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Research Article

Anxiolytic and Anticonvulsant Effects on Mice of Flavonoids, Linalool, and α-Tocopherol Presents in the Extract of Leaves of *Cissus sicyoides* L. (Vitaceae)

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The aim of the present study is to demonstrate the anxiolytic and anticonvulsant effects of a hydroalcoholic extract obtained from the aerial parts of *Cissus sicyoides* L. (CS) (Vitaceae) on male and female mice using several behavioral assays. Groups of males and females treated via intraperitoneal (IP) with doses of 300, 600, and 1000 mg/kg of the extract showed significant action in the elevated plus-maze (EPM), time spent in the open arms, and number of entries in the open arms. The board-hole test also showed a significant increase in the time spent in head-dipping and in marble-burying test of the number of marbles buried. The same treatment increased the duration of sleeping time induced by sodium pentobarbital and also showed a significant increase in protection against pentylenotetrazole-induced convulsions. These results indicate an anxiolytic and anticonvulsant-like action from *C. sicyoides* L. extract on mice, probably due to the action of flavonoid(s), Linalool, and α-tocopherol present in the *C. sicyoides* leaves.

1. Introduction

Cissus sicyoides (CS) belonging to the Vitaceae family comprises of about 165 genus and 1370 species, which are distributed throughout the tropics, mainly in Brazil and the Caribbean. It is popularly known as "insulinas, cipo-pucá, bejuco de porra, bejuco caro, puci, and anil trepador" [1]. Originally from the Dominican Republic [2], it is used in popular medicine as a diuretic, anti-inflammatory, and antidiabetic [3, 4]. It has also demonstrated a vasoconstrictor effect on guinea-pig aorta rings [5] and an antibacterial activity [6]. In Brazil, CS was evaluated for its anticonvulsant property, where it is used against epilepsy andcytotoxic activities [7–9]. The fact that treatment with tea induced an increase in the amount of chromosomal damage in bone marrow cells without altering the cell division cycle was also demonstrated. This plant also presents antibacterial and oxytocic activities [10], and CS contains significant amounts of α-tocoferol, a compound proved to be a useful adjunct to anticonvulsants in clinical medicine [11]. Alpha-tocopherol protects against pentylenotetrazol and methylmalonate-induced convulsions [12] and prevents the occurrence of epileptic foci in a rat model of posttraumatic epilepsy [13]. The central antinociceptive effect of *C. sicyoides* on mice as well as the action of dry leaves extract in pregnant rats and offspring postal development was also demonstrated. [14–16]. Phytochemistry studies identified and isolated from the aerial parts of CS a new coumarin glycoside 5,6,7,8-tetrahydroxycoumarin-5β-xylopyranoside which was obtained together with known coumarin sabandin, two flavonoids kaempferol 3-rhamnoside and quercetin 3-rhamnoside, and two steroids, sitosterol and 3β-O-β-d-glucopyranosylsitosterol [17] (see Figure 1). Leaves of the genus *Cissus* contain sterols, quinones, and phenolic compounds. Anthocyanins, saponins, and flavonoids are also found in the plants leaves and fruits [3]. The effect of

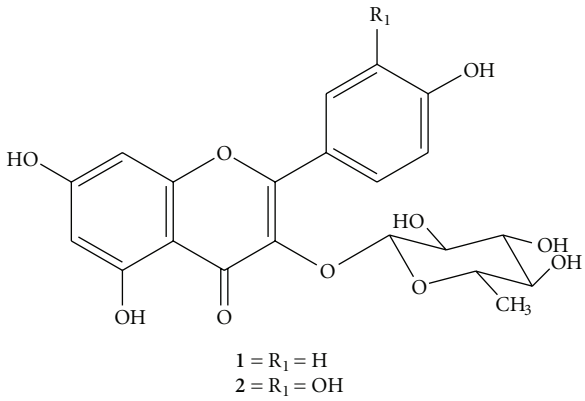

Figure 1: Flavonoids molecular structure present in *C. sicyoides* leaves, (1) canferol 3-a-ramínosideo and (2) quercetina 3-a-raminosídeo.

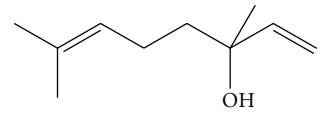

Figure 2: Linalool molecular structure present in *Cissus sicyoides* leaves, 2, 6-dimethylocta-2,7-dien-6-ol; 3,7-dimethyl-1,6-octadien-3-ol.

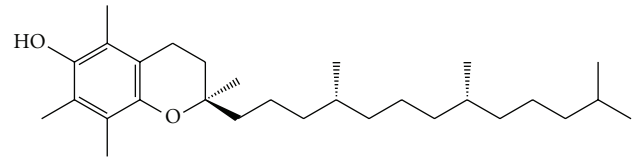

Figure 3: Molecular structure of α-tocopherol, (*2R*)-2,5,7,8-tetramethyl-2-[(*4R,8R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-chromen-6-ol.

linalool present in the leaves of the CS wasdemonstrated in the protection against seizures induced in mice [7, 8, 18] (see Figure 2). However, we found no reference on its activity on the central nervous system (CNS) relating to anxiety as well as information on its acute toxicity. Benzodiazepines (BDZs) are considered safe drugs and are widely prescribed for their anxiolytic and anticonvulsant actions [19–21]. However, they may produce side effects, such as sedation and myorelaxation that are considered as unwanted effects in an anxiolytic drugs [20]. On the other hand, the existence of natural flavonoids that possess anxiolytic effect not associated with myorelaxant, amnesic, or sedative actions has been demonstrated [22]. Although alternative treatments with herbs are increasingly used by the population to alleviate affective disorders, there is a strong rejection among doctors as the use of herbs for treatment of various diseases is still scarce [23]. The antidiabetic action of the CS is in the making of clinical trials (phase II), the results obtained by the authors are promising for the future use in medical clinics [24]. This study also aims to assess the possible effects of flavonoids in the hydroalcoholic extract of the CS leaves in several behavioral tests related to anxiety in mice. The presence of α-Tocopherol has been identified in the leaves of *C. sicyoides*, used in clinical practice as an adjunct in the treatment of seizures [11] (see Figure 3). Our result indicates a new action for use of the *C. sicyoides* which can be related to the presence of the α-tocopherol as an adjuvant of the effect of sedatives together with the linalool and flavonoids present in this plant.

2. Materials and Methods

2.1. Plant Material and Extract Preparation. Aerial parts of CS were collected in the vicinity of the campus of the Federal University of Pernambuco—State of Pernambuco—Brazil in January 2005. The plant was identified by University Prof. Marlene Carvalho de Alencar Barbosa, and a vouched for specimen was deposited in the Geraldo Mariz Herbarium (UFP) under Botanical Department N° 29040. The collected leaves were washed, dried at room temperature (28°C) in the laboratory for 25 days and ground in a mill to a grain size of <1 mm. Then, 360 grams of the powdered plant material were added to 1000 mL of alcohol and water (70:30, v/v). The dry powder yielded 30% of extract. For pharmacological testing, the extract was dissolved in saline plus Tween 80 (0.025%) solution.

2.2. Animals. Male and female two-month-old Swiss albino mice, weighing 20–30 g, were used in this experiment. The animals were housed in groups of ten per cage, with light/dark periods of 12 hours. They were fed and watered ad libitum. All experiments were conducted between 10:00 am and 4:00 pm. Female mice were tested without monitoring the oestrus cycle. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of Laboratory Animals and approved by the Ethical Committee of the Federal University of Pernambuco (UFPE) protocol number 008196/2005-29.

2.3. Drugs. Diazepam (DPZ, 2.5 mg/kg, IP) was used as the standard anxiolytic drug. Pentobarbital sodium (PBS, 55 mg/kg, IP) was used as a hypnotic drug and pentylenotetrazole (PTZ, 55 mg/kg, IP) as a convulsant. All drugs were obtained from Sigma Aldrich, Mo, USA, and Tween 80 was locally purchased.

2.4. Sodium Pentobarbital-Induced Sleeping Time. The mice were divided into four groups (10 animals/group). Three groups received three doses of extract (300, 600, and 1000 mg/kg (IP). After 1 hour, all four groups received 55 mg/kg (IP) of sodium pentobarbital (PBS). The time that elapsed between the loss and recovery of the righting reflex was recorded, for control and drug pretreated animals [25].

2.5. Pentylenotetrazole-Induced Convulsion. The mice were divided into four groups (10 animals/group). The first group received the pentylenotetrazole (PTZ) (55 mg/kg IP)

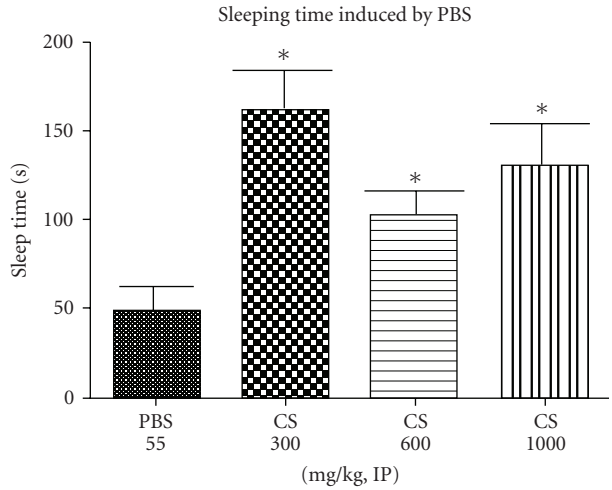

Figure 4: Effect of sodium pentobarbital (PBS-) induced sleep time was evaluated with the extracts (EHDg and EADg). All data cited are the mean ± S.D. The data were evaluated by a one-way analysis of variance (ANOVA) with Duncan's post hoc test. This figure shows that the doses used had a hypnotic effect very significant with relation to the effect of PBS *[F (1.7) = 5.5; $P < .01$]. 10 animals were used in each group.

and served as positive control. The test groups received the CS extract at doses of 300, 600, and 1000 mg/kg (IP). After 1 hour, PTZ (55 mg/kg, IP) was administered to the animals in each group. The number of mice which exhibited convulsions, the lethal time, and the latency to first convulsion was recorded [25].

2.6. Marble-Burying Test. Twenty-five clear glass marbles (20 mm diameter) were used for each individual test. Opaque cages (30 × 36 × 13 cm) were constructed of smooth, opaque plastic with a vinyl ceiling containing air holes, and a 5 cm layer of sawdust. Mice were placed individually in these cages for 15 minutes (habituation trial) and then returned to their home cage. Twenty-five glass marbles were evenly spaced 5 cm apart on a 5 cm layer of sawdust in the habituation cages. Mice were then reintroduced (each test mouse was returned to the same cage in which they had been habituated). 10 animals were used in each group. The test groups received the CS extract at doses of 300, 600, and 1000 mg/kg (IP). After 15 minutes, the test was terminated by removing the mice and counting the number of marbles that were more than two-thirds covered with sawdust. After each trial, the sawdust was replaced, and the test apparatus and glass marbles were washed by water and cleaned with 70% alcohol [26, 27].

2.7. Board-Hole Test. Exploratory behavior was assessed using the board-hole test [28]. The apparatus consisted of a square plastic plate, 40 cm × 40 cm, 1 cm thick, with 16 holes (diameter 2 cm), regularly spaced on the surface, at 3.5 cm from the edges. The apparatus was elevated to the height of 50 cm, in a dimly illuminated room. Mice were placed in the centre of the plate, and the number of head dips was immediately counted during two or three consecutive periods of 5 minutes each.

2.8. Elevated Plus-Maze Test. The elevated plus-maze (EPM) test consisted of two open arms (30 × 5 × 0.25 cm) and two closed arms (30 × 5 × 15 cm) emanating from a common central platform (5 × 5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms. The plus maze was carefully cleaned with a wet towel after each animal test. The mice were divided into four groups (10 animals/group). DPZ (2.5 mg/kg, IP) was used as the positive control and CS extract at doses of 300, 600, and 1000 mg/kg, i.p, in the three remaining groups. All experiments were carried out between 10:00 am and 4:00 pm. After each trial, the EPMapparatus was wiped clean with alcohol (70%) solution [29].

2.9. Statistical Analysis. Statistical analysis was performed using one-way ANOVA with post hoc Duncan's test. $P < .05$ was considered significant. All data are expressed as mean ±S.D.

3. Results

3.1. Sodium Pentobarbital-Induced Sleeping Time. The effect of pentobarbital sodium-induced sleep is shown in Figure 1. The values, up to 1000 mg/kg of CS, were significantly different from the control group *[F (1.7) = 5.5; $P < .01$].

3.2. Effect of CS Pentylenotetrazole(PTZ-) Induced Convulsion. The CS inhibited generalized clonic-tonic convulsions induced by PTZ (55 mg/kg, IP) at doses of 600 and 1000 mg/kg (Figure 5), as in accordance with statistical analysis *[F (1.6) = 5.7; $P < .01$], using analysis of variance one way (ANOVA) and followed by a post hoc Duncan's test.

3.3. Marble-Burying Test. To examine this premise, we studied the effect of the representative of CS on burying behavior. As expected, control exhibited significant decrease in the marble burying behavior. However, CS prompted an increase in marble burying (300, 600, and 100 mg. kg^1, IP. These data were evaluated using the analysis of variance one way (ANOVA) followed by a post hoc Duncan's tests. *[F (1.14) = 5.8; $P < .01$], and **[F (1.12) = 5.7; $P < .05$] (Figure 6).

3.4. Effect of Board-Hole Test. The effect of CS on the board-hole test is shown in Figure 7. At the doses of 300, 600, and 100 mg/kg, IP, a significant increase in the amount of head-dipping behavior was shown *[F (1.13) = 5.7; $P < .001$].

3.5. Effect of CS on the Elevated Plus-Maze (EPM). CS in all the doses (300, 600, and 1000 mg/kg, IP) produced anxiolytic-like effects as determined by the increase in the percentage of open arm entries *[F (1.14) = 5.6; $P < .01$]. Conversely, the number of entries and the time spent in the

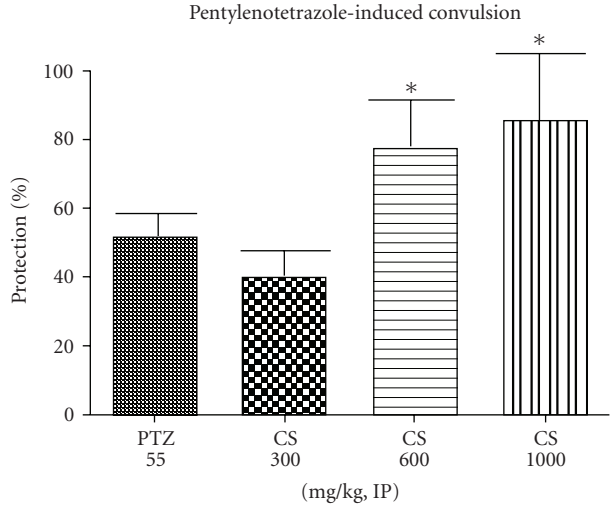

Figure 5: Effect of pentylenotetrazole (PTZ-) induced convulsion was evaluated with the extracts (EHDg and EADg). All data cited are the mean ± S.D. The data were evaluated by a one-way variance analysis (ANOVA) with Duncan's post hoc test. This figure shows that the doses effective in protecting against seizure were 600 and 1000 mg/kg *[F (1.6) = 5.7; $P < .01$].10 animals were used in each group.

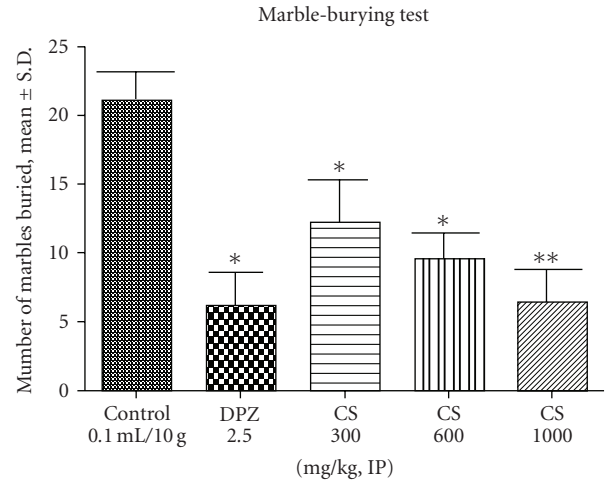

Figure 6: Effect of extracts (EHDg and EADg) in Marble-burying test. The extracts were evaluated in relation to Diazepam. All data cited are the mean ± S.D. The data were evaluated by a one-way variance analysis (ANOVA) with Duncan's post hoc test. The data from this figure show that the effect of fear produced by glass beads was significant compared with the control and the diazepam *[F (1.14) = 5.8; $P < .01$], and **[F (1.12) = 5.7; $P < .05$]. 10 animals were used in each group.

closed arms were reduced by CS treatment [F (1.13) = 5.5; $P < .01$] (Figures 8 and 9).

4. Discussion

C. sicyoides is a plant originating from the Dominican Republic [2]. It is popularly known as "insulina, *cipo-pucá,*

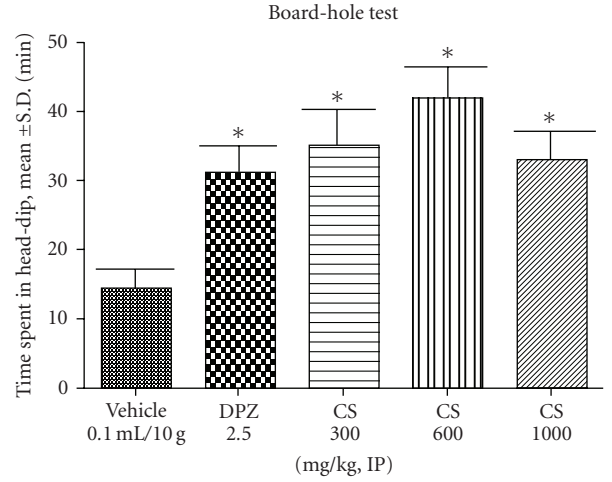

Figure 7: Time spent in head-dip registered in a 5-minute session in the board-hole test performed 1 hour after administered do control, diazepam (2.5 mg/kg), and CS. In this test, all doses used showed a very significant effect on the control and similar to diazepam *[F (1.13) = 5.7; $P < .01$]. 10 animals were used in each group.

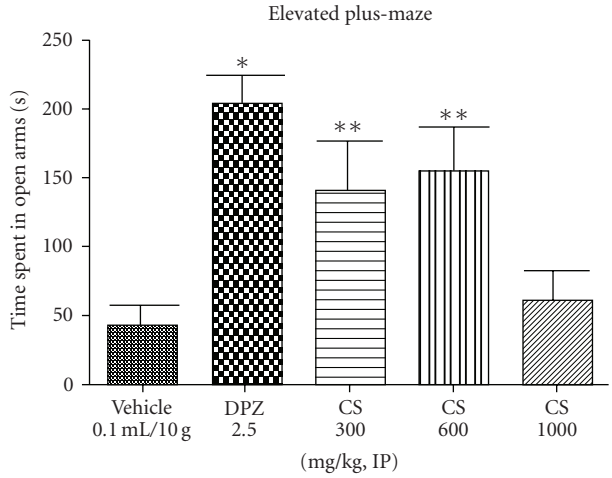

Figure 8: Effect of diazepam and of CS on the values of time spent in the open arms during 5 minutes. The data from this figure show that the dose of 1000 mg/kg was not significant in relation to the vehicle and Diazepam. *[F (1.14) = 5.6; $P < .01$*$P < .01$] and **[F (1.1) = 55; $P < .05$]. 10 animals were used in each group.

bejuco de porra, bejuco caro, puci, and anil trepador" [1]. It is used in popular medicine as a diuretic, anti-inflammatory [4], and antidiabetic [5].

The aim of this study was to analyse the behavioral effects of the crude hydroalcoholic extract of the aerial parts of CS. The results presented here show that CS did not exhibit toxicity in mice and did not induce any significant changes in several behavioral and physiological parameters, and showed a slight decrease in spontaneous locomotor activity and an increase in breathing frequency (data not shown).

Treatment with CS reduced the latency of induction and increased the duration of the barbiturate-induced sleep (see Figure 3) indicating CNS depressant activity, since

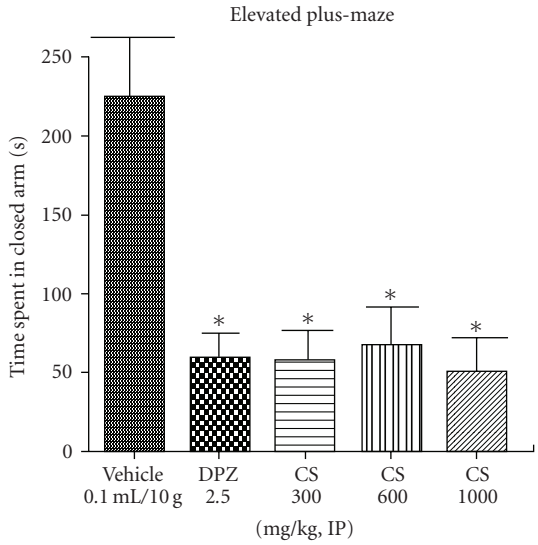

Figure 9: Effect of diazepam and the extract of CS on the values of time spent in the closed arms, during 5 minutes. All doses used in this test had a time of permanence in the closed arm very significant in relation to the vehicle, and similar to diazepam *[F (1.13) = 5.5; $P < .01$]. 10 animals were used in each group.

sleeping time induced by PBS is related to its central depressant properties. These finding suggest that *C. sicyoides*, administered by the intraperitoneal route, has hypnosedative activity due to the potential of barbiturate-induced sleep and might probably be due to pharmacokinetic interactions between CS and PBS through the presence of flavonoids, and mainly by linalool present in CS leaves [7, 8, 19].

In this regard, there are few reports showing pharmacokinetic interaction of other plant species, such as *Smilax* sp., *Piper methysticum*, among others, with therapeutic drugs such as diazepam, alcohol, barbiturates, and other psycho pharmacological agents [30, 31]. PBS is metabolized in the liver by an oxidative pathway that involves cytochrome P_{450}, NADPH, and molecular O_2 [32]. A hypothesis to explain the enhanced barbiturate-induced sleep should be possible as an enzymatic inhibition of liver enzymatic systems, such as CYP 450, by CS, metabolizes intermediate and short-action barbiturates [25, 33]. CS produced a protection against convulsions induced by PTZ in mice *[F (1.6) = 5.7; $P < .01$] (see Figure 5).

CS also increased significantly the number of hidden glass marbles *[F (1.14) = 5.8; $P < .01$], and [F (1.12) = 5.7; $P < .05$] (Figure 6). A significant increase in the exploratory head-dipping behavior was observed after the treatment with 300, 600, and 1000 mg/kg IP of the CS extract, thus reinforcing the anxiolytic-like activity *[F (1.13) = 5.7; $P < .01$] and **[F (1.12) = 5.7; $P < .05$] (Figure 7). On the other hand, several plants increased exploratory behavior of open arms in the EPM test and are generally used to diminish anxiety in folkloric medicine. Among them are *Ginkgo biloba*, *Cassimiroa edulis* [29, 33, 34]. In the EPM test, the effect of CS extract at doses of 300 and 600 mg/kg, IP was observed in the time spent in open arms *[F (1.13) = 5.7; $P < .01$] (see Figure 7).

The number of entries and the time spent in closed arms were reduced by the intraperitoneal treatment with CS (300, 600, and 1000 mg/kg) in comparison to the control values *[F (1.14) = 5.6; $P < .01$] and **[F (1.11) = 5.5; $P < .05$] (see Figure 8). The EPM test is designed to evaluate drugs with anxiolytic-like nonspecific action. Extract or drugs that increase the time spent in open arms are considered anxiolytic by withdrawal of fear in the animals. The same happens with time spent in the closed arms, which are considered to produce fear or anxiety [29].

In this study, diazepam was used as a positive control, and, as expected, it increased the activity in the open arms of the EPM apparatus, confirming anxiolytic-like actions. The presence of flavonoids, linalool, and α-tocopherol in *C. sicyoides* leaves reinforces the anxiolytic-like and anticonvulsant-like effects of this plant found by us in this study.

Acknowledgments

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