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BEHAVIORAL EFFECTS OF *EUPHORBIA HIRTA* L.: SEDATIVE AND ANXIOLYTIC PROPERTIES

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Summary

Lyophilised aqueous extract of *Euphorbia hirta* L. (Euphorbiaceae) has been evaluated for behavioral effects in mice. The extract did not induce any toxic effect when it was administered i.p. and orally. Sedative properties could be confirmed with high doses (100 mg of dried plant/kg, and more), by a decrease of behavioral parameters measured in non-familiar environment tests (activitest and staircase test), whereas anticonflict effects appeared at lower doses (12.5 and 25 mg of dried plant/kg), by an enhancement of behavioral parameters measured in the staircase test and in the light/dark choice situation test. These findings validate the traditional use of *E. hirta* as a sedative and reveal original anxiolytic properties.

Introduction

An ethnopharmacological study of *Euphorbia hirta* L. was undertaken to record its traditional therapeutic indications and to evaluate the pharmacological properties according to the traditional medicine (Lanhers, 1988).

Euphorbia hirta L. (Euphorbiaceae), an herbaceous wild plant native to Australia, is now very common in all tropical countries and has been widely used in traditional medicine in Asia, the Middle East, Africa and the Caribbean, where 183 vernacular names have been recorded (Lanhers, 1988). *E. hirta* has been recommended for various therapeutic indications in traditional medicine, such as diseases of the digestive system (diarrhea, dysentery, constipation, ulcer, parasitosis. . .), of the respiratory system (asthma, bronchitis, emphysema, hay fever. . .), of the urinary apparatus (stones, as a diuretic. . .), of the genital apparatus (metrorrhagia, agalactosis, gonorrhea,

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urethritis. . .), for various ocular ailments (conjunctivitis, corneal ulcer. . .), affections of skin and mucous membranes (Guinea worm, scabies, tinea, thrush, aphtha. . .). Besides these principal indications, other different properties have been mentioned, like hypotensive, tonic, antipyretic, antiinflammatory, hypoglycemic and sedative activities (Watt and Breyer-Brandwijk, 1962; Martin et al., 1964; Ridet and Chartol, 1964; Steinmetz, 1964; De Saqui-Sannes, 1971; Poulet, 1972; Zipcy, 1975; Ndir and Pousset, 1982; Dalil, 1984; Karimou, 1984; Weniger, 1985; Adjanohoun et al., 1986).

Chemical composition of *E. hirta* has been widely studied and a review has been previously published (Lanhers et al., 1987).

The present investigation was undertaken to determine the possible sedative effects of *E. hirta*, effects otherwise suggested by Cabalion after fieldworks in Vanuatu (personal communication). An aqueous extract of *E. hirta* was prepared in accordance to the traditional know-how.

In this aim, we have observed the behavior of mice confronted with several experimental situations. In the first experiment, we have examined the dose-effect relationships by recording the locomotor activity of mice confronted with an activitest.

Experiment 2 was undertaken in order to confirm the sedative properties of the plant, by measuring exploratory behavior with a familiar environment test, the two compartment test, described in rats by Hughes (1965) and adapted for use in mice by Misslin and Ropartz (1981).

Since numerous drugs, known for their sedative properties at high doses such as benzodiazepines, also present anticonflict effects when administered at low doses, we therefore studied in experiment 3 possible anticonflict effects of *E. hirta* with non-familiar environment tests, the staircase test described in rats by Thiébot et al. (1976) and adapted to mice by Misslin et al. (1975), and the two-chambered light/dark test concieved by Crawley and Goodwin (1980) and modified by Belzung et al. (1987).

Material and methods

Plant extract

E. hirta was harvested in Vanuatu (New Hebrides) in 1984. An aqueous extract was prepared in the following manner: powdered whole dried plant (60 g) was infused in boiling water (400 ml) and then macerated for 24 h at 44 °C. The macerate was filtered and lyophilised. The yield of extraction was 14% ± 1.

The characterization of the aqueous extract was limited to the qualitative chemical identification and thin-layer chromatographies of different substances as gallic and catechic tanins, flavonoid compounds, organic acids, amino acids, glucides, saponosides and reducing compounds, according to the literature (Lanhers, 1988).

In this paper, all doses are expressed in terms of dried plant material (mg/kg of body weight).

Animals

Male Swiss mice (Laboratories Janvier, Le Genest, France) weighing 30—35 g (8—9 weeks of age) were used for behavioral tests and male and female Swiss mice for acute toxicity determination.

All animals were conditioned in standard macrolon boxes (five mice per box). They were fed laboratory diet (croquettes Extralabo, Provins, France) ad libitum and allowed free access to drinking water. They were kept in 12/12 h light/dark cycle with lights on at midnight, in order to observe animals during their dark high activity period.

Experiment 1

Activitest: The apparatus consisted of a standard cage (24 × 11 × 10 cm) standing on two wires connected to four quartz crystals. The impulses from the crystal induced by any movement (including breathing) are amplified and transmitted to a counter.

Each subject was placed in the experimental cage immediately before the start of the test. Then, the general activity of the animal was recorded minute per minute, during 5 min.

Plant extract was administered i.p., 30 min before testing, at 50, 100, 200, 400 and 800 mg/kg. The control animals received NaCl 0.9 % solution in the same experimental conditions. Each treated group was composed of 10 mice and control group of 50 mice.

Experiment 2

Two compartment test: The apparatus consisted of a polyvinylchloride box (30 × 20 × 20 cm), subdivided into six equal square exploratory units and covered with plexiglass. It could be divided in half by means of three temporary partitions. The apparatus was kept on a stand in the room which housed the mice. During observation, the experimenter stood next to the box, always at the same place. The observations were made without prior knowledge of the animal's experimental conditions.

Each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor was covered with sawdust and the animal was given unlimited access to food and water. Approximately 24 h after being placed in the apparatus, the animal was exposed to both familiar and novel environments by the removal of the temporary partitions without itself being taken out of the box ('free exploration'). The subject was then observed, in red light, for 10 min. The number of novel and/or familiar units entered by the subject was recorded every minute as locomotor activity (locomotor activities in the familiar compartment, in the novel compartment and total locomotor activity).

Plant extract was administered i.p., 30 min before testing, at 12.5, 25, 50, 100 and 200 mg/kg. The control animals received NaCl 0.9% solution in the same experimental conditions. Each treated group was composed of 10 mice and control group of 20 mice.

Experiment 3

Staircase test: The apparatus consisted of a polyvinylchloride enclosure (47 × 10 × 25 cm) with a staircase of five steps. A light from a 100-w desk lamp above the staircase provides the only room illumination.

The animal was placed singly on the floor of the box with its back to the staircase. The number of steps climbed and rearings were counted minute per minute, during 5 min. A step was considered to be climbed only if the mouse had all four paws on the step. The number of steps descended was not taken into account, in order to simplify the observations. After each test session, the box was cleaned to eliminate any olfactory cue which might modify the next animal's behavior.

Plant extract was administered i.p., 30 min before testing at doses of 6.25, 12.5, 25, 50, 100 and 200 mg/kg. Clorazepate dipotassium, a reference benzodiazepine (Tranxene^{R}, Laboratories Clin-Midy, Paris), was administered at doses of 1, 5, 10, 20 and 40 mg/kg. The control animals received NaCl 0.9 % solution in the same experimental conditions. Each group was composed of 10 mice.

Light/dark choice situation test: The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with plexiglass. One of these was darkened with cardboard. A light from a 100-w desk lamp above the other box provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the light one. During observation, the experimenter stood next to the apparatus, always at the same place.

The subjects were individually tested in 5-min sessions. Testing was performed between 1400 h and 1600 h. Mice were naive to the apparatus and had no previous drug treatment. All animals were placed in the illuminated box to initiate the test session. The amount of time spent in this box was recorded minute per minute, during 5 min after the first entry of mice in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes.

Plant extract was administered i.p., 30 min before testing, at doses 12.5 and 25 mg/kg. The control animals received NaCl 0.9% solution in the same experimental conditions. Each treated group was composed of 10 mice and control group of 20 mice.

Acute toxicity

Groups of 10 mice (5 male and 5 female) received the lyophilised aqueous extract at 3 and 6 g/kg, i.p. and p.o. Mortality and different physiological and behavioral effects (skin state, salivation, whimpering, trembling, locomotion, excrement. . .) were noted after 15 min, 1, 2, 4, 24 h and every day for 14 days. The body weight of each mouse was measured every day.

Statistics

Activitest, two compartment test, light/dark choice situation test: Statistical significances of differences between control and treated groups were

ascertained by a combined analysis of variance and an impaired two-tailed range *t*-test using the Newman-Keuls method.

Staircase test: After the confirmation of the variances homogeneity (Bartlett's test) and an analysis of variance, each treated group was compared with the corresponding control group, by the Student's *t*-test.

ED_{50} were evaluated according to the method described by Litchfield and Wilcoxon (1949).

Results

Experiment 1

Activitest: The results represented in Fig. 1 show that *E. hirta* produced a significant and dose-dependent decrease of general activity from 100 mg/kg. ED_{50} was estimated to 227 mg/kg ± 3.

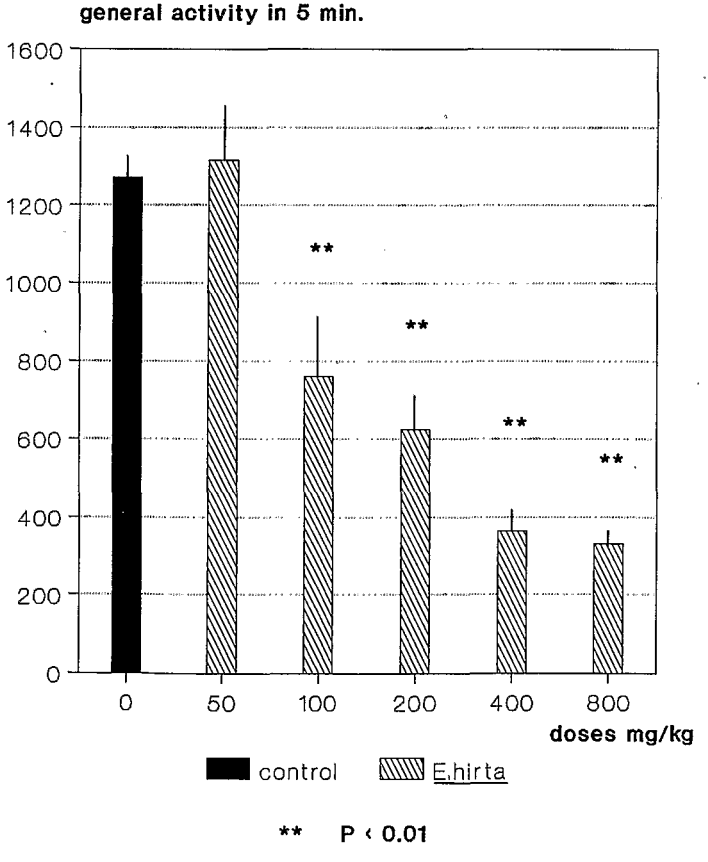

Fig. 1. Influence of *E. hirta* on general activity of mice recorded in the activitest.

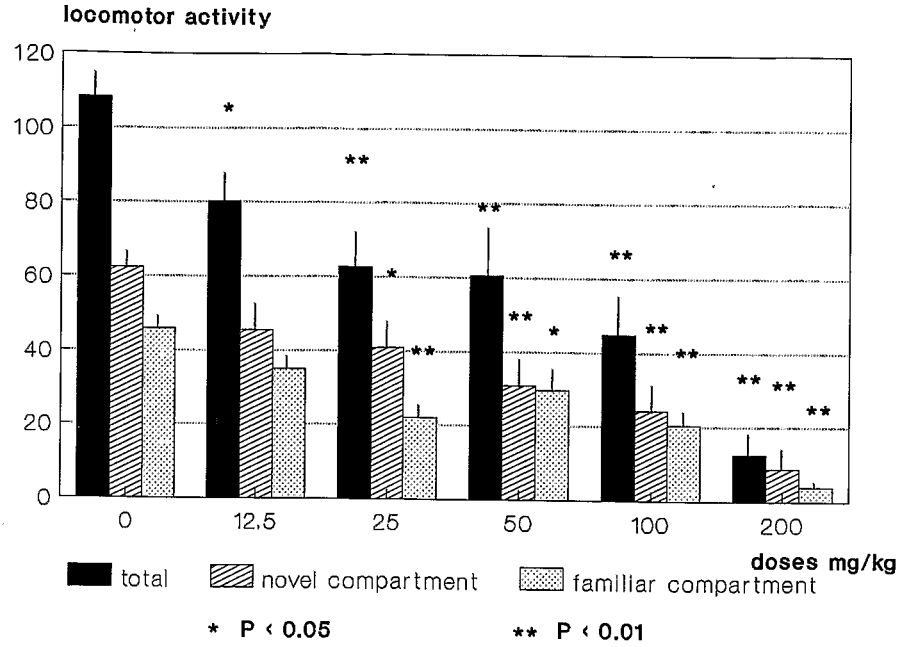

Fig. 2. Influence of *E. hirta* on locomotor activity of mice in the two compartment test.

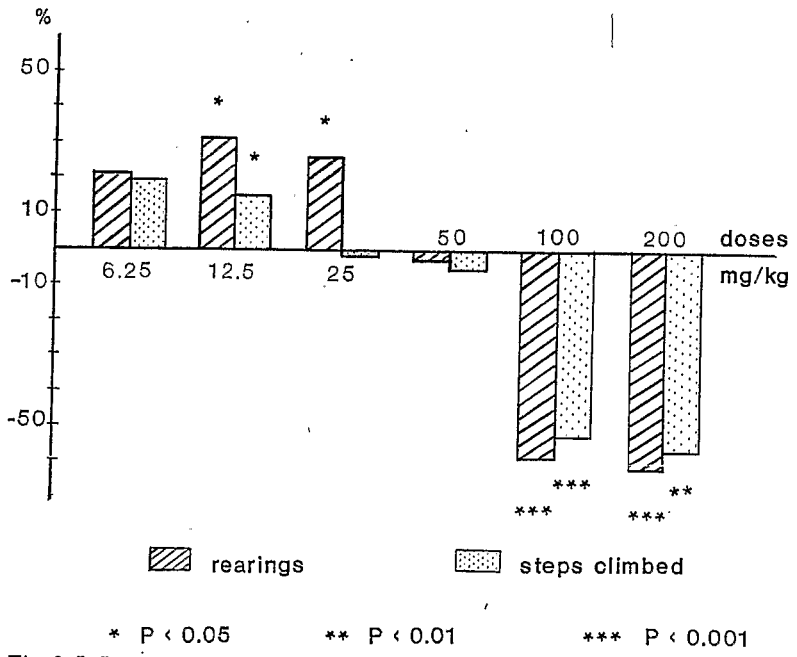

Fig. 3. Influence of *E. hirta* on rearings and steps climbed by mice in the staircase test.

Experiment 2

Two compartment test: As can be seen in Fig. 2, *E. hirta* significantly reduced the total locomotor activity from 12.5 mg/kg, while activities in the familiar and in the novel compartments were significantly affected only from 25 mg/kg. ED_{50} was estimated to 46 mg/kg ± 1.

Experiment 3

Staircase test: The results represented in Fig. 3 demonstrate that *E. hirta* decreased significantly the number of steps climbed and the number of rearings effected by mice, from 100 mg/kg. At lower doses (12.5 and 25 mg/kg), the effects were reversed, the plant extract significantly increasing the number of rearings; however, the number of steps climbed was significantly

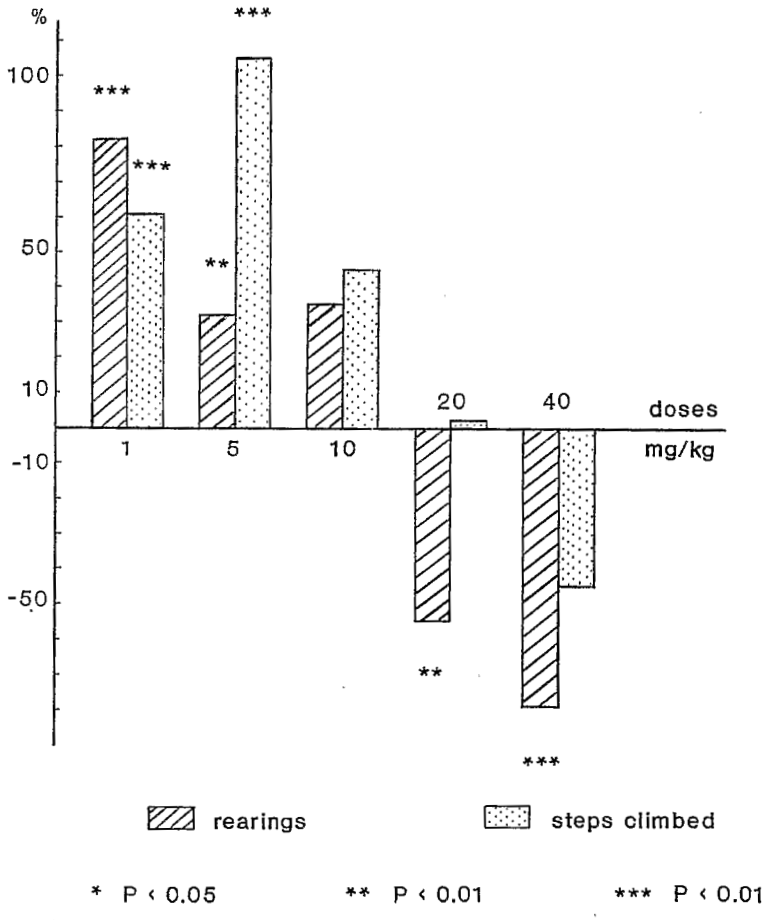

Fig. 4. Influence of clorazepate dipotassium on rearings and steps climbed by mice in the staircase test.

TABLE 1

EFFECT OF *E. HIRTA* ON THE TIME SPENT BY MICE IN THE ILLUMINATED BOX

N: number of mice per group.

Dose mg/kg	Time spent in the illuminated box/s	*N*
Control	30.4 ± 1.9	20
12.5	34.8 ± 2.4	10
25	40.3 ± 2.7*	10

*$P < 0.05$.

affected only at 12.5 mg/kg. Sedative ED_{50} were estimated to 75 mg/kg ± 1 for the rearings and to 99 mg/kg ± 1 for the steps climbed.

The reference compound, clorazepate dipotassium, significantly increased the number of rearings and of steps climbed at 1 and 5 mg/kg, and significantly reduced the former parameter from 20 mg/kg (Fig. 4). Sedative ED_{50} were estimated to 16 mg/kg ±1 for the rearings and to 23 mg/kg ± 1 for the steps climbed.

Light/dark choice situation test: Table 1 shows that *E. hirta* significantly increased the time spent by the mice in the illuminated box, at 12.5 and 25 mg/kg.

Acute toxicity

The aqueous extract of *E. hirta* administrated i.p. and p.o. did not induce the mortality up to the dose of 6 g/kg. The treated animals did not present any toxic manifestation. The evolution of body weight was normal in all treated animals.

Discussion

The present results indicate that the aqueous extract of *E. hirta* L. appears to have sedative properties, since it significantly reduced the general activity of mice in a forcing-test (activitest), as well as the number of steps climbed and rearings effected by mice confronted to another aversive situation (staircase test), the lowest active dose being 100 mg of dried plant/kg. Moreover, this sedative activity appears at lower doses, when mice are confronted with a free-choice exploratory situation (two compartment test) on this particular situation, the locomotor activity was significantly reduced from 12.5 mg/kg.

Furthermore, it is well known that many sedative drugs such as benzodiazepines, phenobarbital, valproate or ethanol, when administered at low doses, have also been found to possess anticonflict properties (Misslin et al., 1975; Treit, 1985). In the present study, we used the staircase test and the light/dark choice situation, in order to observe a possible anxiolytic action of *E. hirta.*

It has been demonstrated that, in experimental situations in which animals are forced into a novel environment, they actually attempt to escape (Misslin et al., 1982; Misslin and Cigrang, 1986). Minor tranquilizers facilitate this behavior, presumably by suppressing behavioral inhibition induced by the aversive properties of forced situations.

In the staircase test, these anticonflict properties found expression in the increase of the number of steps climbed and rearings effected by the mouse, such effects obtained in this study with clorazepate dipotassium from the lowest doses of 1 and 5 mg/kg.

In this test, *E. hirta* produced a significant increase in the number of rearings, at the low doses of 12.5 and 25 mg of dried plant/kg, but its action on the number of steps climbed is clearly slight, and significant only at 12.5 mg/kg. In the light/dark choice situation, anticonflict activity of *E. hirta* found expression in the increase of the time spent by mice in the illuminated box, when it was used at the doses of 12.5 and 25 mg of dried plant/kg.

Thus, the sedative effects of a traditional extract of *E. hirta* (aqueous extract) have been largely demonstrated and so validate one of the various therapeutic indications of this species. These data also reveal a new pharmacological effect for *E. hirta*, its anxiolytic properties.

It will be necessary to clarify the activity profile of this species by researching other benzodiazepine-like properties, as well as other possible psychotropic effects, such as hypnotic, neuroleptic and antidepressant effects. These investigations will be the subject of following publications.

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