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Antidepressant and anxiolytic-like effects of essential oil from *Acantholippia deserticola* Phil in female rats

[*Efecto ansiolítico y antidepresivo del aceite esencial de Acantholippia deserticola Phil en ratas hembras]*

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Abstract
Acantholippia deserticola (Phil.ex F. Phil.) Moldenke is a Verbenaceae that has long been used in traditional medicine in Tarapacá (Chile) as an analgesic, anti-inflammatory and aphrodisiac agent. Since α- and β-thujone were identified as the main constituents (88.4%) of the essential oil from this plant, we investigated its biological properties. The results show that the essential oil from *Acantholippia deserticola* decreased locomotive and rearing activity compared to control group rats, including those treated with diazepam, but the essential oil had no effects on head movements or grooming. The essential oil also had significant anxiolytic and antidepressant effects. This essential oil, therefore, has sedative, anxiolytic and antidepressant actions on the rat central nervous system.

Keywords: *Acantholippia deserticola*, Verbenacea, thujone, anxiolytic and antidepressant effects

Resumen
Acantholippia deserticola es una Verbenaceae de uso en la medicina tradicional como analgésico, antiinflamatorio y afrodisíaco en la región de Tarapacá, Chile. En el aceite esencial se ha identificado α- and β-tuyonas como principales constituyentes (88.4%) de esta planta, que ha llevado a investigar sus propiedades biológicas. Los resultados muestran que el aceite esencial de *Acantholippia deserticola* disminuye la locomoción y el levantamiento en dos patas, en comparación con el grupo control, incluido el tratado por el diazepam, pero el aceite esencial no tuvo efecto sobre la sacudida de cabeza y el acicalamiento. En ambas pruebas, se observa un efecto significativo del aceite esencial en los efectos ansiolíticos y antidepresivos, lo que indica que el aceite esencial tiene actividad sedante, ansiolítica y antidepresiva en el sistema nervioso central.

Palabras Clave: *Acantholippia deserticola*, Verbenacea, tuyona, efecto ansiolítico y antidepresivo

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List of abbreviations: CNS: central nervous system; i.p: intraperitoneally; DZP: Diazepam; FLX: fluoxetine; OFT: open field test; EPM: elevated plus-maze; FST: forced swimming test.

INTRODUCTION

Acantholippia deserticola (Phil) Moldenke belongs to the Verbenaceae genus, popularly named "rika rika" in Chilean folk medicine. The plant grows at 3500 meters level above see (m.a.l.s.) in the Andean region of the north of Chile. The local inhabitants have used this medicinal plant to treat various gastrointestinal, cardiovascular and CNS conditions, and even as an aphrodisiac, although this latter effect is a subject of controversy (Villagran *et al*., 2003).

The chemical composition of the essential oil of *Acantholippia deserticola* was previously determined by Gas chromatography–mass spectrometry (GC-MS) and about 22 compounds have been identified that represent approximately 99% of the total oil (Rojo *et al*., 2006). The main components are α-thujone (10.5%), β-thujone (77.9%) and sabinene (4.9%). It is noteworthy that diastereomers of thujone are considered the main active components and toxic molecules of absinthe (*Artemisia absinthium*), a very popular liquor in the nineteenth century that had undesirable effects on human health but was a source of inspiration to many well-known artists and writers, including Vincent Van Gogh, Oscar Wilde, Pablo Picasso, Ernest Hemingway, Henri de Toulouse-Lautrec and Charles Baudelaire (Vogt and Montagne 1982).

Essential oils containing diverse composition of thujones have been shown to have a wide variety of biological effects, including inhibiting bone resorption in rats (Mülhbauer *et al*., 2003), fungicidal activity (Farzaneh *et al*., 2006), antimicrobial activity (Hayouni *et al*., 2008), antibacterial and antioxidant capacities (Laciar *et al*., 2009) and antidiabetic properties (Alkhateeb and Bonen, 2010). Some of these effects of essential oils may be mediated by thujones, but their mechanism of action has still not been elucidated. Based on structural comparisons, a tempting hypothesis suggested that thujones may exert their neurotoxicity by binding to CB1 cannabinoid receptors (Höld *et al*., 2000). Nevertheless, recent studies have shown that, although thujone exhibits low affinity for cannabinoid receptors, it does not evoke cannabimimetic responses (Meschler *et al*., 1999). Today, thujone is considered to be a non-competitive inhibitor of γ-aminobutyric acid (GABA) receptors (Höld *et al*., 2001; Olsen, 2000).

Recent work with essential oil isolated from *Aloysia polystachia*, which belongs to the Verbenaceae family as does *Acantholippia deserticola*, has reported sedative, anxiolytic and antidepressant effects (Mora *et al*., 2005). Given that this essential oil also contains thujones, we investigated whether the essential oil from *Acantholippia deserticola* would have similar biological properties. To this end, female Sprague-Dawley rats were submitted to different assays exploring the potential effects of the essential oil on general motor activity, using the open field test (Archer, 1973, Herrera-Ruiz *et al*., 2006), anxiolytic activity, using the elevated plus-maze test (Pellow and File, 1986, Lister, 1987), and antidepressant activity, using the forced swimming test (Cryan *et al*., 2002; Mora *et al*., 2005).

The aim of this study was to investigate in a rat model, the neuropharmacological effects of the essential oil of *Acantholippia deserticola* on ambulatory activity, antianxiety and antidepressant response.

MATERIALS AND METHODS

Plant material and oil isolation

Acantholippia deserticola was collected near Colchane at 3500 m.a.l.s, in September 2006, 1st region of Chile. The plant material was identified and authenticated by Professor Roberto Rodriguez, Concepcion University. A voucher specimen (# 158057) is deposited in the Herbarium of the Concepcion University.

The aerial parts of the plant (leaves and flowers) were submitted to hydro-distillation for 3 hours using Clevenger-type apparatus. The oil was protected from direct light and stored at 4 ◦C until its use.

Drugs

The essential oil from *Acantholippia deserticola* was used in all biological tests (dissolved in olive oil). Diazepam (DZP, from Roche Pharmaceutical Co., Ltd, Switzerland) as an anxiolytic drug, and fluoxetine (FLX, from Ely Lilly Co., Ltd, USA), as an antidepressant drug, were used as positive controls.

Animal treatments

Female Sprague-Dawley rats weighing between 175 and 250 g, kept under controlled conditions (12-h dark/12-h light cycle, 23-25 ºC and 50-60% humidity) were used. All experiments were conducted in

accordance with international standards of animal welfare and the experimental protocols were approved by the Neuroscience Society (USA). Groups of eight animals were selected and, to reduce the influence of diurnal variation, all assays were conducted from 09:00 to 13:00 h. in a special noise-free room with controlled illumination. The animals received a standard food pellet and before experiments they were fasted overnight with water *ad libitum*.

Animals received intra-peritoneal (i.p.) the essential oil at different concentrations (ranging from 1 to 15%, in olive oil as vehicle). Selected doses of the extract and time intervals were determined in preliminary tests. Doses lower than 3% were without effects while doses higher than 3% of essential oil provoked toxic reactions, including clonic-tonic movements and convulsions and even death of the animal in some cases. Therefore, a dose of 3% was retained for this study. Drug doses were: diazepam (1 mg/kg body weight, i.p.) and fluoxetine (10 mg/kg body weight, i.p.). All administrations were performed in a dose volume of 1 mL/kg body weight.

Estrous cycle determination and body temperature

Estrous cycle phases were determined by vaginal lavage (Zamorano *et al*., 1994; Marcondes *et al*., 2002) every morning between 07:00 and 08:00 h and female rats with at least two regular 4-day cycles were used.

The rectal temperature of each female rat was measured with a digital thermometer, just before and after i.p. administration of the compounds.

Open field Test (OFT)

The OFT area was made of acrylic transparent walls and a black floor (30 cm x 30 cm) marked with white lines in 10 cm^2 areas. The open field was used to evaluate the exploratory activity of the animal (Archer 1973). The observed parameters were the number of squares crossed (with the four paws) and number of rearing, grooming and defecation activities. After each trial, the open-field apparatus was wiped clean with ethanol (10%) solution.

Elevated Plus-Maze (EPM)

This test has been widely validated to measure anxiety in rodents (Lister 1987; Pellow *et al*., 1986). The apparatus is composed of two open arms (50 x 10 cm each), two closed arms (50 x 10 x 20 cm each) and a central platform (10 x 10 cm) placed in such a way that the arms are opposed; the whole maze is positioned 100 cm above the floor. Each animal was placed in the center of the maze, facing one of the closed arms. The number of entries and the time spent in closed and open arms were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws in the arm. All tests were taped using a video camera. After each test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

Forced swimming test (FST)

The FST is the most widely used *in vivo* model for assessing pharmacological antidepressant activity (Cryan *et al*., 2002; Mora *et al*., 2005). The development of immobility when the mice are placed in a cylinder filled with water, from which there is no escape, reflects the cessation of persistent escape-directed behaviour (Mora *et al*., 2005). The apparatus consisted of a clear Plexiglas cylinder (50 cm high x 20 cm diameter) filled to 30 cm with water (24±1ºC). In the pre-test session, each animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. *Acantholippia deserticola* essential oil and fluoxetine were administered three times: Immediately after the initial 15-min pre-test, 18 h and 0.5 h prior to the swimming test. During the 5-min swimming test, the following behavioural responses were recorded by a trained observer: climbing behaviour (or thrashing), which is defined as upward-directed movements of the forepaws along the side of the swim chamber; swimming behaviour, defined as movement through the swim chamber, which included crossing into another quadrant; and immobility, considered when the rat made no attempt to escape except the movements necessary to keep its head above the water. Increases in active responses, such as climbing or swimming, and reduction in immobility, are considered as behavioural profiles consistent with an antidepressant-like action (Cryan *et al*., 2002).

Statistical analysis

Data were analyzed using the following software: Graphpad INSTAT 3.0 and SPSS 14.0. Results are means ± standard error of the mean (S.E.M.). ANOVA test was followed by Newman-Keuls and $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

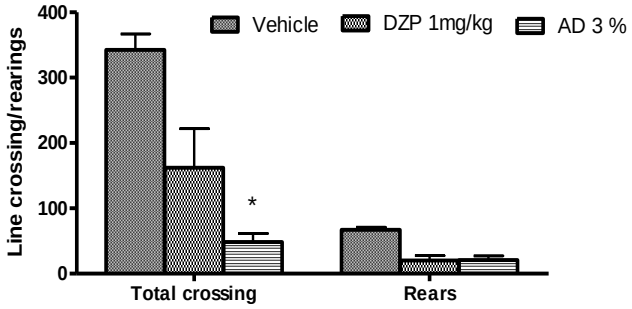
Stress, depression, anxiety and mental disturbances have dramatically increased in the world population (Wong and Licinio, 2001) and the lack of an effective treatment is a permanent challenge for

psychopharmacological research today. Herbal therapies can be considered as alternative/complementary medicines. The search for novel pharmacotherapies for psychiatric illnesses from medicinal plants has progressed significantly in the past decade (Zhang 2004). This progress is reflected in the large number of herbal medicines that have had their psychotherapeutic potential assessed in a variety of animal models. These studies have provided useful information for the development of novel therapeutic agents from medicinal plants to be used in clinical psychiatry. In this context, the aim of this work was to assess the antidepressant and anxiolytic-like effects of essential oil from *Acantholippia deserticola*.

To avoid the influence of ovarian hormone fluctuations across the estrous cycle, only female rats during the diestrus stage were used in the experiments. Vaginal smears were taken daily to determine the different stages of the estrous cycle. Only females exhibiting three or more consistent 4-day cycles were used. Behavioral observations took place in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. Each animal was tested only once. Indeed, several authors have shown that male and female rats behave similarly when females are in the diestrus phase of the cycle (Mora *et al*., 1996; Mora *et al*., 2005; Marcondes *et al*., 2002).

The open-field test is designed to study the exploratory activity of rats and does not involve aversive stimulation. One of the main advantages of this test is that the type and profile of animal behavior are directly observed. Figure 1 shows the effects of essential oil from AD (3%) and diazepam (1 mg/kg), as positive control, on rat general motor activity. Four parameters were recorded: locomotor activity, rearing, head movements and grooming. Among these parameters, only locomotor activity and rearing were influenced by both essential oil and diazepam. Essential oil decreased general motor activity by more than 85% compared to control values ($p < 0.001$), whereas diazepam decreased this parameter by only 50%. Rearing was decreased by both compounds to a similar extent (70%). From these results it can be concluded that the sedative effect of essential oil is similar to that of diazepam. *A. polystachya*, which also belongs to the Verbenaceae family, shows similar activity (Mora *et al*., 2005).

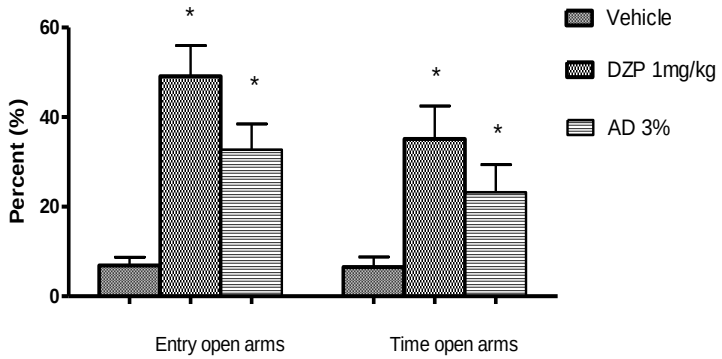
Figure 1
Effects on motor activity and rearing



Effects produced by the i.p. administration of DZP (diazepam) or 3% essential oil from *Acantholippia deserticola* (AD) on spontaneous locomotor activity. The locomotor activity counts (mean ± S.E.M.) were measured over a 15-min period, beginning 30 min after the administration of diazepam or *Acantholippia deserticola*.
***p < 0.05 as compared to control (vehicle-treated).**

The evaluation of the putative anxiolytic activity of essential oil from *A. deserticola* (AD) was performed using the elevated plus-maze (EPM) test (Figure 2). The primary measures in the EPM test are the proportion of entries into the open arms and the time spent in the open arms. According to Barrett 1991, an anxiolytic effect is suggested when the drug increases the percentage of arm entries and time spent in the open arms and decreases the percentage of entries into the closed arms. Compared to control rats, animals treated with either essential oil (3%) or diazepam had significantly increased entries (5- to 8-fold, respectively) and time spent in the open arms (4- to 6-fold, respectively). According to Hall *et al*., 2004, who suggest that thujones interact with GABA receptors, it is thus likely that thujones may be the mediators of the anxiolytic effect shown by essential oil from *A. deserticola*.

Figure 2
Anxiolytic effects of essential oil and diazepam



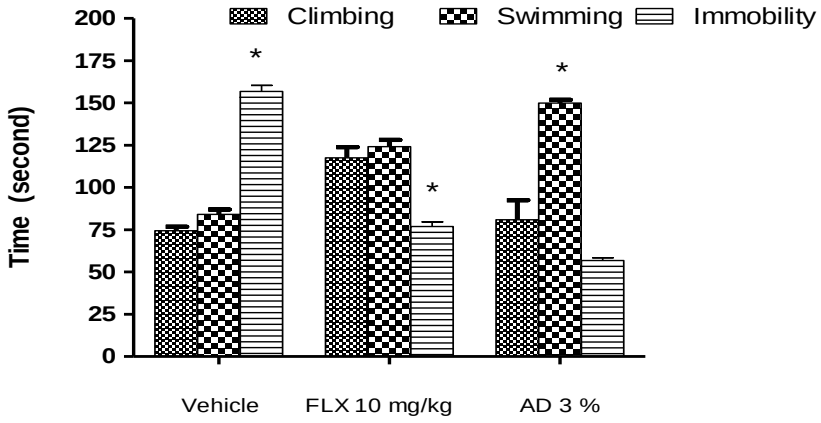
Effect produced by the i.p. administration of 3% essential oil from *Acantholippia deserticola* (AD) on the elevated plus-maze. DZP = diazepam. Data are presented as mean values (± S.E.M.) from a group of five rats.
***p < 0.05 as compared to control (vehicle-treated).**

Finally, the potential antidepressant effect of essential oil was assessed using the forced swimming test (FST), and the results were compared to those of fluoxetine (10 mg/kg), a selective serotonin reuptake inhibitor. Active responses, namely climbing, swimming and reduction of immobility, considered as a behavior profile indicating an antidepressant effect, were recorded during 5 min. Essential oil significantly enhanced the swimming activity of rats and reduced immobility (Figure 3). This profile was similar to that shown by fluoxetine. Indeed, serotoninergic compounds, such as fluoxetine, have been reported to affect swimming whereas tricyclic antidepressants and drugs with selective effects on noradrenergic transmission rather affect climbing (Cryan *et al*., 2005). Although other studies are clearly necessary to elucidate the mechanism of action of *A. deserticola* in the rat CNS, the pattern of effects observed in the FST suggests the involvement of both serotoninergic and catecholaminergic neurotransmitter systems in its antidepressant-like effect. Indeed, antidepressants from different classes produce different effects on active behaviors in the FST (Cryan *et al*., 2005).

CONCLUSIONS

In conclusion, the results obtained after i.p. administration of essential oil from *A. deserticola* in female rats suggest possible applications of this plant in some CNS conditions, such as anxiety and depression. Dosages of the drug seem to be crucial to the type of effect obtained. Because thujones may have sedative, anxiolytic and antidepressant-like properties, as a result of their presence in *A. deserticola* it is likely that they are the main mediators of the observed essential oil activities. However, we cannot exclude the possibility that additional compounds may contribute to these behavioural effects.

Figure 3
Antidepressant effects of essential oil and fluoxetine



The effects of 3% essential oil from *Acantholippia deserticola* (AD) and fluoxetine (FLX) on the forced swimming test (FST). Data represent means ± SEM. Animals were 27 in the solvent group and 8 in the treated groups. Comparisons were made using a one-way ANOVA followed by Newman-Keuls. Comparison test: *p < 0.05 as compared to control (vehicle-treated).

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