

EVALUATION OF ETHANOLIC EXTRACT OF *ALSTONIA SCHOLARIS* AND *BACOPA MONNIERA* AS POSSIBLE ANTIPSYCHOTIC FORMULATION.

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Abstract

*An increased demand has been observed for the use of herbal drug in chronic and incurable diseases. Treatment of psychiatric diseases like Schizophrenia is largely palliative and more importantly a prominent adverse effect prevails with the majority of antipsychotic drugs, which is the extrapyramidal motor disorder. This study was a trial for the ethanolic extracts of two plants *Alstonia Scholaris* and *Bacopa Monniera* with different antipsychotic animal models. Two doses of both the extracts (100 and 200mg/kg) were used for this study with 5 different animal models. The result of the study indicated a significant reduction of amphetamine induced stereotype and conditioned avoidance response for both the extracts compared with the control group, but both did not have any significant effect in phencyclidine induced locomotor activity and social interaction activity. However both the extracts showed increase in catalepsy compared to the control group. The results largely pointed out the fact that both the extract may be having the property to alleviate the positive symptoms of Schizophrenia by altering the dopamine levels of different dopaminergic neurons of the brain. It can also be interpreted from the data that the effect of both the extract was not dose dependent compared to the control group.*

Keywords: *Extrapyramidal, Dopaminergic, Antipsychotic.*

INTRODUCTION

Schizophrenia and psychosis has captured the headlines increasingly for the past 20 years. Incidence of schizophrenia is high which accounts for almost 1 in every 276 people [1] or precisely 2.5 million people, throughout the world today. Anti psychotic drugs available in the markets which are in use today, the safety profile is not so promising considering the fact that it has to be continued for a few years. The main and serious adverse effect of these drugs is the extrapyramidal side effects which includes akathisia, acute muscle dystonia, and tardive dyskinesia [2]. In spite of that, treatment of schizophrenia and allied psychotic disorder is heavily dependent on typical antipsychotic drugs still today [2]. Over the long term, these antipsychotics may cause dopaminergic pathways in the brain permanently dysfunctional [3]. They may lead to severe movement disorders like tardive dyskinesia, tardive psychosis, and global cognitive decline tardive dementia [2]. There has been enough evidence as suggested by the recent MRI scans of Schizophrenia patients that antipsychotics even are responsible for the shrinkage of the basal ganglia region of the brain³. Keeping all the above facts in mind, this study was a trial for exploration of a herbal formulation which can be used for the treatment of schizophrenia patients.

Demand for Herbal drugs is ever increasing. Herbal drugs are known to have very minimal adverse effect and its well worth a therapy for chronic CNS diseases like Psychosis which is virtually incurable. India is a country where ayurveda has been practiced from the Vedic ages successfully. Such a herbal plant is *Alstonia Scholaris* which belongs to the family Apocynaceae found widely in all parts of India. The leaves and the stem extract (ethanolic) and its fraction have been studied for antianxiety [4], antidepressant[4], antitussive[5] , antiasthmatic[5], and expectorant[5] activities. Another herbal plant, Bacopa Monniera which belongs to the family Schrophulariaceae found widely in all parts of India has been studied for various CNS activities. The alcoholic extract of Bacopa Monniera is reported to increase the learning performance of rats and the activity is attributed to saponin mixtures consisting mainly of bacosides A and B [6].The plant is also reported to show anxiolytic [7] , antiepileptic [8] activities. Although both the plant has been studied for various CNS ailments, their potential is still unexplored.

This study will be focused on evaluation of the antipsychotic activity of the two plant extracts on various models of Psychosis.

MATERIALS AND METHODS

Plants

Leaves of *Alstonia Scholaris* and Bacopa Monniera were collected from surrounding areas of Durgapur, West Bengal, identified by the department head of Pharmacognosy in Shri Vishnu college of Pharmacy.

Animals

Wistar Albino Rats of either sexes weighing 150-200 gm were obtained from Ghosh Enterprise Kolkata. They were housed in the Animal house of Shri Vishnu college of Pharmacy. The study was approved by the Institutional animal ethics committee bearing the approval no 439/PO/01a/CPSEA. The rats were acclimatized for 7 prior to the start of the study.

Plant Extracts

300 Gms of dried and powdered leaves of the *Alstonia Scholaris* were extracted on soxhlet extractor with ethanol for 5 days whereas 500gms of dried and powdered leaves of the plant Bacopa Monniera were extracted on soxhlet extractor with ethanol for same no of days. After 5 days both the extract were subjected to Rota evaporator to concentrate the extract. Later it was dried in open air to get the completely dried extracts.

Chemicals

Phencyclidine and amphetamine were obtained from the manufacturing company Sigma Aldrich, USA. Haloperidol sample was gifted by Crescent therapeutics limited, Solan, Himachal Pradesh.

Acute Toxicity Studies

Acute toxicity study was carried out for both the ethanolic extracts following OECD guidelines. The EA fraction suspended in water with 2% w/v gum acacia in the dose of 5 mg kg⁻¹ body weight was orally administered to overnight-fasted, healthy rats (n = 3). The animals were observed individually after dosing at least once during the

first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter for a total of 14 days. The acute toxicity study was repeated with doses of 50, 300 and 2000 mg kg⁻¹ body weight.

Methods

Amphetamine induced Stereotype in Rats [9]- Amphetamine is an indirect sympathomimetic agent. It induces licking, gnawing, grooming, sniffing (stereotype) in Rats which can be successfully prevented by classical neuroleptic agents. This test is predictive of antipsychotic drug D2 receptor antagonism. Two groups (n=6) of adult Wistar rats were taken weighing between 180 to 220gm and were treated with either test or the standard drug (Haloperidol) and then placed in individual cages. They were injected with d amphetamine (5mg/kg ip) after 30 mins. The onset of stereotypic behavior was evaluated at 30 mins interval for 3 hours. The reduction in mean stereotype score is indicative of antipsychotic effect.

Phencyclidine (PCP) Induced Bizarre pattern of locomotor activity [10]- Phencyclidine is a glutamate receptor antagonist. Administration of phencyclidine has been found to induce locomotor hyperactivity in rodents and is antagonized by antipsychotic drugs. Male Wistar rats weighing 150-200gm were housed in a chamber. Animals were divided into 2 groups (n=6), for test or the standard compound. 30 mins before the start of the experiment, the animals were administered with the extract or the standard drug. Phencyclidine (2mg/kg) was administered to the animals of both the groups just before the start of the experiment. Then the locomotor activity of the animals will be measured in photoactometer for a session lasting for 90 mins. Drugs antagonizing the phencyclidine induced activity are expected to act by some other receptor rather than dopaminergic receptors.

Phencyclidine (PCP) Induced Social withdrawal test [10] -This test helps to show the effectiveness of potential antipsychotic drugs against negative symptoms of schizophrenia. Phencyclidine decreases the time of social interaction in the rats .Naïve Male Wistar rats were housed in pairs for 10 days prior to the start of the experiment. During the test one cagemate is removed and a new one is kept in the cage for 20 mins. The amount of social interaction is measured as the total amount of time spent on various elements of interaction i.e. social interaction, and genital investigation. Phencyclidine will be administered 5 mins before the start of the experiment whereas the test or the standard drug will be given 30 mins before the experiment.

Conditioned Avoidance Response in rats [11] - Perhaps the oldest animal model to predict potential antipsychotic drug efficacy is the conditioned avoidance response (CAR) In the conditioned reinforcement model, experimental animals are trained to perform a certain response to avoid a mild shock. Trained avoidance responses may be active (pressing a lever, climbing a pole, or jumping out of a box) .Classical antipsychotic drugs reduce avoidance responding at doses that do not impair natural (untrained) escape. Two groups of rats weighing 150-250 Gms were tested in this model for test drug or standard. 10 days of training period were carried out before the experiment, and a total of 20 sessions of training were imparted to each rat before the experiment. Test or the standard drugs were administered 30 mins before the start of the experiment.

Induction of catalepsy in Rats [9] - Wistar rats weighing between 180 to 200 Gms each are randomly divided in two groups (test or standard). After an appropriate pretreatment time of the drug, each rat is tested for with respect to the right and left front paws which are first put on columns, first 3 cm and then 9 cm high. The cataleptic state was

considered if the rat maintains the abnormal posture for 10 sec or more. The scoring was done according to the following

0- The rat moves normally when placed on a table.

1-Rats move only when touched or pushed.

1+1=2 – Rats placed on a table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 secs. Scored as 1 point for each paw, with a total of 2 for both paws.

1+1=2 – Rats placed on a table with front paws set alternately on a 9 cm high block fails to correct the posture in 10 secs. Scored as 1 point for each paw, with a total of 2 for both paws.

This model predicts the extrapyramidal side effects of the test drug.

Statistical Analysis

All the values will be expressed as mean \pm SEM. Data analysis will be with the help Annova and Students t test as when required.

Groupings of the animals

Control Group – Animals of this group will receive 2% Gum acacia suspension.

Group II- Animals of this group will receive standard drug (Haloperidol)

Group III – Animals of this group will receive *Alstonia Scholaris* (ALS) extract at a dose of 100mg/kg.

Group IV- Animals of this group will receive *Alstonia Scholaris* (ALS) extract at a dose of 200mg/kg

Group V- Animals of this group will receive *Bacopa Monieri* (BM) extract at a dose of 100mg/kg.

Group VI- Animals of this group will receive *Bacopa Monieri* (BM) extract at a dose of 200mg/kg.

RESULTS

Extraction: The amount of the extract obtained for *Alstonia Scholaris* and *Bacopa Monniera* was 22% and 10% respectively of the initial material for each plant.

Acute toxicity Studies: Both the ethanolic extract was found to be safe upto the dose of 2000mg/kg body weight. The dose that was selected for the study was 100mg/kg and 200mg/kg body weight for each of the extracts.

Amphetamine induced stereotypy in rats: Results from this study shows that all the stereotypic activities like sniffing rearing and licking were reduced significantly in all the treatment groups($p < 0.05$) compared to the control groups, but the degree of reduction varied differently among the treatment groups with no significant difference among the different doses of both the extracts. The standard drug haloperidol reduced sniffing, rearing and licking activity by 65%, 55% and 50% respectively. The ethanolic extract of ALS reduced sniffing, rearing and licking

activity by 35%, 43% and 27% respectively, whereas the BM extract reduced sniffing, rearing and licking activity by 22%, 20% and 17% respectively compared to the control groups.

TABLE 1. Inhibition of amphetamine induced stereotype

GROUPS	SMELLING	REARING	LICKING
Control	8.3±1.15	5.52±0.94	3.7±0.28
Group II	2.9±0.28[#]	2.44±0.16[#]	1.62±0.20[#]
Group III	5.23±1.16[#]	3.25±1.60[#]	2.82±0.23[#]
Group IV	5.67±2.20[#]	3.26±1.69[#]	2.74±0.36[#]
Group V	6.62±1.78[#]	4.46±0.77[#]	2.98±0.52[#]
Group VI	6.50±1.99[#]	4.40±0.63[#]	2.69±0.66[#]

N=6; #= P<0.05 when compared with control

Phencycline induced bizarre pattern of locomotor activity

Results from this model are suggestive of no significant change in the locomotor activity for all the treatment groups compared to the control group. . This result suggests that both ALS and BM and the standard drug did not alter the locomotor activity at any of their doses used.

TABLE 2. Phencyclidine induced bizarre pattern of locomotor activity

GROUPS	LOCOMOTOR ACTIVITY SCORES
Control	302±7.28
Group II	300±6.23
Group III	299±12.70
Group IV	303±5.28
Group V	302±6.87[#]
Group VI	299±8.19[#]

N=6; #=p< 0.05 when compared with control.

Phencyclidine (PCP) Induced Social withdrawal test

No animals from the test groups (ALM and BM) or the standard group altered the social exploration and the anogenital inspection activity compared with the control group significantly ($p>0.05$). This model is suggestive of the absence of negative symptoms alleviating property of all the treatment groups.

TABLE 3. Phencyclidine induced social withdrawl test

GROUPS	SOCIAL EXPLORATION	ANOGENITAL INSPECTION
Control	8±0.81	3±0.81
Group II	7 ±1.58 ^{\$}	3±1.63 ^{\$}
Group III	8±0.70 ^{\$}	2±1.92 ^{\$}
Group IV	8±2.29 ^{\$}	2±1.66 ^{\$}
Group V	7±0.78 ^{\$}	2±1.87 ^{\$}
Group VI	7±1.15 ^{\$}	3±0.88 ^{\$}

N=6; \$= $p>0.05$ when compared to control.

Conditioned Avoidance Response in rats

All the groups significantly decreased the escape response compared to the control group ($p<0.05$). Group II reduced the escape response by almost 70%, Group III and IV by 44%, and Group V and VI by 25%. However there was no dose dependent reduction of escape response for both the ALS and the BM extract.

TABLE 4. Conditioned avoidance response in rats

GROUPS	NO OF TIMES ESCAPED
Control	16±0.95
Group II	4.8±1.23 [#]
Group III	9±1.64 [#]
Group IV	9±0.97 [#]
Group V	11±1.98 [#]
Group VI	12±0.73 [#]

N=6;#= $p<0.05$ compared to control

Induction of catalepsy in Rats

All the treatment groups increased the mean cataleptic scores significantly ($p < 0.05$) compared with the control group. However the increase in mean cataleptic score was increased by 200% in case of the test extract where as 300% in case of the standard drug haloperidol. There was no significant difference in cataleptic score among the different dose group of the two test extracts.

TABLE 5. Induction of catalepsy in rats

GROUPS	MEAN CATALEPTIC SCORES
Control	0
Group I	3±0.35 [#]
Group II	2±0.12 [#]
Group III	2±0.12 [#]
Group IV	2±0.27 [#]
Group V	2±0.63 [#]

N=6, #= Significant difference compared to control group

DISCUSSION

Haloperidol and Ethanolic extract of leaves of ALS and BM showed decrease in amphetamine induced stereotype compared to the control group. However the extent of decrease of the stereotypic activity for AS and BM was less as compared to the standard drug haloperidol. This kind of outcome is indicative of a possibility that the test extracts may be decreasing the levels of Dopamine in the brain as is the case for the standard drug haloperidol. Neither of the test extracts or the standard drug altered the phencyclidine induced increase in locomotor activity or the social interaction test. These models are suggestive of the ineffectiveness of haloperidol and the test extracts to alleviate the negative symptoms of schizophrenia. It is once again established that haloperidol has no effect on the negative symptoms of schizophrenia.

Both the extract as well as the standard drug reduced the conditioned avoidance response; however the magnitude of reduction was less for the test extract than the standard drug when they were compared with the control group. This kind of results for the standard and the test extract indicates the alleviating effects of positive symptoms of schizophrenia.

The induction of catalepsy once again pointed out the fact that both the extracts like the standard drug could be acting on the dopaminergic neurons of the brain. Haloperidol is known to decrease the dopamine levels on various dopaminergic pathways of the brain which is the reason for extra pyramidal motor disorders. The ability of the extracts to induce catalepsy indicates that they may be altering the dopamine levels in the dopaminergic pathways.

Further analysis of the data showed that there were no significant dose dependent effects for both the extracts when they were evaluated with all the above models of anti psychotic drugs.

Taking all the above facts into consideration, it may be safe to say that the ethanolic extract of the leaves of *Alstonia Scholaris* and bacopa *Monniera* may decrease the dopamine levels of the brain and can be used for further studies on Schizophrenia.

CONCLUSION

Schizophrenia is a matter of concern for the patients as well as for the psychiatrists. Till now only symptomatic treatment is known to us. Herbal drugs as we know, is safe compared to the synthetic drugs due to its reduced toxic effects. The two poly herbal formulations used in the studies have shown promising effects in this study in reducing the inductive symptoms of psychosis in rats. This poly herbal extracts can be further fractionated and research may be done on isolation of active compounds and evaluates their effects on the dopaminergic receptors.

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