Anxiolytic effect of the homeopathic complex Tepeex®

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ABSTRACT

Aims: Homeopathic complex Tepeex® is a compound of *Actaea racemosa* 4cH, *Natrum muriaticum* 2cH, *Pulsatilla nigricans* 3cH, *Chamomilla* 3cH and *Sepia succus* 5cH. This study evaluated the effect of Tepeex® in pre-clinical models of depression and anxiety. **Methods:** the following tests were performed: elevated plus maze test (EPM); forced swimming test (FST); open field test (OFT) and Rotarod test (RRT). **Results:** In EPM, animals treated with Tepeex® on days 20 and 30 stayed longer in the open arms of the maze than the control group ($p < 0.05$, Dunnett test). In FST, treatment with Tepeex® did not increase swimming time compared to the control group in any phase of treatment. In OFT, crossing increased significantly with treatment with amfepramone, and also with 30-day treatment with Tepeex® ($p < 0.05$, Dunnett test). In RRT, treatment with amfepramone significantly reduced latency time. 30-day treatment with Tepeex® did not affect motor coordination when compared to the control group. **Conclusion:** results suggest that homeopathic complex Tepeex® has anxiolytic properties without affecting motor coordination.

Keywords: Anxiety; homeopathic complex; pre-clinical model; rats

Introduction

Anxiety is a natural response to a threatening situation [1], during which body and mind become awakened and alert, preparing to wither attack or flee from the threat [2]. It can be defined as a vague, diffuse and disagreeable feeling of expectant apprehension or tension, attended by several physical manifestations including breathlessness, tachycardia, muscular tension and tremors. Anxiety becomes pathological when it is excessive, “paralyzing” or involves physical or psychosocial effects [3]. In this way, anxiety – together with phobias – is the main health problem among the Brazilian population, with a global prevalence between 8 and 18%; anxiety disorders are the most common psychiatric condition in both children and adults, with an estimated prevalence during lifetime of 9% and 15% respectively [4, 5].

Some studies have indicated the effectiveness and safety of homeopathic treatment in emotional stress and anxiety [6,7,8,9]. On the other hand, the study of anxiety at the pre-clinical level is limited by the fact that it relates to a subjective state considered exclusive to human beings. For this reason, it cannot be reproduced but at best modeled in animals [10].

A systematic review of the controlled clinical use of homeopathy for mental and psychological problems identified ten attempted applications [11]. Of these, eight results were positive. A subsequent meta-analysis of the clinical placebo-controlled application of homeopathic medications concluded that the positive results of these applications are not related to the placebo effect [12].

“Tepeex” was designed by Almeida Prado Homeopathic Laboratory, Ltda. Sao Paulo, Brazil for the treatment of anxiety (patent required). It is composed of *Actaea racemosa* 4cH, *Natrum muriaticum* 2cH, *Pulsatilla nigricans* 3cH, *Chamomilla* 3cH and *Sepia succus* 5cH.

Pulsatilla nigricans is indicated for anxiety accompanied by crying, sadness and mood swings, which worsens when in a warm room, with tiredness and sick food, and improves in a cool environment, and fresh air [13]. *Natrum muriaticum* is indicated in states of anxiety which lead to severe depression, for individuals who are difficult to console, or whose symptoms worsen with heat and improve with fresh air and cold baths [13]. *Actaea racemosa* is a medication which acts mainly in females, but without excluding its use in males [14]. Stress, sensitivity, anxiety and severe irritability often lead to a prescription of *Chamomilla*. It is indicated for anxious children whose symptoms worsen mid-morning or at night, with heat [15]. In a study of depression treatment with homeopathic medicines, *Sepia succus* was used in a case where there was a reduction in the Montgomery-Åsberg Depression Rating Scale (MADRS) score from 32 to 8 on the last return, thus demonstrating its effectiveness in this case [16].

The mental depression and bipolar disorder are disabling diseases that alter the mood and affect the physical layout, sleep, appetite, libido and functional capacity. Depression differs from schizophrenia, which produces cognitive disorders. Symptoms of depression correspond to the intense feeling of sadness, hopelessness, despair and inability to experience pleasure in usual activities [17].

This study sought to evaluate the effect of complex Tepeex in pre-clinical models of depression and anxiety, by comparison to drugs used in such models, diazepam and amfepramone chloride [18].

Material and methods

Animals

Male Wistar rats were used (n = 5/group), weighing around 180g, from Centro Multidisciplinar para Investigação Biológica (Multidisciplinary Center for Biological Investigation) - CEMIB, of Universidade Estadual de Campinas, São Paulo, Brazil. Rats were kept in a room with controlled humidity and temperature at around 25°C, with a 12-hour light/dark cycle and water *ad libitum*.

All animals were treated daily with 0.5ml of distilled water p.o., for 2 weeks before the experiments, by the same researchers, to reduce stress, thereby reducing the probability of false results.

The initial project was approved by the Ethics Committee of the Federal University of Amapá, and was assigned protocol no. 4A/2008.

Test medication (homeopathic complex Tepeex®)

Samples were produced and supplied by Almeida Prado Homeopathic Laboratory Ltda, Sao Paulo, Brazil, in the form of tablets; with date of manufacture 05/05/2008 and expiration date 05/05/2010, the study was conducted in October/2008.

Behavioral tests

Classic tests were used to determine the possible anxiolytic and antidepressant actions [19,20,21]. Each group of animals was evaluated 3 times by EPM (Elevated plus maze test) and FST (Forced swimming test) on days 1, 20 and 30 of treatment with the test medication. OFT (Open field test) and RRT (Rotarod test) were performed on the last day of treatment. All tests were carried out between 8h00 am and 8h00 pm in a room with controlled temperature (25 ± 1°C), and for subsequent analysis, the tests were filmed using a Sony camera model CCD-TR517.

Elevated plus maze test (EPM)

This test consists of placing the animals (rats) in a wooden cross, with arms of 50 cm in length and 10 cm in width, two of which are open and two closed, with side walls of 40 cm in height, as described by Pellow et al. [19]. The animals were placed individually in the center of the EPM for 5 minutes, and the length of time the animals spent in the open and closed arms is recorded.

In this test, one group of animals (n=5) was treated with the test medication, Tepeex[®], 0.5 ml, p.o., a second group (n=5) with distilled water, 0.5 ml, p.o. (control group) and a third group (n=5) with diazepam, 10 mg/kg, p.o. (positive control group). Thirty minutes after treatment, animals were subjected to the test.

Forced swimming test (FST)

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 described by Porsolt et al., [20] was followed. 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 [21], in which the rat makes only the necessary movements to keep its head above the water.

Treatments were carried out after pre-test, 5 hours, and 1 hour before the definitive 5-minute test, per os, using 0.5 ml of Tepeex[®] (test group n=5) distilled water (control group n=5) and amfepramone, 10 mg/kg, p.o. (positive control group n=5).

Open field test (OFT)

In the open field test animals are subjected to a single 5-minute exposure to an unknown environment. The latter consists of a box (100 cm x 100 cm x 50 cm), painted white and illuminated with a fluorescent bulb placed 50 cm above the center of the box. The box is divided into squares by parallel and perpendicular blue strips 20 cm by 20 cm. Five objects were placed in the central squares, to evaluate the animals' exploratory activity.

Different groups were treated with the doses of 0.5 ml of Tepeex[®], 30-day treatment, twice a day (test group n=5), 0.5 ml of distilled water (control group n=5) and amfepramone 10 mg/kg, was administered three days before the experiment. Parameters evaluated were crossing (number of squares ran across by the animals) and rearing (whether or not the animals supported their paws against the wall). Increase in crossing expresses a stimulant effect on the Central Nervous System. Exploratory activity was analyzed in relation as decrease or increase, suggesting a sedative or psycho-stimulant, but not necessary antidepressant action [22].

Rotarod test (RRT)

This test consists in placing the animals (rats) on a platform with a rotating axis, divided by circular plates into four compartments (Acceler Rotarod - Jones & Roberts for rats, Ugo Basile mod. DS 37) according to the method described by Dunham and Miya [23]. Animals were selected 24 hours before the experiment, and those not capable of remaining on the device for 300 seconds at 32 rpm without falling off, were rejected.

Treatment was carried out 1 hour before the experiment. For Tepeex[®], duration of treatment was 30 days treatment, twice a day (test group n=5); a second group was treated with distilled water, 0.5 ml (control group n=5), and amfepramone, 10 mg/kg, three days before (positive control group n=5). After this period, the animals were placed on the equipment and the latency time to fall off was timed with a chronometer at 60, 90 and 120 minutes, and recorded, considering a cut-off time of 60 seconds.

Treatment

Treatments were carried out for 30 days, starting on the third day after exposure of the animals to training in the open field and forced swimming tests. A Tepeex® tablet was diluted at the time of treatment, in 50 ml of distilled water, and administered per os (0.5 ml/animal) by gavage. In the case of diazepam (Sigma – D0899) and amfepramone chloride (Sigma – M5037) the drugs were administered per os three days before the final experiment. The animals fasted 12h before tests.

Statistical analysis

Data obtained were expressed as mean $\pm$ standard error of measurement; for statistical analysis of results, Dunnett test was used together with ANOVA. Statistical significance was considered $p < 0.05$.

Results

Elevated plus maze test

Treatment with Tepeex® did not increase the time spent in the open arms of the maze until day 20. However, on days 20 and 30 of treatment, the animals treated with Tepeex® stayed longer in the open arms of the maze than the control group (Figure 1). This effect was considered significant when compared with the control group ($p < 0.05$, Dunnett test).

Figure 1. Effect of treatment with Tepeex® (0.5 ml/animal, v.o), distilled water (0.5 ml/animal) for 30 days, and diazepam (10 mg/kg, p.o., 3 days before) 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 $\pm$ SEM of $n = 5$ /group, * $p < 0.05$ Dunnett test.

Figure 2. Effect of treatment with Tepeex® (0.5 ml/animal, p.o.), distilled water (0.5 ml/animal) for 30 days, and amfepramone (10 mg/kg, p.o., 3 days before) on swimming time, recorded on days 1, 20, and 30 of treatment, in the forced swimming test. The bars represent the mean $\pm$ SEM of $n = 5$ /group, * $p < 0.05$ Dunnett test.

Forced swimming test

Treatment with Tepeex® in all measurement times did not increase swimming time, by comparison to the control group (Figure 2). Immobility time was not reduced with the treatment with Tepeex®, a reduction which was observed only with amfepramone on day 30 (Figure 3).

Figure 3. Effect of treatment with Tepeex® (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 $\pm$ SEM of $n = 5$ /group, * $p < 0.05$ Dunnett test.

Open field test

Values for the different parameters observed are described in Table 1. Crossing was significantly increased by treatment with amfepramone, while for the Tepeex[®], there were no differences between these groups.

The other parameter evaluated was rearing (Table 1) which was affected in a similar way in the group treated with amfepramone - increase in the number of rearings significant in Dunnett test ($p < 0.05$), while in the group treated with Tepeex[®] for 30 days, the response was similar to that of the control group.

Table 1. Effect of treatment with Tepeex[®] (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 (v.o.)	Crossing	Rearing (no. of times)
Control	0,5 ml	57 ± 10	$22,2 \pm 2,4$
Tepeex [®]	0,5 ml	115 ± 12	22.0 ± 5.6
Anfepramone	10 mg/kg	$136 \pm 13^*$	$48,3 \pm 6,4^*$

The numbers represent the mean $\pm$ SEM of $n = 5$ /group, * $p < 0.05$ Dunnett test.

Rotarod test

Treatment with amfepramone (10 mg/kg, p.o.) significantly reduced latency time in the rotarod device. 30-day treatment with Tepeex[®] (0.5 ml, p.o.) did not affect motor coordination of animals when compared to the control group, which presented the same behavior (Figure 4).

Figure 4. Effect of 30-day Tepeex[®] treatment (0.5 ml/animal, p.o.), distilled water (0.5 ml/animal) and amfepramone (10 mg/kg, p.o.) on time spent in the rotarod test. The points represent the mean $\pm$ SEM of $n = 5$ /group in the measurement times. * $p < 0.05$ Dunnett test.

Discussion

EPM test is an experimental model that is widely used today, to test substances with anxiolytic or anxiogenic potential. It is based on the natural aversion of some animal species to high, open spaces, which leave them exposed in the EPM. It has been observed that drugs that reduce anxiety cause in the animals to expose more often in the open arms of the maze [19]. Substances with anxiolytic properties, such as diazepam (benzodiazepines) reduce fear and increase the animals' activity in the open arms of the elevated plus maze test [24,25,26].

FST is a classic test used to determine the possible antidepressant action of new drugs [10]. Although forced swimming does not induce in animals symptoms similar to those found in human beings with depression, it is nevertheless an experimental model capable of detecting antidepressant effects in substances [19], since most of the latter cause reduction in immobility time, and this parameter is correlated with the clinical homeopathic dilution of these drugs. Likewise, the specificity of the test allows distinguishing antidepressants from neuroleptics and anxiolytics [27]. According to Porsolt et al., and Porsolt et al., [28], immobility time is reduced by treatment with known antidepressant drugs, a behavior differing from that induced by psychostimulants which cause a reduction in crossings in the open field test.

In the analysis of the results obtained by the oral administration of the homeopathic complex Tepeex®, it was observed that this complex did not reduce immobility time or swimming time in FST (Figures 2 and 3). However, it did increase the time spent in the open arms of the maze from day 20 of treatment onwards in EPM suggesting an anxiolytic action which is dependant on treatment time (Figure 1). In the OFT, motor activity was increased (Dunnett, $p < 0.05$). This indicates a stimulant action on the central nervous system.

RRT determines possible alterations in the motor coordination ability of the animals, often caused by the use of sedatives and antipsychotic drugs, among others [22]. Motor coordination was not significantly altered by Tepeex® but was affected by amfepramone (Figure 4). Various substances have the ability to reduce motor coordination when evaluated by RRT such as ethanol associated with aluminum [29], and derivatives of pyrrolinylnaphthalene [30], amitriptyline and clomipramine [31].

Conclusion

Based on these results, it is suggested that the homeopathic complex Tepeex® has anxiolytic and antidepressant properties without affecting motor coordination.

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Efeito ansiolítico do complexo homeopático Tepeex ®

RESUMO

Objetivos: o complexo homeopático Tepeex[®] é composto de *Actaea racemosa* 4cH, *Natrum muriaticum* 2cH, *Pulsatilla nigricans* 3cH, *Chamomilla* 3cH e *Sepia succus* 5cH. Este estudo avaliou o efeito de Tepeex[®] em modelos pré-clínicos de depressão e ansiedade. **Métodos:** foram realizados os testes de labirinto em cruz elevado (EPM); nado forçado (FST); campo aberto (OFT) e Rotarod (RRT). **Resultados:** no EPM, os animais tratados com Tepeex[®] nos dias 20^o e 30^o permaneceram mais tempo nos braços abertos do labirinto que o grupo controle ($p < 0,05$ teste de Dunnett). No FST, o tratamento com Tepeex[®] não aumentou o tempo de nado por comparação com o grupo controle em nenhuma fase do tratamento. No OFT, os cruzamentos aumentaram de modo significativo no grupo tratado com amfepramona assim como no tratado 30 dias com Tepeex[®] ($p < 0,05$ teste de Dunnett). No RRT, o tratamento com amfepramona reduziu significativamente o tempo de latência. O tratamento de 20 dias com Tepeex[®] não afetou a coordenação motora. **Conclusões:** os resultados sugerem que o complexo homeopático Tepeex[®] tem propriedades ansiolíticas sem afetar a coordenação motora.

Palavras chave: Ansiedade; Complexo homeopático; Modelo pré-clínico; Ratos

Efecto ansiolítico el complejo homeopático tepeex[®]

RESUMEN

Objetivos: el complejo homeopático está compuesto de *actaea racemosa* 4ch, *natrum muriaticum* 2ch, *pulsatilla nigricans* 3ch, *chamomilla* 3ch y *sepia succus* 5ch. Este estudio buscó evaluar el efecto de tepeex[®] en modelos preclínicos de depresión y ansiedad. **Métodos:** fueron realizados los tests de laberinto en cruz elevado (epm); nado forzado (fst); campo abierto (oft) y rotarod (rrt). **Resultados:** en el epm, los animales tratados con tepeex[®] en los días 20^o y 30^o permanecieron más tiempo en los brazos abiertos del laberinto que el grupo control ($p < 0,05$ test de dunnett). En el fst, el tratamiento con tepeex[®] no aumentó el tiempo de nado comparado con el grupo control en ninguna fase del tratamiento. En el oft, los cruzamientos aumentaron significativamente en el grupo tratado con amfepramona así como en el grupo tratado 30 días con tepeex[®] ($p < 0,05$ test de dunnett). En el rrt, el tratamiento con amfepramona redujo significativamente el tiempo de latencia. El tratamiento de 20 días con tepeex[®] no afectó la coordinación motora. **Conclusiones:** los resultados sugieren que el complejo homeopático tepeex[®] posee propiedades ansiolíticas sin afectar la coordinación motora.

Palabras llave: ansiedad; complejo homeopático; modelo preclínico; ratas

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Conflict of interest: Authors declare they had full access to all the data in this study and they take complete responsibility for the integrity of the data and the accuracy of the data analysis.

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