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Efficacy of Aconite and Ignatia as an anxiolytic- In vivo study

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ABSTRACT

Anxiety disorders are the most common form of psychiatric disorders and start at an early age. The homeopathic system of complementary treatment is increasingly used by the general population. Ultra-diluted Aconite and Ignatia are homeopathic medicines used by clinicians for the treatment of anxiety. The objective of this study is to test the efficacy of Aconite 12CH and Ignatia 12CH on experimental anxiety models of Wistar rats subjected to chronic unpredictable stress. 30 Wistar rats were divided into 5 groups of Control, Stress, Vehicle, Aconite and Ignatia group with 6 animals in each group. All the groups were subjected to chronic unpredictable stress except the control group. The last two groups were fed Aconite and Ignatia through oral gavage daily for 5 weeks. Following this, a behavioral and biochemical assessment was done. It was observed that the Aconite and Ignatia treated animals showed better weight gain, but the behavioral and biochemical assessment did not show any significant change. Hence it was inferred that ultra-diluted Aconite and Ignatia though an anxiolytic used clinically, did not decrease anxiety in Wistar rats which were subjected to chronic unpredictable stress.

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INTRODUCTION

Anxiety disorders are highly prevalent and incapacitating psychiatric disorders, which frequently persist unrecognized and untreated. They are common in society and primary health care accompanied by increased use of clinical set up (Lenze, 2000). National studies done in the United States observe that the prevalence of anxiety disorders is 5.1 to 11.9 percent (Kessler *et al.*, 2009; Wittchen and Jacobi, 2005). Anxiety is also probably the most common psychiatric disorder among the elderly population (Munk-Jørgensen *et al.*, 2006). In a nationally demonstrative study, 66 percent of individuals with current anxiety had at least one concurrent disorder (Wittchen *et al.*, 1994).

However, left untreated, anxiety complaints often persevere and can add to the development of other psychiatric disorders (Lenze, 2000). Conventional treatment for mental disorders includes, first and foremost, psychotherapy followed by medications such as benzodiazepines and selective serotonin reuptake inhibitors (Locke *et al.*, 2015). Its adverse effects and also the risk of abuse limits the use of these drugs (Tiller, 2013). Anxiolytic drugs cause an extensive range of adverse effects. As the action of the drugs is primarily on the central nervous system, they have the potential to produce an inconvenient effect on cerebral functions leading to adversial effects on the physiology of the body (Edwards, 1981). So patients turn to alternative systems for treatment to avoid side effects. Complementary and alternative systems are often used to treat mental disorders (Relton *et al.*, 2017). Accordingly, discovering safe, reliable and cost-effective therapeutics to meet the global demand against anxiety disorders has increased. There have been monumental investments without any significant cures for most neuropsychiatric disorders (Onos *et al.*, 2016). Homeopathy is an alternative system of medicine that uses ultra diluted medicinal substances to cure clinical conditions (Fisher, 2012; Milgrom, 2006). A survey of 11 countries over a period of one year shows that there is a significant increase in the use of homeopathy by the general population (Relton *et al.*, 2017). Aconite is used frequently in anxiety disorders with significant clinical improvement in post-surgical anxiety (Alibeu and Jobert, 1990; Oberbaum *et al.*, 2003).

Similarly, Ignatia is a useful drug in clinical practice for treating anxiety (Kent, 2021). Although these medicines are used clinically to treat patients with anxiety, their mechanism of action is still unknown. Animal models have played an important role in drug discovery and preclinical development of a drug. It is also an important tool to identify the underlying cause of the disease and target of the evaluating drug prior to clinical trial. The aim of this study is to try and evaluate the efficacy of ultra-diluted Aconite and Ignatia in chronic unpredictable stress-induced animal models through behavioral and biochemical assessment.

MATERIALS AND METHODS

Animal Model

Healthy female Wistar rats from an inbred colony maintained at Central Animal Research Facility (Reg No 94/1999/CPCSEA) were used for the study. 30 adult rats with an average weight of 250-300 g were maintained with food and water in well-ventilated polypropylene cages containing paddy husk. The protocol for animal use was approved by the Institutional Animal Ethics Committee. (IAEC/KMC/45/2018). 30 rats were divided into the following six groups Group I-Control, Group II-CUS induced stress, Group III- Vehicle, Group IV-Aconite, Group V- Ignatia.

Experimental Design

Chronic unpredictable stress (CUS) model

In this experimental procedure, the animals are subjected to inconstant unpredictable stressor each day. The purpose of this is to prevent acclimatization and adaptation of the animals to the same stress. It is considered to maximize the stress given. One day prior, the animals were caged individually to create social isolation, and the CUS procedure was followed for 5 weeks as outlined in Table 1. Control rats were not given any stress and were not socially isolated. Each stressor was subjected at variable times of the day and in random order (Bondi *et al.*, 2008). Afterwards, the animals were placed in fresh cages with clean bedding and were left alone for the rest of the day. The stressors were applied each day in random order for five consecutive weeks, with the same stressor not administered for two consecutive days. Weight was measured every week.

Administration of drug

Aconite 12cH (1×10^{-24}) high dilution, commercially available from Willmar Schwabe pharmacy, recommended by Homeopathic Materia Medica (Close, 2000), was administered orally. One 2 oz. The vial was taken and filled with 1 oz. of distilled or purified water. One drop of Aconite 12cH was added to it. It was stirred thoroughly. 1ml of this solution was given orally to the animal of group IV through oral gavage daily for 5 weeks. Similarly, Ignatia 12cH (1×10^{-24}) high dilution obtained from the same pharmacy was administered through oral gavage to group V daily for 5 weeks.

Behavioral test

Two behavioral tests, the Light and dark box test and Elevated plus maze test according to the protocol established by researchers previously (Cryan and Sweeney, 2011) was done to assess the induction of anxiety in rats and also to evaluate the efficacy of the drugs in treatment groups.

The animals were euthanized after the behavioral test. Before sacrificing, the blood was collected through cardiac puncture. It was centrifuged for 20 mins at 2000 rpm. The serum collected was aliquoted and stored in -80degrees.

Plasma corticosterone estimation

Table 1: Chronic unpredictable stress chart followed to induce anxiety in wistar rats

	Group I (Control)	Group II (Stress)	Group III (Stress+ vehicle)	Group IV (Stress + Aconite)	Group V (Stress + Ignatia)
1^{st} day	No stress	Food deprivation 24h	Water deprivation 24h	Restrainer 6h	Tail pinch 10 min
2^{nd} day	No stress	Moist bedding-12h	Cage tilt 12h	Alternate dark/light-12h	Food deprivation 24h
3^{rd} day	No stress	Water deprivation 24h	Restrainer 6h	Tail pinch 10 min	Moist bedding-12h
4^{th} day	No stress	Cage tilt 12h	Alternate dark/light-12h	Food deprivation 24h	Water deprivation 24h
5^{th} day	No stress	Restrainer 6h	Tail pinch 10 min	Moist bedding-12h	Cage tilt 12h
6^{th} day	No stress	Alternate dark/light-12h	Food deprivation 24h	Water deprivation 24h	Restrainer 6h
7^{th} day	No stress	Tail pinch 10 min	Moist bedding-12h	Cage tilt 12h	Alternate dark/light-12h

Table 2: Weight in grams of different weeks over the course of 5 weeks

Weight	1^{st} week	2^{nd} week	4^{th} week	5^{th} week
Control	200.83	207.67	212.33	224.17*
Stress	196.00	187.67	184.17	173.00$^{*\#}$
Vehicle	202.83	181.67	178.17	180.50$^{@}$
Aconite	197.50	182.83	191.00	186.50$^{\#@}$
Ignatia	197.33	186.33	186.83	186.00$^{\#@}$

* Significant decrease in weight in stress compared to control (p =>.001).
$^{\#}$Significant decrease in weight in stress group compared to Aconite and Ignatia. (p =>.01)
$^{@}$ Significant decrease in weight in the vehicle compared to Aconite and Ignatia. (p =>.05)

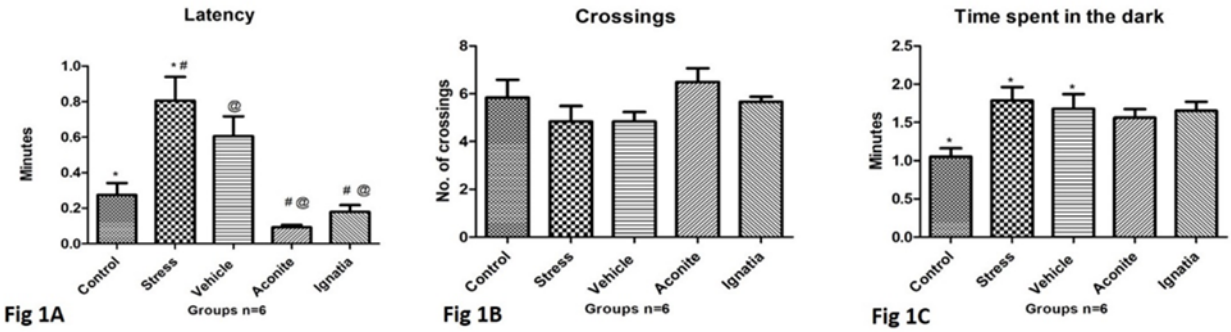

Figure 1: Behavioral characterization between groups in light and dark box test

Rat plasma corticosterone level was analyzed using ELISA kit as per manufacture's instruction given in the manual, purchased from Genetix biotech Asia Cat.No PG-8280Ra.

Statistical analysis

Data were reported as means +/- SEM. Analysis was done using one-way anova followed by Tukey's post hoc test with the Prism Graph pad version 6.0 software. A p-value less than 0.05 was required for results to be considered significant.

RESULTS AND DISCUSSION

The body weight gradually decreased significantly over the course of weeks in all the stress groups compared to the control. There was a drastic reduction in the first 2 weeks and then a gradual decrease

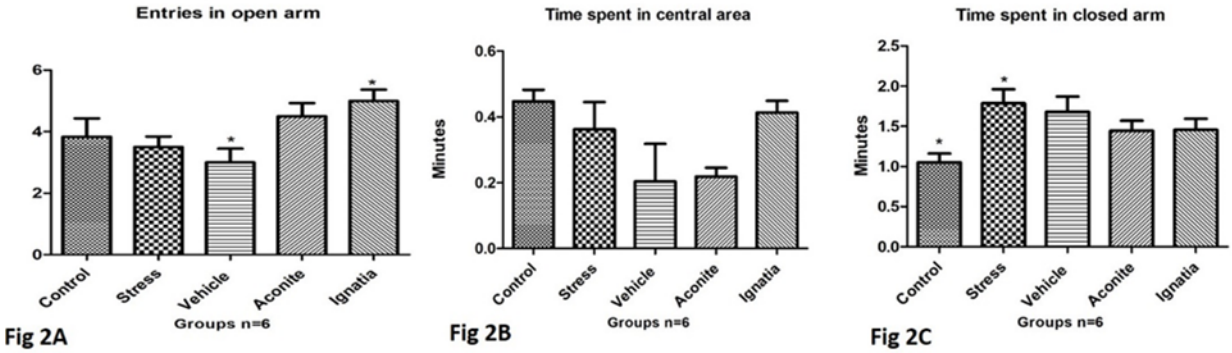

Figure 2: Behavioral characterization between groups in an elevated maze test

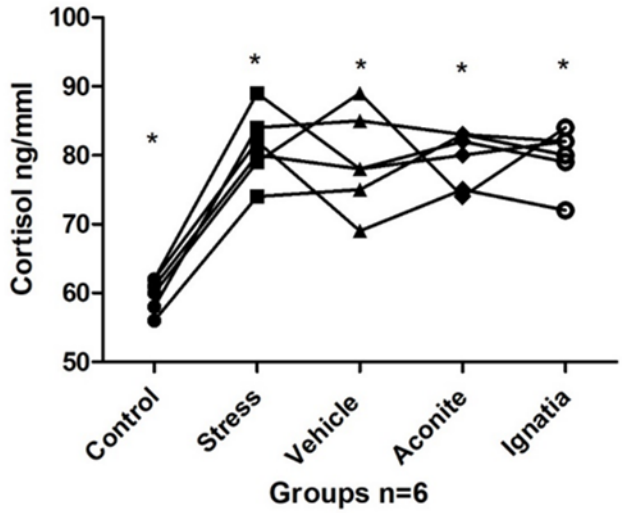

Figure 3: Serum Cortisol level in rats. * Significantdifference between Control (mean 59.83 ± 2.40) Stress (mean 81.33 ±5.04) vehicle (mean 79.00 ± 7.12), Aconite (mean 79.50 ±4.03), Ignatia (mean79.83 ± 4.21)

which then plateaued off, as shown in Table 2.

In the behavioral Light and dark test, the recordings of animals were tabulated, and the difference in means was determined by one-way anova. Tukey's post hoc test revealed that latency was reduced in control (mean 0.27 ± 0.16) compared to stress group (mean 0.80 ± 0.33; p = >.01).

Also, the initial latency to enter the darkroom in both the treatment groups (aconite -mean 0.09 ± 0.03, and Ignatia - mean 0.17 ± 0.09) was less compared to the vehicle group (mean 0.60 ± 0.27; p = >.001) and more like the control group. This indicates that the animals in the treatment group were less stressed compared to the vehicle and the stress group. However there was no difference in the numbers of crossings between the light and dark compartments in different groups control (mean 5.83 ± 1.85); stress (mean 4.83 ± 1.60); vehicle (mean 4.83 ± 0.98); Aconite (mean 6.50 ± 1.37); Ignatia (mean 5.66 ± 0.5; p = > 0.18). Showing that the animals in all groups were active and the motor activity among the animals was not hindered. But the time spent in the dark was significantly less in control (mean 1.05 ± 0.27) than in stress (mean 1.70 ± 0.42) and vehicle groups (mean 1.68 ± 0.46; p = > 0.05) (Figure 1). This infers that the animals in the stress vehicle groups were equally anxious. The treatment group animals showed comparatively less anxiety in terms of latency and time spent in the dark but were not statistically significant. This also indicates that anxiety was established in the stress model as the rats were more anxious in all the groups that underwent CUS.

In the elevated plus-maze test, findings were tabulated and evaluated statistically. It was observed that there was variation among the rats of different groups, as revealed by Tukey's post hoc test. The entries into the open arm in the treatment group was more, especially in Ignatia (mean 5.0 ± 0.89) compared to vehicle (mean 3.3 ± 1.1; p = > 0.05). Nevertheless, there was no difference between vehicle, stress and control group. There was no difference between groups at time spent

in the central area, control (0.45 ± 0.08); stress (mean 0.36 ± 0.20); vehicle (mean 0.20 ± 0.28); Aconite (mean 0.22 ± 0.06); Ignatia (mean 0.41 ± 0.08; p = 0.054). However, anxiety-like behavior is seen in animals of the stress (mean 1.80 ± 0.43) and vehicle group (1.10 ± 0.27) indicated by the significantly increased amount of time spent in the dark arm demonstrating reduced exploration of the open arm (Figure 2). This was not due to reduced locomotor activity or exploratory drive as there was no statistical difference in the total number of entries between treatment, stress and control groups. It was observed through behavioral tests that although the Aconite and Ignatia treated group exhibited some reduced anxiety-like performance, but it was not statistically significant to term them as anxiolytics.

The serum cortisol usually increases during stress due to the Hypothalamo pituitary adrenal (HPA) axis. In fact, psychological stress causes the secretion of hormone from the parvocellular neurons, i.e. corticotropin-releasing hormone (CRH) by the paraventricular nucleus of the hypothalamus. This activates the production of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which consequentially stimulates the cortex of the adrenal gland to secrete cortisol. This influences several physiological processes which affect behavior. (Faravelli, 2012). In this study, the serum cortisol is significantly elevated in all the groups compared to the control. (Figure 3). There was no significant difference between treatment groups, vehicle and stress group, inferring that ultra diluted drugs Aconite and Ignatia did not reduce the cortisol in CUS rodents after 5 weeks of administration.

In a study done previously, it was found that Aconite 12 CH dilution reduced the anxiolytic behavior of rats significantly, as revealed by the results of EPM and open field test. (Haine *et al.*, 2012) Similarly, researchers showed anxiolytic behavior in rats after treating it with ultra diluted Ignatia. (Marzotto *et al.*, 2012; Anser *et al.*, 2020). However, our study showed no such difference in the treatment groups. The reason for this may be that in the present study, the rats were subjected to CUS and an anxiety model was created to study the efficacy of the anxiolytic Aconite and Ignatia.

CONCLUSION

These medicines ultra-diluted Aconite and Ignatia, though clinically very useful in reducing symptoms of anxiety in patients and is a medicine of choice in the medical set up for symptoms of anxiety, were not able to produce the same anxiolytic action on rodents subjected to chronic unpredictable stress.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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