora incarnata*. The extract prolonged the sleeping time induced by pentobarbital and reduced significantly the spontaneous motor activity. These effects were observed with doses of 75 and 150 mg/kg of extract; in doses over 400 mg/kg, the extract produced deep depression and it often caused death. Its LD50 value was estimated as 456 mg/kg.

Table III. Analgesic effect of dry extract of *P. alata* in mice

Group	Before Treatment	After treatment (min)			Analgesic Coefficient
		60	120	180	
Saline (control)	90.0 ± 13.9	92.0 ± 17.0	100.0 ± 11.6	89.2 ± 11.5	1.04
Extract, 75 mg/kg	97.5 ± 18.2	87.5 ± 13.9	91.6 ± 17.1	105.9 ± 14.0	0.89
Extract, 150 mg/kg	87.5 ± 12.1	92.5 ± 11.5	110.0 ± 14.2	148.3 ± 4.8	1.18
Indoprofen, 25 mg/kg	85.8 ± 19.4	147.5 ± 11.8	172.5 ± 14.5	197.5 ± 12.2	2.01*

Each value represents the mean weight ± standard error (g) obtained from 10 animals.
* Significantly different ($p < 0.05$) from the control.

The extract also had an antipentylenetetrazole action. At a dose of 150 mg/kg, it reduced the lethality to 83.3 %, whereas the reference drug, diazepam, reduced it to 66.6 %. At a dose of 75 mg/kg, the extract did not protect against death, but prolonged both onset time and survival period.

In spite of its depressant effect, the extract did not demonstrate significant analgesic action, even with a dose of 150 mg/kg.

Pletscher et al. (13) have reported that harmala alkaloids such as harmine and harmaline isolated from *Passiflora incarnata* inhibit monoamine oxidase, and act as central stimulants. These alkaloids have been used especially as a tremorigenic agent (1).

Aoyagi et al. (2) have atributed to maltol the depressant effects of *Passiflora incarnata*, suggesting that these effects mask the stimulant effects of the harmala alkaloids. Maltol, isolated from the 2N HCl soluble fraction, caused a depression in mice and showed potentiation of hexobarbital induced sleep, anticonvulsant action and inhibition of spontaneous motor activity.

Lutomski et al. (8) have observed that the sedative effect of juice of *Passiflora edulis* fruits is due to the presence of small quantities of alkaloids of the harman group and flavonoids.

The components as well as their amounts are variable among different species and even in the same species. Löhdefink and Kating (7) identified harmane, but they did not find harmine, harmaline and harmole in *P. coerulea, P. decaisneana, P. edulis, P. foetida, P. incarnata, P. supeltata,* and *P. warmingi*. High concentrations, 2.5 to 4.1 mg%, of harman alkaloids in leaves of *P. quitensis, P. lingulani* and *P. mollissima* were determined by Martinod (11). Flavonoid concetrations ranging from 0.2 to 2.0 % in dry extract were found by Löhdefink (6). The leaves of *P. alata* furnished 0.1 % of 2″-xylosylvitexin while *P. pittieri* yielded only 0.002 % (18). Lutomski and Malek (9) determined 0.7 mg %, 0.17 mg % and 0 mg % of alkaloids expressed in harman, respectively, in leaves, stalks and roots of *P. edulis* flavicarpa.

The content of harman-like alkaloids found in this work is similar to that reported by Bennati (3) for *P. incarnata* and by Lutomski and Malek (9) for *P. edulis*. The flavonoid contents are similar to those reported by Löhdefink (6) for several species of *Passiflora*.

These alkaloids and flavonoids may be, at least partially, responsible for depressant effects of *Passiflora alata*.

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