Anxiolytic and antidepressive effects of the homeopathic complex Homeo-pax® (pre-clinical study)

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ABSTRACT

The homeopathic complex Homeo-Pax® has been used as an antidepressant and anxiolytic homeopathic medicine available in Brazil. It is a complex mixture prepared with Aconitum nap. 6cH, Aurum met. 6cH, Phosphorus 6cH, Argentum nitricum 6cH, Arsenicum alb. 6cH, and Valeriana officinalis 3cH. This study had evaluated the behavior in rats after treatment with Homeo-Pax® in pre-clinical models of depression and anxiety. Elevated Plus Maze Test (EPM), Forced Swimming Test (FST), Open Field Test (OFT) and the Rota Rod Test (RRT) behavior assays were used to confirm its activity. In the EPM, the animals treated with Homeo-pax® on the 1st day and until the 20th day of treatment remained longer in the open arms of the maze than on the 30th day. This result was statistically significant compared with the control group (p < 0.05). In the FST, the treatment with Homeo-pax® (0.5 ml, p.o) increased the swimming time, compared to the control group. This effect was dependent on treatment time, resulting in a similar effect to that presented by amfepramone (10 mg/kg, p.o). In the OFT, crossing by the animals was significantly increased by the treatment with amfepramone (10mg/kg, p.o), and also with the 30-day treatment with Homeo-pax®. In the RRT, the 30-day treatment with Homeo-pax® (0.5 ml, p.o) did not affect the animals' motor coordination, compared with the control group, which presented the same behavior. Based on the results obtained, it can be suggested that the homeopathic complex Homeo-pax® has anxiolytic and antidepressant properties without affecting motor coordination capacity.

Keywords: Valeriana officinalis, Behavior, Pre-clinical trial, Anxiolytic, Antidepressant.

Introduction

Psychic depression and bipolar disease are mood-altering, debilitating diseases that affect the physical disposition, sleep, appetite, libido and functional capacity. Depression differs from schizophrenia, which produces cognitive disturbances. Depression includes intense feelings of sadness, desperation and inability to feel pleasure in routine activities. Mania, on the other hand, is characterized by the opposite behavior; enthusiasm, mental and verbal rapidity, extreme self-confidence and decreased critical capacity.

For several decades, antidepressant drugs have been widely used in the treatment of clinical depression and other psychiatric disorders. All clinically-used antidepressant drugs, either directly or indirectly, enable the actions of noradrenalin and/or serotonin in the brain [1].
Different compounds have presented therapeutic effectiveness. These include monoamine oxidase inhibitors, tricyclic compounds, selective inhibitors of serotonin and noradrenalin recapture, and other atypical drugs. However, the molecular mechanism of action of the antidepressants’ therapeutic effect is still not clear, and the use of homeopathic medications is increasing due to their beneficial effects on this pathology [2]. Numerous studies have been carried out to evaluate the effects of antidepressants on the metabolism of the neurotransmitters, their turnover and receptor sensitivity. Various theories have been proposed to explain their mechanism of action. These include inhibition of monoamine transport in the presynaptic membrane [3], regulatory beta-adrenoceptor inhibition, and alterations in the cholinergic, dopaminergic or GABAergic receptors [4].

Anxiety is a natural human response to a threatening situation [5]. It is a manifestation connected to the response controlled by the sympathetic nervous system. During this response, the body and mind become awakened and alert, preparing to either attack, or flee from the threat [6].

Anxiety and phobias are the main mental health problems among the Brazilian population, with global prevalence varying from 8% to 18%. Even so, less than 30% of all individuals who suffer these disturbances seek treatment.

The homeopathic complex Homeo-Pax® contains *Aconitum nap.* 6cH, *Aurum met.* 6cH, *Phosphorus* 6cH, *Argentum nitricum* 6cH, *Arsenicum alb.* 6cH, & *Valeriana officinalis* 3cH.

*Aurum metallicum* has been used to general mind diseases, including depression and anxiety. The patient usually refers to death, and seeks methods to commit suicide, feeling that there is no hope for himself [7]. The symptoms of *Argentum nitricum* generally appear in cases of nervous apprehension, fear, or strange psychological situations [7]. The clinical manifestation of the emotional reaction is an overreacting gastrointestinal system, causing an organic depressive effect, avoiding eating and generally taking the patient to a sub nutrition state, interacting with the immunological system and allowing the occurrence of infections and other diseases conjugated to the depressive mood [8]. *Phosphorus* has been indicated to anxious patients with feelings that something will happen. These patients usually feel anxious at twilight or when alone. They are apprehensive and have fear of death. Also, apathy or indifference are common symptoms of these patients, taking to a depressive condition [8].

The *Aconitum* patient is anxious, restless, agitated, and has a fear of death. Fear is always present in this patient, who is irrational and afflicted by exacerbated anguish. *Aconitum* has been used primarily for acute conditions caused by shock or sudden exposure to changes in temperature. It is indicated for sudden discomfort following external exposure to cold or external heat, which is common in people who must travel around the world for working reasons [7-9].

*Valeriana officinalis* affects the central nervous system, producing a high degree of irritation. Hence, the patients are intellect clouded, with mild delirium with trembling excitement, headaches with violent pressure in the forehead. Moreover, at night the patient is restless, sleeping only toward the morning [8,9]. Arsenic has a specific action on almost every organ, tissue, and secretion of the body. It acts directly upon the blood composition, upon the tissues, and upon the nervous system. Asthenia and exhaustion of vital power, producing symptoms of impeded functional activity amounting in some cases to important paralysis. This exhaustion gives rise to lassitude, prostration, weakness. The leading feature of this remedy is the nervous restlessness, especially delirium at night, with great restlessness that resembles *Aconitum* or *Rhus toxicodendron* [9,10].

Based on the individual indications of each component of this homeopathic medicine, Homeo-pax® complex, this study aims to evaluate its effect on behavior in pre-clinical models of depression and anxiety.

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Material and Methods

Animals

Groups of male Wistar rats (*Rattus norvegicus*, n=5), weighing around 180g, from the Centro Multidisciplinar para Investigação Biológica (CEMIB - Multidisciplinary Center for Biological Investigation) of the Universidade Estadual de Campinas were used. The animals were kept in a room with controlled humidity (55 ± 5 %) and temperature (21 ± 1 °C), with a 12-hour light-dark cycle and water *ad libitum*.

All the animals were treated daily with 0.5 ml of distilled water, for two weeks prior to the experiments, by the same researcher, to avoid any kind of stress in the animals and thereby, the probability of false results. The test medicine and placebo (vehicle) were randomized by the senior researcher of the laboratory, which had assigned the groups.

The initial project was approved by the Ethics Committee of the Federal University of Amapá, and was assigned protocol #4A/2008.

Test medication (Homeopathic Homeo-pax® complex)

The samples were sent by the company Farmácia e Laboratório Homeopático Almeida Prado Ltda., in the form of tablets with a date of manufacture of 05/05/2008 and expiry date of 05/05/2010.

Treatments

The treatment with Homeo-pax® was carried out over a 30-day period, beginning on the 3rd day after exposure of the animals to the training in the open field and forced swimming tests. The treatments were prepared by one researcher that did not participate in the experiments (senior researcher). They were labeled as A, B, C and D. All following procedures were blind to the researchers involved in the experiment itself. Homeo-pax® was administered orally twice a day (0.5 ml/animal). Diazepam (Sigma – D0899) and amfepramone chloride (Sigma – M5037) were administered daily (10 mg/kg, p.o.) during three days before the experiment. In this case, all animals received the placebo (vehicle 0.5 ml, p.o.) twice a day during all period (except during the three days before the treatment) to standardize the treatment with Homeo-pax®. The animals were fasted for 12h before the experiments. The control group received 0.5 ml of placebo twice a day. The placebo was prepared just as Homeo-pax® but with no active ingredients.

Behavioral tests

Each group of animals was evaluated three times in the maze test and forced swimming test on the 1st, 20th and 30th days of treatment. The open field and rota rod tests were performed on the last day of treatment (30th day).

The behavioral assays were carried out between 7 am and 11 am in a room with controlled temperature (21 ± 1°C), and for subsequent analysis, the tests were recorded with a Sony camera model CCD-TR517.

Elevated plus maze test
This test consists on placing the animals in a wooden cross, with arms with 50 cm length and 10 cm width, two of which are open and two closed, with side walls of 40 cm height, as described by Pellow et al [11]. The animals were individually placed in the center of the EPM for 5 minutes, and the time spent in the open and closed arms was recorded for further analysis.

In this test, the animals were treated according to the statement above and diazepam (Sigma – D0899, 10 mg/kg, p.o, positive control group) was administered during three days before the experiment. The animals were submitted to the experiment 30 minutes after the treatment.

**Forced swimming test**

The forced swimming test consists of two swimming episodes, in a cylindrical glass aquarium of 40 cm height and 18 cm diameter, containing 15 cm of water at 25°C. The technique was described by Porsolt et al [12]. The first swimming episode was carried out by placing the animal in the aquarium for 15 minutes (pre-test). After 24 hours, the test was carried out again, placing each animal in the aquarium to swim for 5 minutes and recording the immobility time. Immobility was based on the technique of Borsini & Meli [13], which is attained when the animal makes only the necessary movements to keep its head above water.

The animals were treated with 0.5 ml of Homeo-pax® (p.o.), distilled water (0.5 ml, control group) as stated above and amfepramone hydrochloride (Sigma – M5037, 10 mg/kg, p.o., positive control) was administered during three days before the experiment. The animals were submitted to the experiment 30 minutes after the treatment.

**Open field test**

Animals were introduced in an arena (100 cm x 100 cm x 50 cm) with white ground. The ground was divided with black ribbons set in both parallel and perpendicular way with 20 cm spaces among them. Five objects were placed in the central squares to evaluate the animals’ exploratory activity.

The treatment consisted in doses of 0.5 ml of Homeo-pax® (during a 30 days period, twice a day) and 0.5 ml of distilled water (control group). The control drugs, diazepam (10mg/kg) and amfepramone (10 mg/kg) were administered during three days before the experiment. The animals were submitted to the experiment one hour after the last treatment. The experiment was recorded with a video camera and the motion activity (number of squares crossed) and rearing (the animals put hind paws leaning or not on the wall) were counted.

**Rota rod test**

This test consists of placing the animals on a platform with a rotating axis, divided by circular plates into four compartments (Acceler rota rod - Jones & Roberts for rats, Ugo Basile mod. DS 37) according to the method described by Dunham and Miya [14]. The animals were selected 24 hours before the experiment, and those capable of remaining on the device for 300 seconds at 32 rpm, without falling down, were used.

The treatment of Homeo-pax® and the control group (distilled water, 0.5 ml) followed the established protocol for 30 days (twice a day). The positive control was treated with amfepramone (three days, 10 mg/kg, p.o.). After this period, the animals were placed in the equipment, and the latency time until the fall was measured with a chronometer at 60, 90 and 120 minutes after treatment, considering a cut-off time of 60 seconds.

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Statistical analysis

The data obtained were expressed as Mean ± Standard Deviation. The results were analyzed by ANOVA followed by Tukey-Kramer post-hoc [15] test using the GraphPad Instats software (version 3.01). Results with p<0.05 were considered statistically significant.

Results

Elevated plus maze test

The treatment with Homeo-pax® (0.5 ml, p.o) produced an anxiolytic effect on the 1st and 20th days. The animals remained longer in the open arms compared to the 30th day (Figure 1). It is emphasized that the effect presented by the group treated with diazepam (10 mg/kg, p.o) was similar to that presented by Homeo-pax®, on the 1st and 20th days. This effect was considered significant when compared to the control group (p < 0.05).

![Figure 1](https://example.com/image1.png)

**Figure 1.** Effect of treatment with Homeo-pax® (0.5 ml/animal, p.o), distilled water (0.5 ml/animal) for 30 days, and diazepam (10 mg/kg, p.o) on time spent in the open arms, recorded on days 1, 20 and 30 of treatment in the elevated plus maze test. The bars represent the mean ± SD of n = 5/group, *p<0.05 ANOVA followed by Tukey – Kramer post-hoc test.

Forced swimming test

Treatment with Homeo-pax® (0.5 ml, p.o) resulted in an increase in the swimming time, compared to the control group (Figure 2). In this group, it was observed a dependent treatment time, resulting in a similar effect to that presented by the amfepramone group (10 mg/kg, p.o) on 30th day after treatment with Homeo-pax®. The time of immobility was significantly reduced, also in a treatment presenting a time-dependent manner.
Figure 2. Effect of treatment with Homeo-pax (0.5 ml/animal, p.o.), and distilled water (0.5 ml/animal) for 30 days, and amfepramone (10 mg/kg, p.o.) on swimming time, recorded on days 1, 20 and 30 of treatment, in the forced swimming test. The bars represent the mean ± SD of n = 5/group, *p<0.05 ANOVA followed by Tukey – Kramer post-hoc test.

Figure 3. Effect of treatment with Homeo-pax (0.5 ml/animal, p.o), distilled water (0.5 ml/animal) for 30 days, and amfepramone (10 mg/kg, p.o) on immobility time, recorded on days 1, 20 and 30 of treatment, in the forced swimming test. The bars represent the mean ± SD of n = 5/group, *p<0.05 ANOVA followed by Tukey – Kramer post-hoc test.

Open field test
The values of the different parameters observed are described in table 1. Crossing by the animals was significantly increased by the treatment with amfepramone (10mg/kg, p.o), and also with the thirty-day treatment with Homeo-pax*. The other parameter evaluated was rearing (Table 1). It was affected as well as the crossings for the group treated with amfepramone. The number of rearing was increased significantly compared to control group (p < 0.05). The group treated with Homeo-pax* for thirty days did not show a significant effect.

Table 1. Effect of treatment with Homeo-pax* (0.5 ml/animal, p.o.), distilled water (0.5 ml/animal) for 30 days and amfepramone (10 mg/kg, p.o.) in the open field test.

| Group       | Dose      | Crossing     | Rearing (no. of times) |
|-------------|-----------|--------------|------------------------|
| Control     | 0.5 ml, p.o | 58.6 ± 10.34 | 22.2 ± 2.39            |
| Homeo-pax   | 0.5 ml, p.o | 109.3 ± 13.86* | 31.75 ± 6.80          |
| Amfepramone | 10 mg/kg, p.o | 136 ± 12.84* | 48.3 ± 6.37*          |

The numbers represent the mean ± SD of n = 5/group, * p < 0.05 ANOVA followed by Tukey – Kramer post-hoc test.

Rota rod test

The treatment with amfepramone (10 mg/kg, p.o) significantly reduced the latency time in the apparatus. The thirty-day treatment with Homeo-pax* (0.5 ml, p.o), did not affect the motor coordination of the animals when compared with the animals of the control group, which presented the same behavior (Figure 4).

![Figure 4](https://doi.org/10.51910/ijhdr.v10i34.420)
The elevated plus maze test has been an experimental model widely used to test substances with anxiolytic or anxiogenic potential. It is based on the natural aversion of some animal species to height and open spaces, which leave them exposed. It has been observed that drugs able to reduce anxiety cause the animals to expose themselves more often in the open arms of the maze.

Substances with anxiolytic properties, such as diazepam (benzodiazepinic) and buspirone (selective receptor 5HT<sub>1A</sub> agonist), reduce fear and increase the animals’ activity in the open arms in the elevated plus maze test [16-18].

The forced swimming test is a classic test used to determine the possible antidepressive action of new drugs. According to Porsolt, et al [12,18] the fact that the animals spend more time immobile in the second exposure to the swimming is due to the state of desperation, generated by the impossibility of escape. O'Neill et al [19] have tested groups of animals which, in the pre-test, could or not escape from the aquarium, demonstrating that there is no relationship between inability to escape and immobility of the animals. However, Handley [20] have observed that the animals' fear in the second exposure to swimming was decreased if compared with the first, which suggests that immobility is an adaptive response of the animal for stress situations.

Although forced swimming does not induce similar symptoms in the animals to those found in humans with depression, it is an experimental model capable of detecting antidepressant substances [12] since the majority of these substances cause a reduction in immobility time, and this parameter is correlated with the clinical potency of these drugs [21]. Likewise, the specificity of the test is capable of distinguishing antidepressants from neuroleptics and anxiolytics.

In the forced swimming test, drugs that cause an increase in motor activity can lead to false positive results, and should therefore be conducted with the open field test, to evaluate this parameter. This relation is not clear, since substances like phenylethylamine increase motor activity, without affecting immobility time[22] or substances that reduce motor activity exert anti-immobility effects.

In the analysis of the results obtained by the oral administration of the homeopathic complex Homeo-pax®, it was observed that this complex caused a reduction in immobility time and an increase in swimming time in the forced swimming test, indicating an antidepressant activity (Figure 2 and 3). It has also caused an increase in the time spent on the open arms of the elevated plus maze, suggesting an anxiolytic action (Figure 1).

The open field test has been used as a valid experimental method for therapeutical analysis but not for pathogenetic effects [23]. In the open field test, motor activity and number of rearings (complex stereotyped behavior), were increased, though in the latter, this increase was not significant. This observed behavior, associated with the result of the forced swimming test, indicates antidepressant activity of the homeopathic complex Homeo-pax®. Pinto et al [24] have performed a pre-treatment in mice with Chamomilla 6cH and submitted to the forced swimming test. Chamomilla 6cH treated animals did not show excitatory behavior when compared to ethanol or amitriptyline.

The motor coordination of the animals was not significantly altered by the treatment with Homeo-pax®, but it was affected by treatment with the drug used as positive control (amfepramone) (Figure 4). Several substances have the ability to reduce motor coordination in the rota rod test, such as ethanol associated with aluminum[25], derivatives of pyrrolinynaphthalene [26], amitriptyline and clomipramine [27].

Conclusion
Based on the results presented, it can be suggested that the homeopathic complex Homeo-pax® has presented anxiolytic and antidepressant properties, without affecting motor coordination ability. The mechanism of action by which it leads to these effects requires further investigation, through specific models, comparing the data obtained here with specific blocking drugs, and their interactions with those drugs.

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**Efeitos ansiolíticos e antidepressivos do complex homeopático homeo-pax (estudo pré-clínico)**

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RESUMO

O complejo homeopático Homeo-Pax® tem sido usado no Brasil como um medicamento homeopático de ação ansiolítica e antidepressiva. O Homeo-Pax® é um complexo preparado com Aconitum nap 6C, Aurum met 6C, Phosphorus 6C, Arsenicum alb 6C e Valeriana officinalis 3C. Este estudo avaliou o comportamento de ratos após o tratamento com Homeo-Pax® em modelos pré-clínicos de depressão e ansiedade. Testes de labirinto em cruz elevado (EPM), nado forçado (FST), campo aberto (OFT) e Rotarod (RRT) foram usados para avaliar a atividade dos animais. No EPM, os animais tratados com Homeo-pax® permaneceram mais tempo nos braços abertos do labirinto, durante os 20 primeiros dias de tratamento, em relação ao 30º dia. Este resultado foi estatisticamente significativo quando comparado com o grupo controle (p<0.05). No FST, o tratamento com Homeo-pax® (0.5 ml, p.o) aumentou o tempo de nado, comparado ao grupo controle. Este efeito foi dependente do tempo de tratamento, resultando similar ao efeito da anfepramona (10 mg/kg, p.o). No OFT, o movimento dos animais foi significativamente aumentado pelo tratamento com anfepramona (10mg/kg, p.o) e também no 30º dia de tratamento com Homeo-pax®. No RRT, o tratamento por 30 dias com Homeo-pax® (0.5 ml, p.o) não afetou a coordenação motora dos animais, em relação ao grupo controle. Baseado nesses resultados, pode ser sugerido que o complexo homeopático Homeo-pax® tem propriedades ansiolíticas e antidepressivas sem afetar a coordenação motora.

Keywords: Valeriana officinalis, Comportamento, Ensaio pré-Clinico, Ansiedade, Depressão.

Efectos ansiolíticos y antidepresivos del complejo homeopático homeo-pax (estudio preclínico)

RESUMEN

El complejo homeopático Homeo-Pax® viene siendo usado en Brasil como un medicamento homeopático de acción ansiolítica y antidepressiva. El Homeo-Pax® es un complejo preparado con Aconitum nap 6C, Aurum Met 6C, Phosphorus 6C, Arsenicum nitricum 6C, Arsenicum alb 6C y Valeriana officinalis 3C. Este estudio evaluó el comportamiento de camondongos después del tratamiento con Homeo-pax® en modelos preclínicos de depresión y ansiedad. Testes de laberinto en cruz elevado (EPM) nado forzado (FST), campo abierto (OFT) y Rotarod (RRT) fueron usados para evaluar la actividad de los animales. En el EPM los animales tratados con Homeo-pax® permanecieron más tiempo en los brazos abiertos del laberinto durante los 20 primeros días de tratamiento en relación al 30º día. Este resultado fue estadísticamente significativo si comparado con el grupo control (p<0.05). En el FST, el tratamiento con Homeo-pax® (0.5 ml,p.o) aumentó el tiempo de nado, comparado al grupo control. Este efecto fue dependiente del tiempo de tratamiento, resultando similar al efecto de la anfepramona (10 mg/kg, p.o). En el OFT, el movimiento de los animales fue significativamente aumentado por el tratamiento con anfepramona (10mg/kg, p.o) y también en el 30º día de tratamiento con Homeo-pax®. En el RRT el tratamiento por 30 días con Homeo-pax® (0.5 ml, p.o) no afectó la coordinación motora de los animales, en relación al grupo control. Basado en estos resultados puede ser sugerido que el complejo homeopático Homeo-pax® tiene propiedades ansiolíticas y antidepressivas sin afectar la coordinación motora.

Palabras-llave: Valeriana officinalis, comportamiento, ensayo preclínico, ansiedad, depresión.