Assessment of homeopathic medicine *Aconitum napellus* in the treatment of anxiety in an animal model

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ABSTRACT

Background: *Aconitum napellus* is a classic resource of complementary medicine for the treatment of patients exhibiting neurological symptoms of anxiety. **Aim:** To assess the action of homeopathic medicine *Acon* in the treatment of generalized anxiety in an experimental model using rats. **Methods:** 48 adult (two to three months old) male Wistar rats (*Rattus rattus*) were randomly divided in six groups (n= 8/treatment) and given the following treatments by gastric tube along 10 days: 1) control (diazepam 1 mg/kg/day); 2) negative control (0.15 mL saline solution/day); 3) ACH6 (0.15 mL *Acon* (6cH/day); 4) ACH12 (0.15 mL *Acon* 12cH/day); 5) ACH30 (0.15 mL *Acon* 30cH/day); and 6) ALC30 (0.15 mL 30% cereal alcohol/day). Behavioral effects were blindly and randomly assessed in elevated plus maze (EPM) and open field test. **Results:** *Acon* in dilutions 12cH and 30cH exhibited possible anxiolytic effects on the central nervous system (CNS) since they increased the number of entries in the EPM open arms (12cH and 30cH) and the permanence time in the EPM open arms (30cH only). In the open field test the homeopathic preparations did not show effects on the locomotor system of rats. **Conclusion:** Dilutions 12cH and 30cH of *Acon* exhibited anxiolytic effects on the CNS in an animal experimental model.

Keywords: Anxiety, homeopathy, *Aconitum napellus*, animal model, rats

Introduction

Anxiety is an emotional state comprising psychological and physiological components; it is a part of the normal scope of human experience and enhances performance. Anxiety becomes pathological when its intensity bears no proportion to the triggering situation or lacks a specific target. In the latter context, it is one of the most frequent psychiatric disorders in the overall population, with a prevalence of 12.5% [1].

Anxiety is a feeling stemming from excessive excitation of the CNS; it is a reaction of the organism to a perceived physical or psychological threat that triggers the so-called general adaptation response involving release of adrenalin, cortisol and other hormones that once in the bloodstream activate the body defenses to prepare the organism to face and resist physical or psychological trauma [2]. Anxiety encompasses feelings of fear, insecurity, and apprehensive anticipation, the mind is dominated by catastrophic ideas or of personal unfitness, increase of the state of wakefulness and alert, increased arterial blood pressure, increased heart rate, increased breathing rate, urinary and evacuation urgency, muscular tension causing pain, trembling and restlessness, besides a wide scope of physical discomforts secondary to the hyperactivity of the vegetative nervous system [3].

Behavioral cognitive therapy, first-line pharmacological agents (anxiolytics) and selective monoamine reuptake inhibitors are effective therapeutic strategies. The best-known anxiolytics or tranquilizers are benzodiazepines such as diazepam, which act by modulating the GABA receptors. However, their use in clinical practice is not free from risks mainly due to potential adverse effects such as psychomotor deficit, or the augmentation of other CNS depressants [4]. Evidences show that the effects of behavioral cognitive therapy last longer.

Complementary and alternative medicine (CAM) represents a further therapeutic strategy. Homeopathy is one of the most widely spread CAM worldwide; anxiety and depression are among the most frequent reasons for patients to seek homeopathic treatment [5, 6]. It was initially formulated at the beginning of the 19th century by physician Samuel Hahnemann, who represented it as a safe and effective healing method grounded on observation and experiment, and able to achieve its aim in a fast, smooth and long lasting manner. He further stressed that treatment must systematically be less painful and noxious than the state of disease it seeks to heal. Homeopathy is based on the law of similarity (*similia similibus curantur*) [7] according to which substances that cause some symptoms are used to stimulate healing in patients exhibiting similar clinical manifestations [8].

To understand the relationship of similarity between homeopathic medicine *Aconitum napellus* and anxiety disorders, the following description in Charles J. Hempel's materia medica and therapeutics (1880) is illustrative: "Aconitum napellus is inside us, not actively, but in potential state, waiting for a chance to irrupt powerfully against the destruction of the organism. Under the influence of an accidental cause, the sleeping power of aconite becomes a rebellious disease" [9].

The traditional homeopathic materia medica describes *Acon* as having a wide field of action comprising a large number of illnesses. Its main action consists in increasing the activity of arteries, thus causing remarkable hyperemia in the brain and medullar centers, which is translated by mental tension and high levels of anxiety. This state of agitation is as violent as intense and thus constitutes one of the keynote of this medicine; as a fact, together with *Rhus toxicodendron* and *Arsenicum album* it composes the classic homeopathic "restless trio" [10].

Studies on the action of homeopathic medicines performed by means of behavioral animal models evidenced the anxiolytic effects of *Ignatia amara* [11] and *Gelsemium sempervirens* [12,13]. The present study sought to assess the effect of homeopathic medicine *Aconitum napellus* in the treatment of generalized anxiety in an experimental model using rats.

Methods

Preparation of the homeopathic medicine

Homeopathic medicine *Aconitum napellus* was prepared in dilutions 6cH, 12cH, and 30cH in 30% cereal alcohol from a source acquired in the Brazilian pharmaceutical market following the guidelines in the second edition of Brazilian Homeopathic Pharmacopoeia for the centesimal scale (c) according to Hahnemann's method (H) in liquid form for oral route [14].

Animals

The present study used male Wistar rats (*Rattus rattus*) aged two to three months old and weight between 200 and 350 g supplied by the vivarium of Anglo-American Faculties (Foz do Iguaçu – Paraná) and Bela Vista Biological Reserve (Foz do Iguaçu – Paraná). The animals were housed in groups (n= 8) in plastic cages in a room kept at 22 ± 1°C, 60 to 80% humidity, 12-hour dark-light cycle, light was on between 7 am and 7 pm; animals were fed commercial ration and water *ad libitum*.

Treatment

The sample comprised 48 rats that were randomly divided in six groups (n= 8/treatment), which were given: diazepam (1 mg/kg/day); control (0.15 mL saline solution/day); ACH6 (0.15 mL Acon 6cH/day); ACH12 (0.15 mL Acon 12cH/day); ACH30 (0.15 mL Acon cH30/day); and ALC30 (0.15 mL 30% cereal alcohol 30%/day).

Each group was given its corresponding treatment daily for 10 consecutive days. Treatments were administered by one and the same person every day at the same time by gastric tube. The behavior of animals was assessed one hour after treatment on the 10th day. Animals were subjected only once to each pharmacological model.

Elevated plus maze

Based on the method described by Pellow et al., [15] elevated plus maze (EPM) is a widely employed anxiety model because it is grounded on two conflicting tendencies: the ability of rodents to explore new environments and their aversion to high and open places.

EPM consists in a wooden device comprising two closed arms perpendicular to two open arms. The closed arms have lateral and an end wall, whereas the open arms have no walls. The total rate of exploration of open arms is a measurement of anxiety, thus, increase of the permanence time and number of entries in the open arms is considered an index of anxiolytic action of drugs.

The animals were individually placed at the center of a maze raised 51 cm above the ground comprising two open arms (114.8 x 10 cm), two closed arms (114 x 10 x 43 cm) and a central intersection (10 x 10 cm). The behavior of animals was tested in a room illuminated by a 40W lamp and filmed for five minutes; the data were recorded by software PlusMZ. The number of entries in the closed and open arms and well as the permanence time in each arm was assessed for five minutes.

Open field

The open field test is based on the method described by Sielgel [16] and validated by Archer [17]. Rats are placed in a previously unknown sand square divided in smaller squares that allow assessing the exploratory activity of animals to observe their locomotor activity.

The animals were individually placed at the center of the open field (100 x 100) surrounded by walls (43 cm). The field floor is divided in 25 squares (20 x 20 cm). The behavior of animals was tested in a room illuminated by a 40W lamp and filmed for five minutes; the data were recorded by software OpenFLD. This test assessed the number of crossed squares.

Statistical analysis

The results are expressed as mean $\pm$ standard error. Statistical differences among groups were detected by one-way variance analysis (ANOVA) followed by Tukey's test. Significance was established as p-value < 0.05.

Ethics

All tests complied with the guidelines in the Guide for the Care and Use of Laboratory animals of the United States National Institutes of Health (NIH) [18]; the study was approved by the Animals Use Ethics Commission of Assis Gurgacz Faculty, ruling n° 015/2011.

Results

Elevated plus maze

Treatment with *Acon* induced anxiolytic effect, but did not exhibit linear progression according to dilutions. The number of entries in the open arms increased with dilutions 12cH and 30cH compared to the control (Figure 1). The animals treated with dilution 12cH also exhibited higher permanence time in the open arms (Figure 2).

Figure 1 – % entry in EMP open arms. Significant values: * $p < 0.001$, † $p < 0.01$ and ‡ $p < 0.05$ compared to control group

Figure 2 – % permanence time in EMP open arms. Significant values: * $p < 0.001$ and † $p < 0.05$ compared to the control groups

Diazepam, namely the positive control used for comparison of anxiolytic effect induced definite anxiolytic effect as shown by the increase in the number of entries and permanence time in the open arms of EPM (Figures 1 and 2), thus validating the method used to establish the anxiolytic effect of the investigated medications. The effect of diazepam measured as number of entries in the open arms was significantly higher compared with *Acon* 6cH, and also compared to dilutions 12cH and 30cH concerning the permanence time in the open arms. Also when compared to 30% alcohol diazepam exhibited significant increase of both investigated parameters. These results (Table 1) show that the anxiolytic effect of *Acon* in the investigated dilutions have moderate intensity compared to diazepam.

Table 1 – Comparison among groups after 10 consecutive days of treatment

	% entries in open arms	% time in open arms	% time in closed arms	Number of crossings
Control	21 ± 13 [§]	4.7 ± 6.6 [§]	6.4 ± 2.3 [§]	74 ± 20 [§]
Diazepam	52 ± 15	24.3 ± 6.7	6.5 ± 2.2 [§]	68 ± 28 [§]
ACH6	*32 ± 18 [§]	14 ± 13 [§]	6.6 ± 2.4 [§]	50 ± 38 [§]
ACH12	39.5 ± 8.4	*15.4 ± 8.6	8.00 ± 0.53 [§]	73 ± 12 [§]
ACH30	38 ± 12	†11 ± 10 [§]	6.6 ± 2.6 [§]	62 ± 15 [§]
ALC30	†30 ± 11 [§]	‡8.6 ± 4.4 [§]	8.0 ± 2.2 [§]	55 ± 32 [§]

Means followed by same symbols in a same column so not exhibit statistical difference. Significant values: *p<0.05; †p<0.01; ‡p<0.001 compared to group diazepam

The number of entries in the closed arms is a complementary parameter used to assess whether the response of animals indicates anxiolytic effects of the investigated treatments. In this study, it exhibited significant difference in the groups treated with *Acon* 12cH and 30cH and the positive control diazepam (Figure 3). These results suggest that the anxiolytic effect did not interfere with the mobility of the animals in the EPM.

Figure 3 – % entry in EMP closed arms. Significant values: *p<0.001, †p<0.01 and ‡p<0.05 compared to control group

Open field

Treatment with *Acon* did not show any alteration of the locomotion of animals compared with both saline solution and diazepam controls (Figure 4).

Figure 4 – Number of squares crossed by animals in open field during five-minute observation

Discussion

The present study used the EPM model to assess the anxiolytic effect of homeopathic medicine *Aconitum napellus* in rats. As expected, diazepam induced significant increase of the number of entries and permanence time in the open arms. These findings agree with the results of classic studies where diazepam and other benzodiazepines exhibited anxiolytic effects in a variety of procedures of anxiolytic screening [19] including conflict models [20], EPM procedures [21], and other non-punitive models [22]. On the grounds of their pharmacological effect, benzodiazepines monopolize the market of anxiolytic drugs for about 40 years, however, their potential to induce tolerance and dependence has awoken an interest in other options [23].

Anxiety and depression are among the most frequent motives for patients to seek CAM [13]. In this regard, *Aconitum napellus* is one of the classic resources used in CAM to treat patients presenting with neurological symptoms of anxiety [24]. Indeed, this effect was observed in the present study, since animals treated with *Acon* and subjected to EPM were more active and exhibited less anxiety compared to the animals in the control group. However, this effect did not exhibit linear progression as a function of the dilution, the most evident therapeutic effects were induced by dilution 12cH (Figure 1), since variable entry in open arms is the most representative of anxiolytic activity [25]. This group also exhibited the best anxiolytic effect, since also the permanence time in EPM open arms increased (Figure 2). Homeopathic experience since Hahnemann's times indicated that definite dilutions have distinctive effects [26], whence the importance homeopathy attributes to experiment.

Although the investigated homeopathic medicine exhibited positive effects for the treatment of anxiety, diazepam exhibited greater statistical difference compared to the control (Figure 1 and 2), perhaps suggesting that *Acon* might be effective in cases of mild anxiety. Comparison between *Acon* 12cH and diazepam showed no significant difference in the number of entries in the open arms, but diazepam exhibited significant difference in the permanence time (Table 1), because the standard error of means was very high in the group treated with *Acon* 12cH. This discrepancy might be related with the interaction between genetic and environmental factors that might occur despite standardization and that thus lead to experimental differences [27]. Also the direct effects on the motor activity induced by diazepam should be taken into account, because they make animals remain quiet longer; however, the anxiolytic effect prevailed above all others.

Homeopathic dilutions are usually prepared using alcohol [28], which exhibits depressant effects on the CNS [29], for this reason also a control group treated with 30% cereal alcohol was included in the present study. This group showed no modification of the investigated parameters of anxiolytic (Figures 1 and 2) and locomotor activity (Figure 4) compared to the control treated with saline solution. These results suggest that in the homeopathic preparations, *Acon* rather than alcohol is accountable for the improved quality of life of patients with anxiety.

The alterations observed in the anxiety-related behavior together with the lack of effects on locomotor activity and entry in the closed arms suggest that the anxiolytic effect observed was specific and did not depend on a sedative action that might favor longer permanence time in the EPM open arms [30].

Although homeopathy is indicated for the treatment of neuropsychiatric diseases, the number of corresponding studies in the literature is small. The few studies published in peer-reviewed indexed journals were the target of much criticism stressing the possibility of placebo effect.

On the other hand, in studies performed with species where homeopathy proved to be successful such as Martins *et al.* [31] (veterinary medicine) placebo effect was ruled out, since the experimental subjects lack the neurophysiological processes required for autosuggestion [32]. Behavioral studies with animal models showed that homeopathy has anxiolytic effects [11-13], however, a systematic review of studies performed in humans showed that the effects of homeopathy in the treatment of anxiety are limited [33], or absent [34].

Conclusion

The main effects of *Aconitum napellus* may be detected by means of the same specific pharmacological models used for the reference drugs, since in dilution 12cH it exhibited anxiolytic properties without affecting motor coordination. The mechanism of this action requires thorough investigation by means of specific models comparing the results obtained with specific medications and their interactions with other drugs.

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Avaliação do medicamento homeopático *Aconitum napellus* no tratamento da ansiedade em modelo animal

RESUMO

Introdução: O *Aconitum napellus* é uma das soluções clássicas utilizadas na medicina complementar para tratar pacientes que apresentam sintomas neurológicos de ansiedade. **Objetivo:** Avaliar a ação do medicamento homeopático *Aconitum napellus* no tratamento da ansiedade generalizada em um modelo experimental com ratos. **Material e Métodos:** 48 ratos (*Rattus rattus*) machos, adultos de 2 a 3 meses da linhagem Wistar foram divididos randomicamente em 6 grupos (n=8/tratamento) e receberam o tratamento por gavagem no período de 10 dias: 1) grupo controle (Diazepam 1mg/kg/dia); 2) controle negativo (0.15 mL solução salina/dia); 3) ACH6 (0.15 mL *Aconitum napellus* 6cH/dia); 4) ACH12 (0.15 mL *Aconitum napellus* 12cH/dia); 5) ACH30 (0.15 mL *Aconitum napellus* 30cH/dia); e 6) ALC30 (0.15 mL álcool de cereais 30%/dia). Os efeitos comportamentais foram avaliados no teste do Labirinto em Cruz Elevado (LCE) e Campo Aberto, ambos de forma cega e randomizada. **Resultados:** As potências 12cH e 30cH apresentaram um possível efeito ansiolítico sobre o sistema nervoso central (SNC), visto que foram capazes de aumentar a frequência de entrada nos braços abertos do LCE (12cH e 30cH) e o tempo nos braços abertos do LCE (apenas CH30). No teste do Campo Aberto observou-se que as potências homeopáticas não foram capazes de afetar o sistema locomotor. **Conclusão:** As potências 12cH e 30cH do *Aconitum napellus* demonstraram efeito ansiolítico sobre o SNC em modelos experimentais.

Palavras-chaves: Ansiedade, homeopatia, *Aconitum napellus*, modelos animais, ratos.

Evaluación del medicamento homeopático *aconitum napellus* en el tratamiento de la ansiedad en un modelo animal

RESUMEN

Introducción: *Aconitum napellus* es un recurso clásico de la medicina complementar para el tratamiento de pacientes con síntomas neurológicos de ansiedad. **Objetivo:** evaluar la acción del medicamento homeopático *Acon* en el tratamiento de ansiedad generalizada en un modelo experimental con ratones. **Métodos:** 48 ratas (*Rattus rattus*) adultas (2 a 3 meses de edad) raza Wistar fueron divididas aleatoriamente en 6 grupos (n=8/tratamiento), que recibieron los siguientes tratamiento por sonda gástrica: 1) control (diazepán 1 mg/kg/día); 2) control negativo (0,15 ml solución fisiológica por día); 3) ACH6 (*Acon* 6cH 0,15 ml/día); 4) ACH12 (*Acon* 12cH 0,15 ml/día); 5) ACH30 (*Acon* 30cH 0,15 ml/día); 6) ALC30 (0,15 ml alcohol de cereales por día). Los efectos en la conducta fueron evaluados en ciego y aleatoriamente en laberinto elevado en cruz (LEC) y prueba del campo abierto. **Resultados:** *Acon* en dilución 12cH y 30cH produjo efectos posiblemente ansiolíticos en el sistema nervioso central (SNC), porque aumentó el número de entradas en los brazos abiertos del LEC (12cH y 30cH) y el tiempo de permanencia en ellos (sólo 30cH). Este medicamento homeopático no produjo efectos en el sistema locomotor de los animales en la prueba de campo abierto. **Conclusión:** *Acon* en dilución 12cH y 30cH produjo efectos ansiolíticos en el SNC en un modelo animal experimental.

Palabras clave: Ansiedad, homeopatía, *Aconitum napellus*, modelo animal, ratas

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