Original Article

Anxiolytic Activity of Ultra-diluted *Aconitum napellus* on Wistar rats

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Abstract

Background Anxiety disorders are highly prevalent that affect millions of people worldwide. Homeopathic system is gaining popularity due to its efficacy in chronic diseases. Ultra-diluted *Aconitum napellus* is a homeopathic medicine used by clinicians for treatment of acute anxiety. **Aim** Objective of this study is to test the efficacy of *Aconite* on experimental anxiety models of Wistar rats. **Methodology** 18 Female Wistar rats were divided into Control, Anxiety, and Anxiety + *Aconite* (ANX+ACO) groups. Acute stress was induced by restraining the rats for six hours daily for five days in Anxiety and ANX + ACO groups. After five days of stress, a single dose of *Aconite* 200cH was given to ANX + ACO group through oral gavage, whereas control and anxiety group rats were maintained with a normal diet. Following this, behavioral assessment was done. Histological sections of the hippocampal area were taken and stained with Hematoxylin and Eosin and also Cresyl violet stain. **Results** It was observed that the *Aconite* treated group had more number of entries and also the percentage of time spent in the open arm was higher than the stressed anxiety group. Histological study of the brain tissue procured from all three groups was analyzed. **Conclusion** It was inferred that ultra-diluted homeopathic *Aconite* has decreased anxiety in Wistar rats which were subjected to acute restrain stress. However, the decrease was not statistically significant to deduce *Aconite* as an anxiolytic.

Keywords: Anxiolytic, Ultradiluted, Homeopathy, *Aconite*, Anxiety

Introduction

Anxiety disorders are exceedingly predominant globally and signify a leading purpose for consultation in health care [1, 2]. Large number of psychotropic drugs for disorders are prescribed in conventional treatment but due to their adverse effects its consumption is limited [3, 4]. The occurrence of anxiety disorder cases in alternative system of treatment is also ever growing [5]. Homeopathic management one of the alternative systems is shown to be increasingly effective in the treatment of anxiety disorders [6]. Homeopathic system of medicine was established by Samuel Hahnemann in the late 18th century, though comparable conceptions existed earlier [7,8]. There is a

noteworthy body of clinical research comprising randomized clinical trials and meta-analyses of such trials which propose that ultra-diluted medicines used in homeopathy have activities which are curative in nature [9,10]. A number of countries include homeopathy in their publicly funded healthcare systems and physiochemical research projects [11, 12].

To know the efficacy of different types of anxiolytics several animal models have been developed and described especially in rodents [13]. Pyramidal neurons of Cornu ammonis-3 (CA3) area of hippocampus is known to undergo atrophy in rodents in response to physical or psychological stress [14, 15]. Previous research shows different constituents of plant and animal origin which have been investigated as anxiolytics in such stress induced rodent prototypes [16]. However, the effects of homeopathic medicines on experimental animal models are lesser known and such studies are rarely conducted. *Aconite* the choice of an anxiolytic was suggested by homeopathic Materia Medica, reporting a number of neurological and anxiety-related symptoms for this medicine, and in part by pre-existing literature [17]. *Aconite* is a classic homeopathic medicine for the treatment of patients exhibiting neurological symptoms of anxiety. The main characteristic feature indicating *Aconite* is physical as well as mental tension. Manifestation of mental tension is in the form of anxiety and fear. Literature suggests that acute anxiety, which is the feature of this deadly poison, will be relieved when dosed ultra-diluted [18].

A double-blinded study on 50 children with post-operative agitation, where *Aconite* was administered, proved to be effective with 95% reduced anxiety [19]. *Aconite* ultra-diluted to 200cH, demonstrated to be useful for orthopaedic patients with post-traumatic anxiety [20]. Although *Aconite* is used to clinically treat patients with anxiety, its mechanism of action is unknown. Through this study we attempt to provide necessary evidence for homeopathic medicine *Aconite* by testing its efficacy in stress induced animal models through behavioral assessment and histological analysis of hippocampus.

Materials and Methods

Animal Model: Healthy female Wistar rats from inbred colony maintained at Central Animal Research Facility (Reg No 94/1999/CPCSEA) were used for the study. 18 adult rats with average weight of 250-300 g were maintained in well ventilated polypropylene cages containing paddy husk. Animals were given food and fresh water ad libitum. The protocol for animal use was approved from the Institutional Animal Ethics Committee. (IAEC/KMC/28/2018). 18 rats were divided into the following three groups Control, Stress induced anxiety (ANX) and Anxiety + *Aconite* (ANX+ACO).

Experimental Design

Induction of stress

The rats of anxiety and treatment group's six animals each were placed within the appropriate restrainer, and the entrance was closed by sliding the door. The dimensions of each compartment of this restrainer were 35 cm (L) X 35cm (B) X 30 cm (H). These rats remained within the restrainer for 6 h on each day (10.00 AM-4.00 PM) for 5 days. The restrainer with the rats was kept on a table in a room with sufficient ventilation. After 6 h of stress, rats were released from the restrainer and were kept in their respective home cages. The restrainer was washed and dried after use [21, 22, 23].

Administration of Homeopathic Drug

Aconite 200cH (1×10^{-200}) high dilution, commercially available from Willmar Schwabe pharmacy, recommended by Homeopathic Materia Medica, AYUSH [24] was administered orally. One 2 oz. vial was taken and filled with 1 oz. of distilled or purified water. One medicated globule of size No. 10 of *Aconite* 200cH was added to it. It was stirred thoroughly. 1ml of this solution was given orally to the animal through oral gavage [25].

Behavioral test: The elevated T maze (26)

This device is in the shape of a cross which is raised to a height of 40 cm from the floor. The device has two open arms without walls of the size 50 x 10 x 59 cm. Perpendicular to the open arms are two other arms closed by walls.

Procedure

The test takes a total of 5 minutes per animal. The rat was placed in the center of the maze, facing an open arm. Anxious rats are averse to heights and open spaces. A rat exploring the open arms was designated as "less anxious" and a rat that is confined to the closed arms of the device was described as "anxious." Measured variables were a) Entries of the head, nose, and forepaw in the open arm, b) Number of full body entries in the open arm, and c) Percentage of time spent in the open arm [26]. The assessment was done by an unprejudiced observer.

Collection of Brain

Post-behavioural study, the rats were transcardially perfused with heparinized saline through the left ventricle followed by 10% formalin at the rate of 1ml/min and the brains were fixed with 10% formalin. Subsequently, these were dehydrated using ascending grades of ethanol and embedded in paraffin wax and blocks were prepared and labelled.

Histological technique

5µm thickness sections were cut using rotatory microtome of the prepared tissue blocks. The sections were fixed on slides and stained using Haematoxylin and Eosin (H & E) according to the protocol given in Principles and Techniques in Histology Microscopy and Photomicrography by Singh et.al [27]. Further sections were also used to stain with cresyl violet. The slides were dipped in the 0.1% cresyl violet solution for 20-25 min at 60°C in accordance of protocol explained by Shetty et.al [28]. The nucleoproteins and nissel substance is stained using a basic dye, cresyl violet. Also the view detailing the cell bodies can be obtained with the help of this dye.

Histological assessment

Stained sections were studied under Olympus Research Microscope (model cx21i) and photographed at 10X and 100X magnification. Cornu Ammonis-1(CA1) and Cornu Ammonis-3 (CA3) region of hippocampal formation were considered in at least three random microscopic fields from each animal by an expert pathologist blinded to the treatment groups.

Statistical analysis

One-way ANOVA followed by Bonferroni's post hoc test using the statistical software, Graph Pad Prism was used for data analysis. Results were expressed as mean $\pm$ SD and p-value ≤ 0.05 was expressed as significant.

Results

Behavioral assessment

Twenty-four hours after administration of *Aconite* 200cH, treatment group animals showed increase in the open arm entry compared to stress group in the elevated plus maze test. The mean $\pm$ SD are as follows, Control- 2 ± 0.8 , Anx- 0.8 ± 0.6 , Anx + Aco- 1.6 ± 0.8 (**Figure 1**). The forepaw, nose, head entries in the open arm are increased compared to the animals in the stress group. Control- 9.83 ± 1.47 , Anx- 5.16 ± 3.31 , Anx + Aco- 10.16 ± 4.40 (**Figure 2**). Also the percentage of time spent in the open arm is comparatively increased in the treatment group; Control- 6.16 ± 5.84 , Anx- 1.45 ± 2.39 , Anx + Aco- 5.03 ± 2.5 (**Figure 3**). In all these parameters although there is an alteration between the

three groups, nevertheless there is no statistical significance ($p = \geq 0.05$) seen after applying one way anova followed by Bonferroni's post hoc test.

Figure 1- Effect of Aconite on Behavior of Wistar rats in Elevated plus maze. No. of open arm entries in Control, Stress induced (Anx) and Aconite treated group (Anx+Aco).

Figure 2 - Effect of Aconite on Behavior of Wistar rats in Elevated plus maze. No. of the fore paw, head, nose entries in the Control, Stress induced (Anx) and Aconite treated group (Anx+Aco).

Figure 3-Effect of Aconite on Behavior of Wistar rats in Elevated plus maze. Percentage of time spent in open arm in the Control, Stress induced (Anx) and Aconite treated group (Anx+Aco).

Qualitative analysis of neural cells in Hippocampus

CA1 region

Microscopic examination of CA1 sub region of hippocampus showed almost equal number of viable neurons in all three groups. In H & E stain no difference was found in treatment group compared to control and stress group (Figure 4). In cresyl violet stain the number of viable neurons was similar in all three groups (Figure 5). In all three groups, neurons showed clear cytoplasm and distinct nucleus.

Figure 4 – 10X Haematoxylin and Eosin stain of CA1 and CA3 sub region of the Hippocampus of control, stress induced (Anx) and treatment groups (Anx + Aco).

CA3 region

Detailed microscopic examination of the CA3 region too did not show any significant difference in the quality of neurons. In H & E stain all three groups demonstrated similar histological presentation of CA3 region (Figure 4). In cresyl violet stain, the cells appeared similar in all three groups taking uniform stain. With no notable difference between treatment and stress groups (Figure 5).

Figure 5- 10X Cresyl violet stain of CA1 and CA3 sub region of the Hippocampus of control, stress induced (Anx) and treatment groups (Anx + Aco).

Discussion

The objective of this study was to obtain behavioral profiles after homeopathic treatment and to evaluate the strengths of ultra-diluted *Aconite* in experimental anxiety induced rat models. We explored behavioral and histological parameters to come to a conclusion. In the elevated plus maze test the number of entries and time spent in the open arm is suggestively more in treatment group than stress group animals which hints the efficacy of ultra-diluted *Aconite* 200cH, but cannot be concluded as an anxiolytic as it is not statistically significant. Similar studies done by Haine et al. on Wistar rats, ultra-dilutions of homeopathic 12cH and 30cH of *Aconite* exhibited anxiolytic effects revealed by improved percentage of time and the number of entries in elevated plus maze open arms [29]. The difference between our experiment and the previous research is that they have used *Aconite* 12cH and 30cH whereas we have used further dilution i.e 200cH.

Bellavite et. al have validated that both low and high dilutions of extract of *Gelsemium* conventionally used for patients with anxiety disorders, decrease anxiety and panic in confirmed mice behavioral models [30, 31]. Similar anxiolytic activities have been demonstrated by another Homeopathic remedy Ignatia in ultra-diluted 3cH and 9cH potency on experimental rat models. These researchers conclude that ultra-diluted homeopathic medicines act more than placebo and efficacy is shown in anxiety induced rat models [32].

A previous study conducted by researchers showed an anxiolytic effect of homeopathic preparation of *Pulsatilla nigricans* 3X on animal model comparable to that found with a psychotropic standard

drug diazepam [33]. Similarly, *Argentum nitricum* 30cH a homeopathic drug was found to possess a potent anxiolytic activity in two species rats and mice in comparison with the standard drug escitalopram, and it is suggested that high dilutions of *Argentum nitricum* should be administered because of its potential to produce toxic effects [34].

There are confirmed reports in the literature of chronic stress causing apoptosis in hippocampal region as well as cerebral cortex [35, 36]. There are numerous variances seen in studies due to differences in the species of rodents, type and duration of stress, housing conditions, type of behavioral tests etc. Recognition of stress in laboratory animal models necessitates an understanding of these inconsistencies. [37, 38]

In our research according to the protocol we have observed changes in behavioral study. However, we haven't perceived any noteworthy changes in histology of brain, with no significant neuronal death in hippocampal region. The reason for this maybe that the stress induced on the rats were for short duration of 6h for 5days. It was not sufficient to cause any pathological changes in the brain as demonstrated by the normal architecture of the CA1 and CA3 areas of the hippocampus.

Conclusion

In the present study we evaluated the efficacy of ultra-diluted *Aconitum napellus* as an anxiolytic. We conclude that though Aconite shows some anxiolytic activity, it does not appear to be significant as demonstrated by the results of the behavioral tests in the stress induced rats. Further studies with diverse experimental stress models need to be done on large number of animals with immunohistochemistry test for fortification of its anxiolytic action.

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