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Evaluation of psychopharmacological activity of ethyl acetate extract of *Sarcostemma acidum* (Roxb).Voigt

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Abstract

Herbal medicines are gaining growing interest because of their cost- effective, eco- friendly attributes and true relief from disease condition. *Sarcostemma acidum* was documented in many folklore practices for various psychiatric conditions. It has been dealt with in detail in "SHRUSHRUTHA SAMHITHA". Ethyl Acetate Extract of the whole plant *Sarcostemma acidum* (EASA) was evaluated for psychopharmacological effects, Anti Psychotic, Anxiolytic and CNS inhibitory activity. Anti psychotic effects of EASA was assessed by Condition Avoidance Response and cataleptic Scoring test using pole climbing and Bar test respectively. Elevated Plus maze (EPM) and Hole Board Apparatus (HBA) was employed for the anxiolytic activity while Actophotometer was used to assess the CNS inhibitory activity. EASA (650mg/kg), Haloperidol (5mg/kg) and 1% CMC was administered to the test, standard and control group respectively for Antipsychotic activity, while For Anxiolytic and CNS depressant studies test, standard and control group receive EASA (650mg/kg), Diazepam (2mg/kg) and 1% CMC respectively. It was found that EASA significantly enhance the latency period to climb the pole and the cataleptic score which indicates its suppression on CAR activity, which clearly confirms its Anti Psychotic activity, might be due to blockade of dopaminergic pathway. It was observed that EASA at a dose of 650mg/kg significantly increases the no: of entries in to the open arm in EPM as well as no: of head poking in HBA, which reflects its increase in exploratory behaviour which indicates the anxiolytic activity. Reduction in the loco motor activity in actophotometer indicates CNS depressant property of the drug.

Keywords: *Sarcostemma acidum*, Psychosis, Psychopharmacology.

Introduction

Human brain is a wonder in itself and is still being unexplored fully. It is a complex assembly of interacting neurons and nuclei that regulate their own and each other's activities in a dynamic fashion, generally through chemical neurotransmission. Understanding the relationship between the brain and the mind is a great challenge. Due to this complex nature medical science is in an unfortunate situation of attempting to prevent/ cure any pathological processes that affects brain.

Psychopharmacology is the scientific study of the effects drugs have on mood, sensation, thinking and behaviour. It is distinguished from neuropsychopharmacology, which emphasizes drug-induced changes in the functioning of cells in the nervous system.[1]

Mental health can be seen as an unstable continuum, where an individual's mental health may have many different possible values. Psychiatry refers to a field of medicine

focused specifically on the mind, aiming to study, prevent, and treat mental disorders in humans.[2]

Public concern on mental health has noticeably increased given the high prevalence of neuropsychiatric disorders. According to the new concept of measuring disability "DALY" mental disorders constitute a significant part (8.1%) more than the disability caused by several well-recognized disorders such as cancer (5.8%) and heart diseases (4. 4%). WHO reports approximately 450 million of people suffer by mental or behavioral disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020.[3]Two-thirds of the anxious, depressed or psychotic patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Most of the drugs for these conditions used nowadays have adverse side effects so the need for newer, better-tolerated and more efficacious treatments is remaining high.

Herbal medicines are gaining growing interest because of their cost- effective, eco- friendly attributes and true relief from disease condition. Since ancient time the herbal medicines are effective in the treatment of various ailments. Many plants have folklore claim in the treatment of several dreadful diseases but they are not scientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern medicine. The plant used in present study was *Sarcostemma acidum* (Somalatha) for its psychopharmacological effect and CNS studies of the plant are sparse. In Ayurveda it is useful in vitiated conditions of Pitta, dipsia, viral infection, hydrophobia, psychopathy and general debility.[4] Sōmā is believed to possess powers to overcome old age and death. It has been dealt with in detail in'स्वभावव्याधिप्रतिषेधनीयंरसायनम्' in सुश्रुतम् (svabhavavyadipradisedhaniyamRasayanam) in SHRUSHRUTHA SAMHITHA. Sarcostemaacidum(somalatha) is one of the main ingredient of the famous product MANASAMITRAVADAKAM of KottakkalAryaVaidyaShala used in various mental disorders.

The present study was carried out from January 2013 to August at Postgraduate & Research Laboratory, Department of Pharmacology, University College of Pharmacy, Cheruvandoor, Mahatma Gandhi University, Ettumannoor, Kottayam, Kerala, India.

Materials and methods

Plant material

The plant materials of *Sarcostemma acidum* (whole plant) was collected from Maruthumalai, Coimbatoredist, Tamilnadu and authenticated by Dr Jomy Augustine, H.O.D of Botony, ST. Thomas College, Palai (Ref. no. 2256). The plant was collected in the months of January to March and shade dried at room temperature and subjected to extraction procedures.

Drugs& Chemicals

Haloperidol, Diazepam, Imipramine, Carboxy methyl cellulose, DPPH (Diphenylpicrylhydrazyl) solution, Gallic acid (standard), Methanol, Distilled water, Hydrogen peroxide, Griess reagent, ascorbic acid, DMSO, sodium nitroprusside.

Animals

Wistar albino rats weighing 180-220g of either sex maintained under standard husbandry conditions (temp 23± 2 ^{o}C, relative humidity 55 ± 10% and 12 hr light dark cycle) were used for the screening which was obtained from the animal house of the University College of Pharmacy, Cheruvandoor. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after getting the approval for experimental protocol from the institutional animal ethics committee, India 2013 under the IAEC no: 010 /MPH/UCP/CVR/13.

Experimental Details

Preparation of Ethyl acetate extract

Plant materials were dried in shade for 2 weeks and coarsely powdered. About 450 mg of plant powder in a porous bag made of muslin cloth was placed in a round bottom flask under reflux at a temperature of 80ºC. The solvent used for extraction was ethyl acetate and it was extracted for 24 hrs. After extraction, ethyl acetate was distilled out and extract was concentrated and dried at 40-50°C to obtain a green semisolid sticky mass.

In vivo Assays

CNS Inhibitory Activity-

Actophotometer

The animals were numbered and weighed. The actophotometer was switched on and the animals were placed individually in the activity cage for 10 min. The basal locomotor activity score of all the animals were noted. Standard, Test and Vehicle were injected on each animal of proposed groups and after 30 min each animal was retested for 10 min. The differences in activity before and after treatments were noted and the percentage decrease in locomotor activity was calculated Diazepam (2mg/kg, p.o), EASA (650mg/kg, p.o), CMC (1%, p.o) served as standard, test and control respectively.[5]

Anti Psychotic Activity-

Pole Climbing Test

A chamber which consist a 2.8 kHz speaker situated on the top, stainless steel pole 2.5cm in diameter is suspended, and condition stimulus (buzzer sound) is given for 10seconds, followed by an unconditional stimulus-a scrambled shock delivered to the grid floor. Animals were trained to avoid the unconditional response (shock) following the conditional stimulus.

The rats were weighed and grouped as standard, test, control and were treated with Haloperidol (5mg/kg, p.o), EASA (650mg/kg, p.o) and CMC (1%, p.o). The conditioned stimulus was given for 10 seconds and unconditioned stimulus, foot shock delivered through the grid of floor applied for 10 seconds. Animals kept in the chamber jumped on the pole on hearing the buzzer tone to avoid electric shock. Failure to do so result in foot shock applied for 10 seconds.[5] trials were conducted. The latency period to climb the pole was noted for standard, EASA and control treated group.[6]

Catalepsy Scoring

The rats were weighed and grouped as standard, test, control and were treated with Haloperidol (5mg/kg, p.o), EASA (650 mg/kg, p.o) and CMC (1%, p.o) respectively. The fore paws of the rats were placed on a wooden bar elevated at 9cm above the ground. Duration for which the animal maintained the imposed posture was noted as the time required for removing the fore paws from the bar. Duration of catalepsy was measured at 0, 30, 60, 90,120 min and was assessed by scoring technique of Costall and Naylor.

Anxiolytic Activity-

Elevated Plus Maze

Elevated plus maze consisted of wood with two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm). The maze was elevated to a height of 40 cm. The rats were placed individually at the end of one arm facing away from the center of the elevated plus maze (locally constructed).The time taken by rat to move from open arm to either of the closed arms(transfer latency, TL) was recorded. On the first day, the rats were allowed to explore the plus maze for 20 sec. After the measurement of TL, rats were returned to their home cages. Twenty four hours later, again rats were placed on the elevated plus maze individually as before and TL was recorded. TL measured on 1st and 2nd day served as parameters for acquisition and retrieval respectively. Diazepam (2mg/kg, p.o), EASA (650mg/kg, p.o), CMC (1%, p.o) served as standard, test and control respectively.[5]

Hole Board Test

Exploratory behaviour of rats in a novel environment was measured using a hole-board test (locally constructed). This method is used for measuring the response of the rat to an unfamiliar environment. The apparatus consisted of a grey cardboard box (50×50×50 cm) with 18 equidistant holes 3 cm in diameter in the floor. 30 minutes after proposed treatment with Std/samples, head-dipping behaviours were checked for 20 minutes with intervals of 5 minutes. Diazepam (2mg/kg, p.o), EASA (650mg/kg, p.o), CMC (1%, p.o) served as standard, test and control respectively.[5]

Statistical analysis

The results of the studies were expressed as mean ± SEM (standard error of mean). The difference between the control and treated means were analyzed using one-way analysis of variance (ANOVA). P-values < 0.05 were taken to be statistically significant. Dunnett's post hoc test was used for multiple comparisons. The statistical analysis was done using the software Graph pad prism version no: 5.0. Results were presented as Figures.

Results

Percentage yield

The percentage yield of ethyl acetate extract of *Sarcostemma acidum* (EASA) leaves was approximately 12% w/w.

In vivo Assay

CNS Inhibitory Effect-

In the method for evaluating CNS inhibitory activity, the percentage change in locomotor activity was increased to 55% on EASA treated animal when compared to normal control (untreated animals).

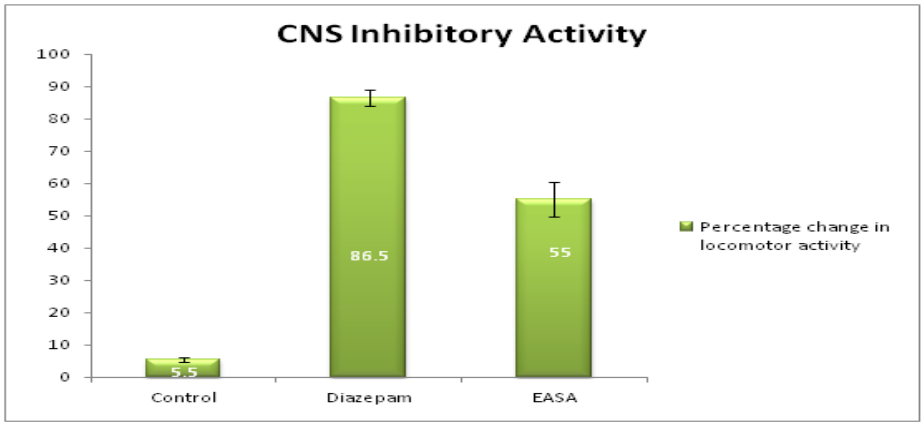

Figure 1: Graph plotting percentage change in locomotor activity

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Dunnet's multiple comparison test. xP value < 0.0001, yP value < 0.001, zP value < 0.01 F = 139.3, DF= 17(2,15)

Anxiolytic Activity-

Elevated Plus Maze

From the result of EPM it was evident that EASA treated animals exhibit an increased number of entries into open arm (38.8) when compared to normal control, which shows the anxiolytic activity of EASA.

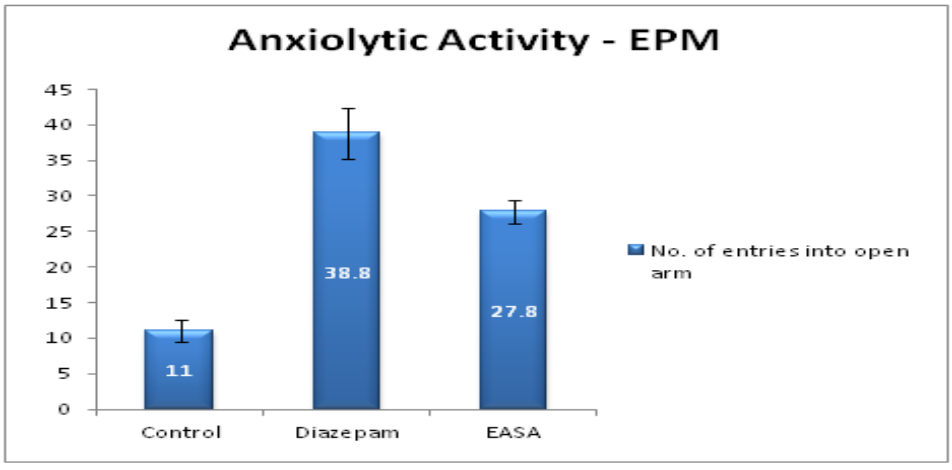

Figure 2: Graph plotting No: of entries in to the open arm in Elevated Plus Maze

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Dunnett's multiple comparison test. xP value < 0.0001, yP value < 0.001, zP value < 0.01 F = 32.77, DF= 17(2,15)

Hole Board Apparatus

Increased no. of head pokes shows the exploratory behaviour of the animal indicating anxiolytic activity. Here EASA had shown increased no. of head pokes (9.5) when compared to control.

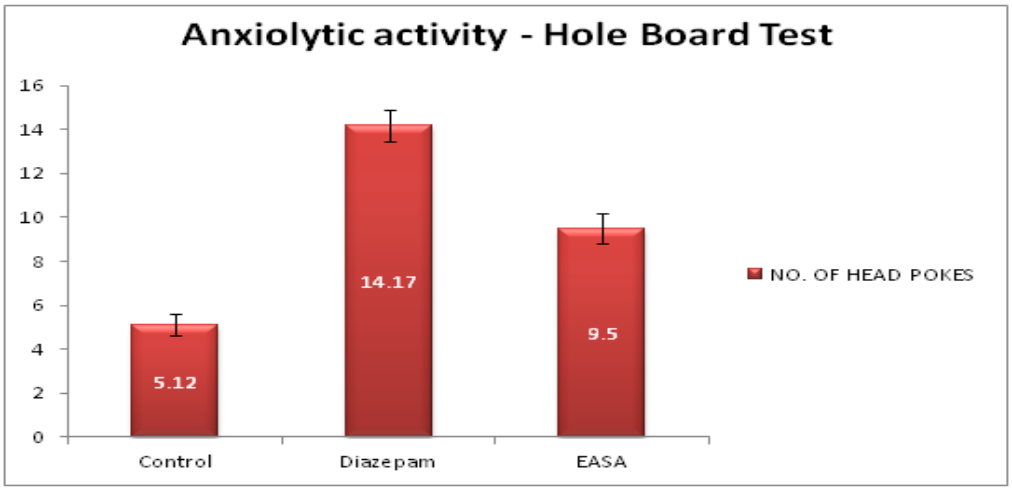

Figure 3: Graph plotting No: of head pokes in Hole Board Apparatus

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Dunnett's multiple comparison test.. xP value < 0.0001, yP value < 0.001, zP value < 0.01 F = 42.51, DF= 17(2,15)

Antipsychotic Activity-

Pole Climbing Method

In the method latency period for pole climbing in EASA treated group is increased when compared to the normal, depicts the inhibition of CAR by the EASA treated group and so EASA was considered as antipsychotic.

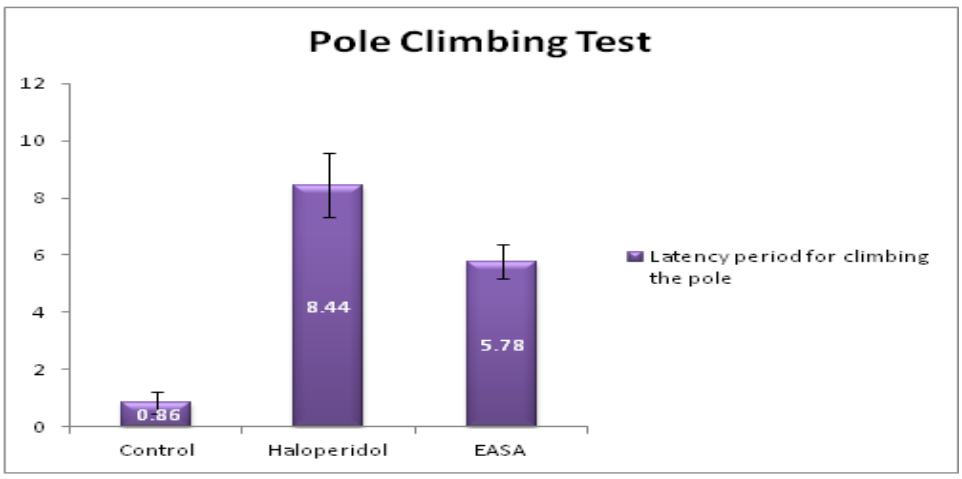

Figure 4: Graph plotting latency period for climbing the pole in Pole Climbing Apparatus

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Dunnett's multiple comparison test. xP value < 0.0001, yP value < 0.001, zP value < 0.01 F = 28.36, DF= 17(2,15)

Bar Test

From the results of Bar test, it was found that EASA treated groups exhibited a higher degree of catalepsy on each 30' upto 120' respectively when compared to normal control. This indicates that EASA possess a significant antipsychotic activity.

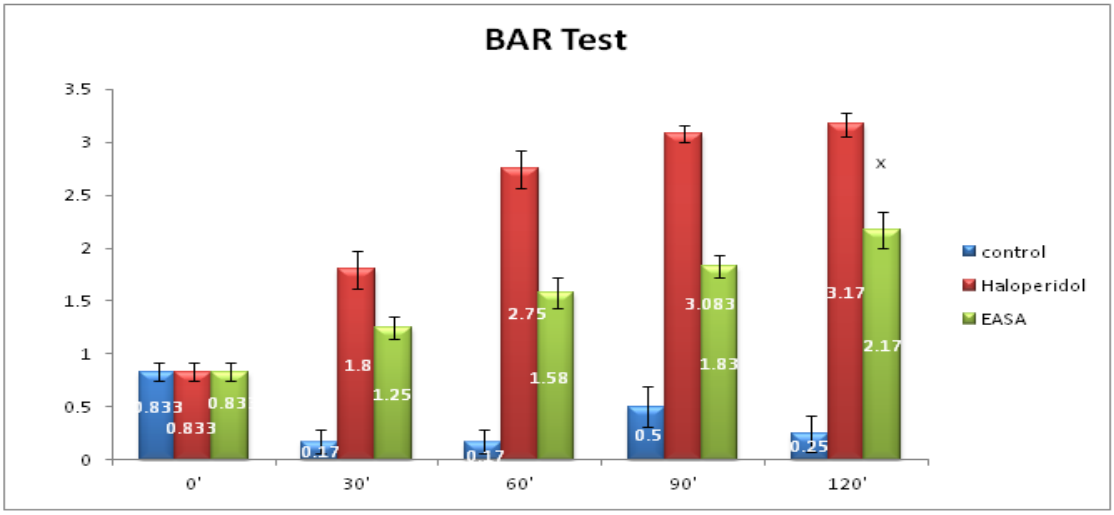

Figure 5: Graph plotting cataleptic score in Bar Test

Values expressed as Mean ± SEM, n=6 in each group. One way ANOVA followed by Dunnett's multiple comparison test. *P value < 0.05, **P value < 0.01, ***P value < 0.001, ****P value < 0 .0001

Discussion

In the present study ethyl acetate extract of *Sarcostemma acidum* was evaluated for the Psychopharmacological activity. Despite intensive efforts to develop novel psychiatric drugs for anxiety, depression and Psychotic disorders over the past two decades, all drugs have got marked side effect. In this respect, herbal medicines could be an attractive candidate as the therapeutic strategies for this conditions.[8, 9]

Reduction in the locomotor activity indicates C.N.S. depressant property of the drug. The percentage change in locomotor activity of vehicle treated animal is 5.5% while the EASA shows 10 times more activity (55%) than the control and standard drug diazepam shows 86% reduction in locomotor activity. In nutshell, EASA shows a significant CNS inhibitory activity when compared to control.

In antipsychotic test both conditioned avoidance response and catalepsy scoring were evaluated using Pole climbing test and BAR test. Latency period to climb the pole was 4.96 ± 0.42, 8.3 ± 0.62 and 0.85±0.26 for EASA, Haloperidol and control groups respectively. This clearly indicated the significant inhibition of CAR activity ($p<0.001$). Results of pole climbing method indicate that haloperidol affected the process of acquisition of conditioned avoidance response in rats when compared to control. Various authors have supported this evidence in different studies.[10, 11] EASA also produces the same effect when compared to haloperidol, only the intensity of the response differs. From this it is evident that EASA has got significant antipsychotic activity.

Haloperidol induced catalepsy is one of the animal models to test extra pyramidal side effects of antipsychotic drugs.[12] This is due to the blockade of dopamine receptors in striatum. In case of BAR test, catalepsy scoring is evaluated. This cataleptic result revealed that EASA had significantly increased the cataleptic activity, when comparing to the control, it might be due to the blocking effect of EASA on D2 receptor activity.

EPM test is one of the most frequently used animal models in behavioral Neuropsychopharmacology for screening drugs with potential anxiolytic effects.[13] In general, the reduction or increase in the number of entries and times spent in the opened arms induced by a given substance had been regarded as good indicators of its anxiogenic or

anxiolytic effects respectively.[14] The results of the present study demonstrate that administration of EASA could produce the anxiolytic like effect in this paradigm. This may be due to modulation of GABA receptors by the EASA extract.

Conclusion

The studies confirmed that the ethyl acetate extract of *Sarcostemma acidum* possess significant CNS depressant, antipsychotic and anxiolytic, activity. The results are encouraging to pursue further studies to propose the underlying pharmacological mechanism and also to isolate and characterize probable bioactive molecule responsible.

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