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Evaluation of anxiolytic and antidepressant effect of Saraswata Churna in the pilocarpine induced rat model of epilepsy

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***Abstract*---**The aim of our present study was to assess Saraswata Churna's anxiolytic and anti-depressant efficacy in a Pilocarpine-induced epilepsy rat model. Depression and anxiety are major psychological symptoms in epilepsy patients, and they have a significant impact on health-related quality of life. Many cases of neurological problems have been treated using Saraswata Churna, an ayurvedic medicine. The elevated plus maze (EVPM) was used to assess anxiolytic action, while the forced swimming test (FST) was used to assess antidepressant activity. The EVPM test has been shown to be effective in determining the anxiolytic effects of pharmaceuticals and various steroid as well as non-steroid hormones, as well as identifying the brain locations and mechanisms that underpin anxiety-related behaviour in rodents. Saraswata churna was reported to be effective in reducing anxiety and depression in rats at a dosage of 500 mg/kg.b.w by increasing open arm time and decreasing closed arm entrance in the EVPM paradigm and increasing the duration of immobility in the FST model. As a result, Saraswata Churna could be used as an effective therapeutic agent for anxiety and depression.

***Keywords*---**anti-depressant, anxiolytic, elevated plus maze, forced swimming test, Saraswata Churna, phenytoin, pilocarpine.

Introduction

Mental illness has existed since the dawn of time. Anxiety and depression have been linked in studies, as has the presence of subthreshold psychological distress[1]. Stress may predispose to depression, or anxiety and depressive disorders may be surface manifestations of the same underlying cause. As a result, dealing with depression and anxiety difficulties is challenging enough without having to worry about side effects or cost. Although various medicines are available, many of them have drawbacks, and there are relatively few drugs that can treat both anxiety and depression.

Saraswata churna is an Ayurvedic compound formulation referenced in the Bhaishajya Ratnavali book for treating various psychiatric illnesses[2]. Plants with established psychotrophic action, such as Acorus calamus Linn, are included in this composition[3, 4]., Saussurea lappa (Falc.) Lipsch.,[5] Withania somnifera (L.) Dunal.,[6] Carum carvi Linn.,[7] Convolvulus pluricaulis Forsk.,[2, 8-10] Bacopa monnieri(Linn.) Pemmell.[11-14] Zingiber officinale Rosc.[14] etc., are the other components of Saraswata Churna used in various animal models. Despite the fact that some of the constituents in this formulation have a lot of research behind them, there is no information on the pharmacological evaluation of anxiolytic and anti-depressant characteristics. The goal of this study was to see the effect of Saraswata churna in affecting the depression and anxiety in experimental animal models.

Objective

The objective of this study is to assess the effect of Saraswata Churna (SC) in anxiety and depression through EVPM and FST in the lithium-pilocarpine induced epilepsy of rat model.

Materials and Methods

Methods

Ethical Statement

Ethical approval has been obtained from the Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Manipal Academy of Higher Education,(IAEC/KMC/25/2019), India for conducting this study.

Animals

Four-month-old male Albino rats of the Wistar strain (n=24) weighing 180g - 250g were used in this investigation. All of the animals were acquired from the Manipal Academy of Higher Education's Central Animal House Facilities in Karnataka, India. All of the animals were housed in in the animal cage in a controlled environment (22°C and 1212 light-dark cycle, lights on at 7 a.m.). The animals were provided with pellet meal and water. All protocols for animal research were compliant with the CPCSEA.

Experimental Design

All the animals (n=24) were divided into four experimental groups (n = 6 per group), Normal Control group (NC), Pilocarpine group (PI), Phenytoin group (PHE), Saraswata Churna group (SC), and the control group (n=6) was maintained in the home cage under normal conditions. Four month old adult male Wistar rats (n=24) were randomly divided into four groups (n= 6/group) as Normal Control (NC), Pilocarpine Group (PI), Phenytoin Group (PH) and Saraswata Churna (SC). Epilepsy model was created by a single intraperitoneal injection (270mg/kgbw) of pilocarpine 18-24 hours after Lithium chloride (127 mg/kg.b.w.) injection. At the end of 24 hours and 48 hours post first seizure occurrence, Phenytoin (30mg/kgbw.i.p.) and SC (308 mg/kgbw oral) were given to the respective groups. After 14 days of inducing seizure Elevated Plus Maze Test and the Forced Swim Test is done for all the animals of all the four groups.

Establishment of Rat Model of Status Epilepticus

Lithium-pilocarpine (LIP) Treatment

Epilepsy model was created by a single intraperitoneal injection (270mg/kg.b.w) of pilocarpine, 18–24 hours after obtaining a lithium chloride injection (127mg/kg.b.w, i.p.) to all the three groups (PI group, PHE group, SC group). Status Epilepticus (SE) was reached in 20-30mins. SE was characterized as a condition associated with long-term seizures lasting more than 30 minutes. As per the Racine scale, seizure behaviors were classified into five stages as follows: Stage I, oral and face movements; Stage II, nodding of head; Stage III, clonus of the forelimbs; Stage IV, ascending (rearing); and Stage V, rearing and falling. If convulsive behaviors persisted, diazepam (10 mg/kg.b.w., i.p.) was injected one hour after status epilepticus. The animals were given 2.5 ml (i.p.) of 5% dextrose multiple times a day. At the end of 24 hours and 48 hours post first seizure occurrence, Phenytoin (30mg/kg.b.w.i.p.) and SC (308 mg/kg.b.w oral) were given to the respective groups.

Methodology

Assessment of Anxiety using EVPM Test

This is a widely used rodent behavioural assay that has been evaluated to assess the anxiolytic effects of therapeutic approaches, as well as to identify the brain locations and mechanisms that underpin stress behaviour. The anxious behaviour of mice was examined using the ratio of time spent on open arms to time spent on closed arms through the EVPM device after a 14-day experimental period (from the day of first status epilepticus). This apparatus is shaped like a cross and stands at a height of 40 cm off the ground. The apparatus has two open arms that are 50 x 10 x 59 cm in size. Two further arms, perpendicular to the open arms, are closed by walls. The test takes 5 minutes per animal in total. The rat was positioned in the maze's centre, facing an open arm. Height and open areas frighten anxious rats. A rat exploring the device's open arms was labelled "less worried," while a rat confined to the device's closed arms was labelled "anxious." The variables that had been measured were a) the amount of time the

rats spent in the open arm and b) the number of times the rats enters into the closed arm. The data was reported as mean ± standard deviation and statistical data analysis was performed by using SPSS 16.0 software.

Evaluation of Depression using Forced Swim Test (FST)

This test is often used to diagnose depression in rodents[15] (Petit-Demouliere et al 2005). This experiment was done using a glass cylinder tank. The water will be filled at a constant temperature of 23-25°C. The water depth is kept constant so that the animal does not touch the cylinder's bottom or jump out. A five-minute trial session will be conducted, with the entire experiment being videotaped for later analysis. Because the majority of the rats will be active at the start of the experiment, the effects of the drug will be difficult to detect within the first two minutes. Therefore, the first two minutes will not be analysed. The remaining three minutes will be analysed to see how much time was spent on mobility (swimming, climbing, and struggling) and how much time was spent immobility (floating with no body movement). At the end of the 5th minute, the mice will be taken out of the water and carefully wiped with tissue paper before being returned to their home cages.

Statistical analysis

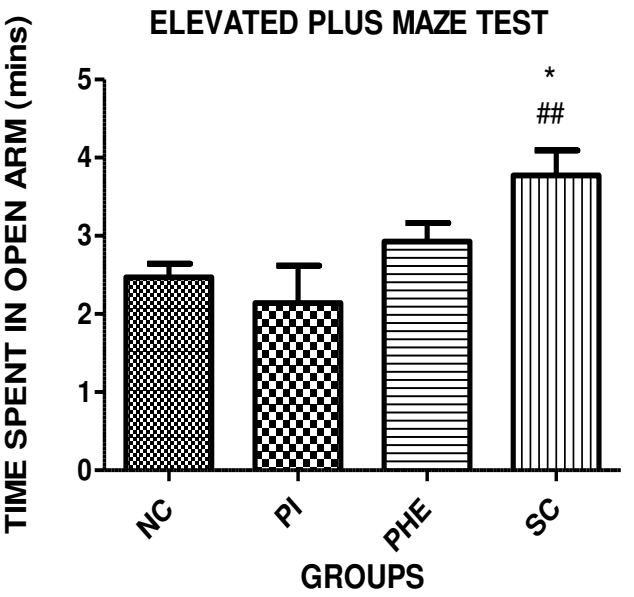
Version 16.0 of IBM's Statistical Packages for the Social Sciences (SPSS) was used to analyse the data for the histopathological comparison between groups after Cresyl violet staining. The structural alterations in the CA1 and CA3 regions neurons were compared across groups using the One-way ANOVA to compare the mean across groups for neuronal degeneration and healthy neurons, with the data displayed as Mean ± SEM. Tukey's for the post-hoc analysis was done to report significance between the groups, if any found.

Result

Elevated Plus Maze Test (EVPM)

Time Spent by the Rodents in the Open Arm

The time spent in the open arm of the elevated plus maze apparatus by the animals are: Normal Control (NC) group (49.4%), Pilocarpine Group (PI) group (42.8%), Phenytoin (PHE) treated group (58.6%), and the drug Saraswata Churna (SC) treated group (75.4%). This result shows that SC treated group showed significant difference ($p \leq 0.01$) in spending more time in open arm in comparison to the PI and ($p \leq 0.05$) in comparison to the PHE group which shows the anxiolytic effect of SC is significant in reducing the anxiety Graph 1.

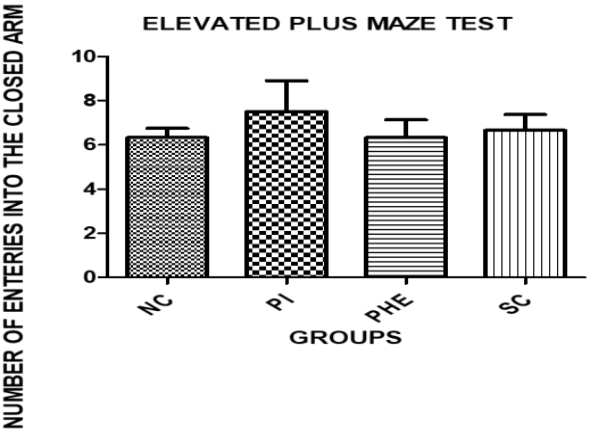


Graph: 1. The above graph shows the time spent by the animals of all the four groups in the open arm: Normal Control (NC), Pilocarpine group (PI), Phenytoin treated group (PHE), and the drug Saraswata Churna (SC) treated groups.

NC- Normal Control Group, PI- Pilocarpine Group, PHE- Phenytoin Group, SC- Saraswata Churna Group, *- In Comparison with NC Group, #- In Comparison with PI Group, @- In Comparison with Phenytoin Group

Number of Times the Rats Entered Into the Closed Arm

Number of entries attempted by the rats in all the four groups are: Normal Control group (38 times), Pilocarpine Group (45 times), Phenytoin treated group (39 times), and the drug Saraswata Churna treated group is (40 times). This result suggests that the animals in the PHE treated group and SC treated groups' showed less number of entries into the closed arm and is significant when compared to PI group Graph 2.



Graph: 2. The above graph shows the number of times entered by animals of all four groups into the closed arm: Normal Control (NC), Pilocarpine group (PI), Phenytoin treated group (PHE), and the drug Saraswata Churna (SC) treated groups.

NC- Normal Control Group, PI- Pilocarpine Group, PHE- Phenytoin Group, SC- Saraswata Churna Group

Forced Swim Test (FST)

Analysis

The amount of time each rat stays mobile is measured throughout the behavioural analysis. The mobility time is computed by subtracting the entire amount of movement time from the 180 seconds of testing time. While it is feasible to directly quantify immobility time, we have discovered that detecting and measuring active movements rather than the absence of such movements is easier in our laboratory.

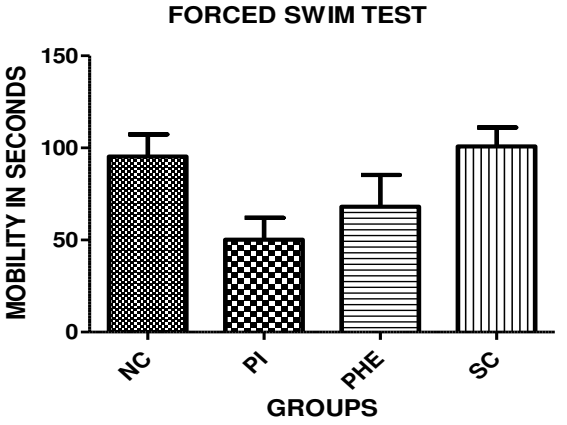
(1) Definition of mobility:
Any movements other than those required to balance the body and keep the head above the water are considered mobility in the FST[16]. Rats float easily in water, but they nevertheless make modest movements to keep their bodies balanced and their heads above the water. These actions aren't an attempt to flee, and therefore shouldn't be included as mobility. Furthermore, even though rats are virtually immobile after a single bout of mobility, they can still drift in the water due to momentum. These actions should also not be counted as mobility.

(2) Definition of immobility:
When a rat was floating and/or making only the movements required to maintain body balance or keep its head above water, it was deemed immobile.

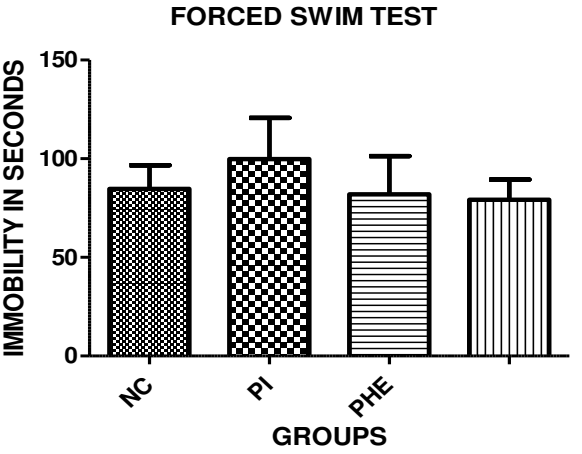
(3) Scoring of the Forced Swim Test:
The first two minutes of the normal FST (5 minutes) were used to allow the animals to investigate and adapt to the setting. Mobility was scored for the

entire 5 minutes, however only the last 3 minutes' data were used to calculate mobility.

The mobility duration in the control group is (52.9%), treatment with Saraswata churna (SC) increased the mobility of the rats in the SC groups (56.0%) when compared to the Phenytoin treated group (37.8%) and Pilocarpine group (27.9%) Graph.3 and decreased the immobility time (44.0%) of rats when compared to the control group (47.1%), Pilocarpine group (55.4%), Phenytoin group (45.5%). These results show that Sraswata Churna non significantly increases the mobility duration and non-significantly reduces the immobility duration Graph.4.



Graph: 3. The above graph shows the mobility shown by animals in all the four groups in the Forced Swim Test: Normal Control (NC), Pilocarpine group (PI), Phenytoin treated group (PHE), and the drug Saraswata Churna (SC) treated groups



Graph: 4. The above graph shows the immobility shown by animals in all the four groups in the Forced Swim Test: Normal Control (NC), Pilocarpine group (PI), Phenytoin treated group (PHE), and the drug Saraswata Churna (SC) treated groups

NC- Normal Control Group, PI- Pilocarpine Group, PHE- Phenytoin Group, SC- Saraswata Churna Group

Discussion

The elevated plus maze is a paradigm that takes advantage of rodents' inherent fear of open and high spaces[17]. The GABA-benzodiazepine combination in the traditional EVPM is particularly vulnerable to the effects including both anxiolytic and antidepressant medications[18]. Naive rats will often spend the majority of their time in the closed arms in this circumstance. This preference stems from a fear of large expanses, which manifests itself as an aversion to open arms. Medicines that boost open arm exploration are known as anxiolytics, while drugs that inhibit open arm exploration are known as anxiogenics[2]. Pre-treatment with Saraswata churna significantly increased the amount of time the rodents spent in the open arm while having no influence on the rate of entries into the closed arm.

The FST (also known as the Porsolt swim test) was developed for rats in basic and preclinical research to investigate the potency of antipsychotic medications and the influence of various behavioural and neurobiological alterations [15, 19-21]. It's been described as creating a circumstance in which the animal experiences "behavioural despair," in which the animal loses hope of escaping the stressful setting[19]. The anti-anxiogenic impact on a forced swim test paradigm provides a quick and accurate anti-depressant behavioural screening test. This methodology is applicable to a wide range of anti-depressants, primarily tricyclics and monoamine oxidase (MAO) inhibitors, which reduce the duration of immobility in FST considerably[22]. The development of passive behaviour that disengages the animal from active modes of dealing with stressful stimuli is assumed to be the cause of immobility[23]. Several antipsychotic drugs reduce the impairment of mobility after forced swimming[24]. Saraswata churna considerably reduced the duration of immobility of rats in the FST research. The observed impact could be due to 5-hydroxytryptamine receptor blockade, serotonin receptor reuptake inhibition, or MAO inhibition. Because the FST's behavioural outcome is one-dimensional, it can only reflect the antidepressant efficacy of chemical or experimental manipulations, not distinguish between mechanistic differences[25]. Based on the aforementioned, Saraswata churna possesses anxiolytic and anti-depressant effect that is commensurate to industry standards. The reported activity profile could be associated to one or more phytochemical constituents identified in the various plants because this is a hetero medicinal herbs formulation. More study is needed, however, to pinpoint the exact process.

Conclusion

Supplementation of SC has the potential to mitigate the functional/behavioural alteration in the epilepsy caused by pilocarpine. The current study demonstrates that SC has the efficiency to improve anxiolytic effect of rodents in the lithium-pilocarpine induced rat model of epilepsy.

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Availability of data and materials

All data and materials are presented in this manuscript. No additional materials are available.

Acknowledgment

Not Applicable

Competing interests

Authors declare no competing interest

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