Anxiogenic-like effects of Uncaria tomentosa (Willd) DC aqueous extract in an elevated plus-maze test in mice: a preliminary study

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Abstract

The purpose of this study was to examine by the administration orally of *Uncaria tomentosa* aqueous extracts (Willd) DC. (Rubiaceae) during 7, 15, 30 and 90 days of treatment of on the expression of anxiety, as expressed in the elevated plus-maze test in male Albino swiss mice.

In our study UTE revealed an anxiogenic effect in relation to the control group at 15 and 30 days, but was reversed after 90 days of administration, without affecting the locomotor activity, or any deleterious effects on the overall performance of the animal, either for its ambulation, or clinical status and body weight and organ weight/body weight, from liver, lung and kidney were unaffected. These biphasic effects are usually indicative of heterogeneity in sites of action due to the presence of many alkaloids (speciophylline, uncarine F and E) and flavanols (catechin, epigallocatechin) identified and isolated from aqueous extract from UTE.

Keywords: *Uncaria tomentosa*, anxiety, plus-maze, mice

1. Introduction

Anxiety disorders are currently the most predominant psychiatric diseases in USA and in the Europe, affecting between 10-30% of the general population and for these countries represent a serious and ever-increasing spending (Wittchen, Schuster, & Lieb, 2001). The elevated plus-maze (EPM) is a sensitive test and in the animal models, the EPM is one of most popular animal tests used for research into the behavioural pharmacology of anxiety and neurobiological anxiety mechanisms (Treit, Menard, & Royan, 1993). Anxiolytic agents increase, whereas anxiogenic compounds decrease the percentage of time spent on open arms. In the present work, it was demonstrated that the administration on different days of the aqueous extract of *Uncaria tomentosa* in murine was able to induce temporary anxiogenic effects, without modifying the clinical status or locomotion.

2. Results and Discussion

Medicinal plants have their use as medicament based simply on a traditional folk use that has been perpetuated along numerous generations in anxiety disorders (Carlini, 2003; Cryan & Sweeney, 2011; Newman & Cragg, 2007). In according with Santa María et al. (1997) and Roque et

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al. (2009) the administration on different days did not induce overt clinical signs and any symptoms of intoxication or abnormalities in tissues in mice (and no difference in water consumption was noted for control versus treatment during all experiments (ml/Kg/hs; n=8; 5.59 ± 0.27 versus 5.58 ± 0.41 respectively); or in weight initial and final in respectively (g, n:6): Control 34.09 ± 0.92, 33.65 ± 1.42; UTE-7 32.96 ± 085, 32.75 ± 1.04; UTE-15 34.17 ± 0.92, 33.50 ± 1.21; UTE-30 34.36 ± 1.03, 35.33 ± 1.27; UTE-90 34.88 ± 0.88, 33.6 ± 0.69; or in effects of oral administration of UTE on relative organ weights (n:6) ((organ weight/body weight) x 100) Kidney: Control 0.51 ± 0.02, UTE-7 0.43 ± 0.03, UTE-15 0.45 ± 0.03, UTE-30 0.41 ± 0.03, UTE-90 0.46 ± 0.02; Lung: Control 0.38 ± 0.02, UTE-7 0.32 ± 0.03, UTE-15 0.44 ± 0.02, UTE-30 0.40 ± 0.01, UTE-90 0.38 ± 0.01; Liver: Control 1.95 ± 0.16, UTE-7 1.79 ± 0.12, UTE-15 1.98 ± 0.13, UTE-30 2.01 ± 0.18, UTE-90 2.01 ± 0.09.

In this experimental design, the animal is forced to drink a decoction (which might not be liked by the animal) because it has no other liquid to drink. This itself might cause the anxiety seen in days 15 and 30, but when we compare the amount of water drinked with the intake of the aqueous extract of UTE all experiments, not difference was found, demonstrating the aqueous extract does not produce any stress effects per se in the range of concentration tested. The oral administration of 15 or 30 days of UTE extract in mice exposed to the EPM significantly decreased the percentage of time spent in open arms and also the percentage of open arm entries compared with the control ($p \leq 0.05$, Table 1). However, treatment for 7 or 90 days with UTE had no significant effect. Different levels of expression of percentage of time spent in open arm and the percentage of open arm entries were equal to all controls at different times. This data indicates that UTE exerted anxiogenic effects at 15 and 30 days post oral administration which was reversed after 90 days without change in the locomotor activity during the experimental elapsed time.

Very few studies have examined the long-term consequences of UTE aqueous extracts administration in laboratory animals and moreover, there are no reports about the effects of UTE on anxiety-like behavior in mice. Nevertheless, others authors describe that *Uncaria rhynchophylla* (Miq.) Havil has an anxiolytic-like effect mediated by the stimulation of receptors 5-HT_{1A}, one specie very close to *Uncaria tomentosa*, which apparently has the same complex and specialized opening mechanism (Jung et al., 2006; Shi, Yu, Chen, & Xu, 2003). While the purpose was not to identify the compounds of this plant, , *Uncaria tomentosa* have many components and could be responsible for CNS actions of this plant (Senatore, Cataldo, Iaccarino, & Elberti, 1989; Wagner, Kreutzkamp, & Jurcic, 1985) and a phytochemical analysis of UTE

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revealed these effects have been attributed to components such as plant phenolics, flavonoids, and phenylpropanoids among others, the presence of triterpenes, quinovic acid, glycosides, and the effect of total alkaloids, was shown to be partly due to major such as pteropodine, isopteropodine, mitraphylline, isomitraphylline, speciophyline and uncarine, which that could have effects on the CNS (Aquino, De Feo, De Simone, Pizza, & Cirino, 1991; Wagner, et al., 1985). Also and in accordance with other authors, our experiments confirmed that the water extract of UTE acts on the SNC (Jurgensen, Dalbo, Angers, Santos, & Ribeiro-do-Valle, 2005; Mohamed et al., 2000).

3. Conclusions

In conclusions, our data presented here demonstrate that the UTE have an transient anxiogenic effect, as demonstrated by the plus maze test. Additional studies to refute or confirm the effects UTE are necessary to investigate the possible mechanisms involved.

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Keywords: *Uncaria tomentosa*, anxiety, plus-maze, mice

Materials and methods

Animals and clinical observations

The animals used in the experiments were kept and handled in accordance with our University regulations, and were acclimatized to our laboratory animal facilities for at least 1 h before the start of the elevated plus- maze test (EPM).

Young adult male Swiss albino mice of 12-14 weeks age weighing about 25 ± 4 g were selected. The animals were randomly distributed individually and were housed in standard polycarbonate cages (27 × 13× 13 cm) under a 12h light/dark cycle (with lights on 20:00-8:00) at a constant temperature (20ºC ± 2 °C) with continuous access to food (Control, UTE), and water (Control) and UTE administered *ad libitum*.

The general health proposed by Crawly (Crawley, 1999)for screening was evaluated. Thus, control and UTE pre-treated mice were observed. Home cage observations recorded the activity of all mice for approximately 15 minutes at two different daily time points (between 9:00 and 18:00 h.) for clinical signs and symptoms of intoxication in mice (i.e. mortality, sedation, excitation, stereotypy, aggressiveness, piloerection, muscle tone, convulsions, and reactivity to touch, motor incoordination).

Experimental design

The following treatment was given to animals of different groups, except during behavioral tests (equivalent dose at 0.75 g/kg):

(1) Group Control: Water only and served as control.

(2) Group UTE-7: UTE during 7 days.

(3) Group UTE-15: UTE during 15 days.

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(4) Group UTE-30: UTE during 30 days.

(5) Group UTE-90: UTE during 90 days.

***Uncaria tomentosa* preparations and drink intake measure**

Previous studies conducted in our laboratory suggested that in a dose of UTE pre-treatment for the administration on different days have biological activities in aqueous extract (Roque, 2009). The chronic administration and doses used in mice were approximately parameters used by others authors (Aquino, De Feo, De Simone, Pizza, & Cirino, 1991[Sandoval, 2000 #2390; Sandoval et al., 2000; Santa Maria et al., 1997; Sheng, Li, Holmgren, & Pero, 2001). Water and UTE consumption was measured by weighing the bottles daily between 12:00 p.m. and 15:00 p.m. and the absolute drink intake was expressed as mL/Kg/hs.

The UTE was obtained from a local market, and the extract was prepared freshly by decoction of the bark at a 20 g/L concentration. This involved the extract being purified for 45 minutes in boiling water, before being filtered and then rested for 12 h before use. The bottles were cleaned and filled with fresh beverage and was protected from direct light by brown bottle.

Voucher specimens have been deposited in the Herbarium of Museo Botánico de Córdoba (CORD), Universidad Nacional de Córdoba, Argentina.

Anatomical analyses

All experimental mice were weighed just before they were placed in the cage (Initial), and again after different treatments days (Final). Control mice were weighed on the same days as the experimental mice.

Animals were killed using anaesthetic ether. After mice were sacrificed, liver, lung and kidney were quickly excised and placed in a drying oven at 55°C and weighed (absolute organs weight). Relative organs weights [(organs weight/body weight) × 100)] were also calculated.

Apparatus and behavioural test

Control and UTE groups were tested on the EPM (7, 15, 30 and 90 day's post-treatments) and observed for anxiety-related behaviors. The EPM has been examined in a variety of investigations and has been proposed to reflect measures of anxiety because it uses natural stimuli (fear of a novel, brightly-lit open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans (Dawson & Tricklebank, 1995; Lister, 1987; Wall & Messier, 2000).

The plus-maze was constructed of transparent Plexiglas and consisted of two open arms (30 x 5 cm, surrounded by a 0.25 cm-high border) and two closed arms (30 x 5 cm, surrounded by 40 cm high walls), with two pairs of identical platforms, that emerged from a central platform (5 x 5 cm), which were, positioned opposite each other. This apparatus was elevated 40 cm above the floor by a wooden support and was situated in a darkened room. The mice had never encountered the elevated plus-maze prior to their use and each animal participated in only one experiment and

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were tested on the maze in a random order. The testing room was kept at the same temperature as the colony room.

The test was initiated by placing a mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Every effort was made to prevent acoustic and visual disturbances during this time. Each session was registered by one experimenter who was isolated by the special wall and blind to the treatment conditions and away approximately 1 m from the apparatus. Any feces or urine were first removed with paper towels and then the arms, central platform, and inner walls, after each test, a weak cider vinegar solution was used to clean the apparatus. After toweling off the solution, the apparatus was further allowed to air dry for about 2 min before another animal was tested.

A number of classical parameters were collected during the session, with the following spatio-temporal measures being recorded during a 5-min test: Percentage of time spent in open arm [(time in open arm/Total time) x100] and percentage of open arm entries [(Open arm entries/Total entries) x 100]. Arm entries were only counted when all four paws had entered either a closed or an open arm. These were used as anxiety indices and closed arm entries were used as locomotor activity (Dawson & Tricklebank, 1995; Lister, 1987; Wall & Messier, 2000).

Data analyses

Data are expressed as mean ± standard errors of the means (SEM). The statistical significance of differences between treatments and control groups was determined by factorial analysis of variance (ANOVA) by Kruskal Wallis test followed Dunn multiple comparation for three or more variables. Differences were considered statistically significant for p values of < 0.05. Statistical analyses were performed using Info-Stat software (Cordoba, Argentina, 2008).

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Table 1. Effects of oral administration of *Uncaria tomentosa* extracts (UTE) on arm entries in the elevated plus-maze test in Albino Swiss mice.

	Treatments days							
	7		15		30		90	
	Control	**UTE**	**Control**	**UTE**	**Control**	**UTE**	**Control**	**UTE**
TOA	3.19±1.84	6.13±1.54	6.28±2.95	1.21±0.61*	4.96±2.41	0.74±0.57*	2.14±0.67	4.48±2.07
OAE	5.84±1.93	9.72±1.29	13.42±2.51	2.58± 0.8*	10.21±1.44	2.31±1.04*	8.36±2.57	6.12 ± 1.5
CAE	11.43±1.51	9.93±1.06	12.00±1.84	11.69±0.65	12.13±0.91	8.47±0.90	14.14±2.05	17.00 ±1.11

TOA: Time spent in the open arms, as a percentage of total time spent in open arms. Overall, UTE-pre-treated group different from control group ($P<0.05$) with post-hoc analysis demonstrating a statistical difference on days 15 and 30 (*$P<0.05$).

OAE: Open arm entries, as a percentage of total arm entries. Overall, UTE-pre-treated group different from control group ($P<0.05$) with post-hoc analysis demonstrating a statistical difference on days 15 and 30 (*$P<0.05$).

CAE: Close arm entries. UTE pre-treated group not different from control group on any of the days tested.

Mice were administered (0.75 g/kg) for 7, 15, 30 or 90 days. Bars represent the mean ± S.E.M. for 8–10 mice. *$P < 0.05$ vs. water-treated controls.

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