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The antipsychotic potential of Ethanol Extract of Leaf of Piper betle Linn on Ketamine induced rats

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ABSTRACT

Piper betle Linn has been traditionally used as medicine , possess antiulcerogenic, anti-inflammatory and analgesic effect and the underlying mechanism of action has been estimated to be antioxidant.
The present study aims to investigate the antipsychotic and antischizophrenic effect of Piper betle Linn based on its antioxidant effect in ketamine induced experimental model.
The experimental rats [n=45] received a dose of ketamine at 30 mg/kg body weightvia intraperitoneal injection for 5 consecutive days.They were then allocated into their respective treatment groups and given risperidone [APD] and the ethanol extract of leaf of Piper betle Linn at the dose of 6 mg/kg body weight and 150 mg/kg body weight. For 7 consecutive days.
Ketamine produced reduced weight gain in rats.Dopamine and Acetylcholine concentration levels in brain were high in brains with treatment with risperidone and Piper betle Linn.These findings proved the positive antipsychotic action of Piper betle Linn similar to risperidone.

Keywords*: Piper betle Linn, Antipsychotic Activity, Ketamine-Induced Schizophrenia, Antioxidant Activity, Risperidone*

INTRODUCTION

Piper betle Linn which is also known as Tambula [Sanskrit] or Pan (Hindi and Bengali) is being widely cultivated in tropical humid climate in South East Asia.It is reported to possess wound healing ,antimicrobial, antifungal and anti-inflammatory activities and the underlying principle has been pinpointed towards free radical scavenging action that has been reported .As the principle mechanism of many diseases appears to be free radical neutralizing action , the present study aims to investigate into antipsychotic action of ethanolic extract of leaf of Piper betle Linn ..Elimination of side effects of ant psychiatric drugs including antipsychotic and ant schizophrenics, can be of high significance pathologically and biochemically, if herbal plants are offered by the l health practitioners for elimination of neuro psychiatric diseases including in the treatment of schizophrenia .

This study is attributed to the investigation of the effects of ethanolic extract of leaf of Piper betle Linn on ketamine induced animal models on social interaction, novelty and modulation of motivation .

It is also aimed to find out, and , explore into the novelty and efficacy of use of the herbal extract on motivation in adult male rats . The study , thus, explores into the potential of resperidone and the herbal extracts viz. ethanolic extract of leaf of Piper betle Linn to reverse impairments that are induced by ketamine .

Experimental rats (n=45) received a dose of ketamine at 30 mg/kg body weight , intraperitoneal injection for 5 consecutive days .They were then allocated into their respective treatment groups and given risperidone (APD) and the herbal extract viz ethanolic extract of leaf of Piper betle Linn at a dose of 150 mg/kg body weight for 7 consecutive days .

Social behaviour were tested using 3 chambered sociability test and anhedonia was tested using the sucrose preference test. Ketamine induction elicited social withdrawal and reduced social novelty which were later successfully

reversed by risperidone and ethanolic extract of leaf of Piper betle Linn.

The rats showed reduced preference to sucrose post induction and post treatment.
Dopamine and Acetylcholine concentration levels were high in groups which received risperidone and groups treated with ethanolic extract of leaf of Piper betle Linn.

These findings highlight that the antipsychotic potential of ethanolic extract of leaf of Piper betle Linn is also good compared to risperidone.

Approximately 1 in every 8 individuals are affected by mental disorders globally , with approaximately 1 in 300 or almost 25 million individuals affected by Schizophrenia [World Health Organisation 2022] [de Barlolomeis et al 2023]1. Schizophrenia is classified as one of the most serious mental disorders due to its complicated behavioural and cognitive symptoms. (Gao et al 2023)2

Western treatment of Schizophrenia include medication , psychosocial rehabilitation , family interventions and psychoeducation 3.

The medication includes antipsychotic drugs which are commonly classified into typical [First Generation] and Atypical [2 nd Generation].4

Typical antipsychotic drugs attenuate the undesirable symptoms whist lacking the ability to improve the ability to improve the negative and cognitive symptoms of Schizophrenia.

Although atypical antipsychotic drugs are reported to result in less severe side effects compared to the typical drugs , all antipsychotic drugs are associated with adverse effects .

Heral medicines are used in India to treat various diseases , both chronic and acute.5

Being a herbal formulation, the ethanolic extract of leaf of Piper betle Linn is reported to possess significant antimicribial, antiulcerogenic , antiinflammatory and wound healing activity.6,7,8

It would have been great if the herbal formulation is shown to possess antipsychotic effect .The present study is aimed to observe any efficacy of the herbal formulation in treatment of neuropsychiatric disorder including schizophrenia and psychosis.9

Various models for the pathophysiology of schizophrenia exist .However dysregulation of Dopamine [DA] is regarded as the primary neurotransmitter Landmark and the nigrostriatal , mesolimbic , mesocortical and tuberoinfundibular pathways are the four major dopaminergic pathways .Dopamine plays a primary role in reward processing and a dysregulation in the reward circuit resulting in anhedonia .

In the mesolimbic pathway , excess production of Dopamine [DA] from the ventral tegmental area (VTA) to the Limbic areas results in the development of the positive symptoms of schizophrenia 10.
On the other hand , the negative symptoms of schizophrenia are associated with low levels of DA from VTA to the cortex in the mesocortical pathway.

Resperidone (RIS)11 one of the four atypical antipsychotic drugs is established to successfully treat negative symptoms of Schizophrenia through 15 antagonistic role on DA and 5 hydroxy tryptamine (5 HT) 2A receptors .However , the side effects of this drug include elevated prolactin levels , akathisia and extrapyramidal symptoms .

Hence , the exploration of alternative medicine is needed.
The dysfunction in cholinergic systems is also linked with the pathophysiology of schizophrenia as acetylcholine plays a significant role in functions such as memory and learning process [Which are impaired in the disorder] handled in the hippocampus.

MATERIALS AND METHODS
The study has been divided into multiple phases—
1. Adaptation[Pre induction]
2. Induction
3. Treatment

A total of 66 Sprague -Dawley rats (Weighing between 150-200 g at the beginning of Ketamine injection regime) were obtained .
The rats were divided into two main groups

1. Control [CTRL] group which received sterile saline .2. Experimental Group which received Ketamine during the induction phase .

From these groups , the rats were further subdivided into six groups for the treatment phase
1]CTRL SAL
2] CTRL APD
3] EXP SAL
4] EXP APD
5]EXP APD
6] EXP PIPER BETLE [PB]
CTRL= CONTROL
EXP=EXPERIMENTAL
APD =ANTI PSYCHOTIC DRUG
SAL=SALINE
PB =PIPER BETLE

The animals were single housed in order to avoid contact with each other . However , the cages were placed close to each other to maintain auditory and olfactory contact between the animals , thus minimising stress and aggression.
The housing conditions were set at a 12 hour light-dark cycle with lights on from 6 AM -6 PM and the room temperature was kept constant .All experiments were conducted during the light phase.

The rats were afforded seven days of acclimatization , monitored and gently managed by the primary investigator daily.
All stipulated research protocol were adhered to in accordance to the guidelines approved by the Animal Research Ethics Committee.

Plant Metrials -Leaves of Piper betle Linn were collected from the local market during the period of June July and identified by Botanical Survey of India as Piper betle Linn of Piperaceae family. The leaves were cut into small pieces and made into a paste in 95% ethanol forming a slime .The slime was then percolated at room temperature of 3 7degree celsius with 95% ethanol for 10 days using an aspirator and the solvent was filtered through a nylon mesh.

The alcohol soluble portions were taken and evaporated under vacuum condition (0 cm of Hg) in a rotary evaporator.
The final concentrated solution , devoid of alcohol, was dried by a lyophilizer to obtain a solid amorphous brownish mass that was stored in vacuum dessicator.

Animals and treatment-
Healthy , pathogen free , colony bred, male Charles foster rats (150-200g) as described earlier were maintained in laboratory condition with 12:12 hour light and dark cycle and average room temperature of 32 degree celsius for 10 consecutive days and provided with food pellets and drinking water.
They were housed in standard cages and acclimatized .All chemicals used were of analytical grade.

Treatment and Drug Preparation
After acclimatization to laboratory conditions , behavioural tests were conducted prior to the inducement of schizophrenia symptoms with ketamine.

Ketamine12 at 30 mg/kg was diluted in 0.9 % saline and administered intraperitoneally to the experimental animals for five consecutive days to induce symptoms of Schizophrenia .

The ratio of ketamine -saline solution was calculated based on the weight of each rat for the duration of the induction phase.

This solution was freshly prepared daily with the dose determined according to the weight of the rat.
The control group received sub chronic doses of 0.9% sterile saline (0.5 ml/kg) as the experimental group via intraperitoneal injection for 5 days.
The animals were given 48 hours of withdrawal after induction.

The antipsychotic drug , risperidone [RIS]10 and one of the herbal plants , Piper betle Linn 13,14,15[Ethanol Extract as described above) was used .

The duration of treatment was a week [Seven Days] based on the findings by Potkin et al [2003]16 which indicated that improvements of symptoms of Schizophrenia can be observed in the first week of treatment with RIS at a dose of 6 mg/day. The animal equivalent doses for risperidone was calculated based on the weight of the animals on each day of treatment . The stock solution for RIS was prepared by dissolving 5 mg of powdered RIS in 1000 microlitre of 1% DMSO, the

required dose was then diluted with sterile phosphate buffer saline [PBS] or RIS , which was insoluble and formed instant precipitation in water and normal saline Ethanol Extract of leaf of Piper betle Linn was used at a dose of 150 mg/kg body weight , as earlier found to have antiulcerogenic effect with antioxidant mechanism of action in earlier studies.

BEHAVIOURAL TESTS

The 3 chambered sociability test and sucrose preference tests were used to test the rats for associability and anhedonia respectively .

This was conducted pre-induction [Before inducement with ketamine on the experimental group] , post induction [24-48 hrs after the last ketamine injection on the day five] and post treatment with saline [SAL]

Crawley's Sociability Test-17

Crawley's sociability Test also known as 3 chambered paradigm test was used to assess social interaction behaviour.(Sanavi et al 2022) .

The Ugo Basile sociability apparatus (With transparent walls , partitions and Lids -180x40x40 [h] cm with 2 grid cages was used and the protocol was adapted from Sanavi et al (2022) protocol.

All rats , viz test and 2 strangers were of the same weight , age and sex.

The apparatus were cleared using tap water and 70% ethanol between each test .

Phases of the Sociability and Novelty Phase was indicated as follows-

Time spent by the test animal near or interacting [Sniffing, Touching , Biting and/or Scratching] with the caged animals or empty cage as recorded as Time [F].

And the total time spent in each chamber with the caged animals and the empty cage was also recorded.

The Object Recognition Index [RI] for sociability was calculated as follows

$$RI = \frac{\text{STRANGER 1[TN]}}{\text{STRANGER 1 [TN] -EMPTY CHAMBER [TN]}}$$

Where --

STRANGER 1[TN] was the time spent by the test animal interacting with the caged STRANGER 1 animal and Empty Chamber [TN] was the time spent by the test animal exploring the empty cage during the sociability phase.

The RI for novelty was calculated as follows –

$$RI = \frac{\text{STRANGER 2[TN]}}{\text{STRANGER 2 [TN] + STRANGER 1[TN]}}$$

Where –

STRANGER 1 [TN] was the time spent by the test animal interacting with the caged STRANGER 1/familiar animal from the sociability phase ,

STRANGER 2 [TN] was the time spent by the test animal interacting with the novel animal /Strangers 2 introduced during the novelty phase.

The closer the RI to 1, the more social the animals are and the more they slow preference for a novelty .

Sucrose Preference Test18

The two bottle methodology for assessing sucrose preference is an effective test that can be used in the investigation of anhedonia [Eagle et al 2016]

The two bottle experiment allows for a comparison between behavioural preferences for water to a preference for the sucrose solution.

Two 100 ml bottles were placed on either side of home cages , one with normal drinking water and one with 1% sucrose solution.

The bottles were weighed for 4 consecutive days and the side of each bottle was alteranted every day to avoid bias.

The sucrose preference percentage was calculated using the formula as below.

% SUCROSE PREFERENCE =

$$\frac{\text{CONSUMED SUCROSE SOLUTION}}{\text{TOTAL LIQUID CONSUMPTION}} \times 100$$

Dopamine and Acetylcholine ELISA 19, 20,21

Brain tissue samples [Pre frontal cortex [CORTEX] , ventral tegmental area [VTA] and hippocampus were weighed and rinsed and homogenised using PBS. Thereafter , the homogenates were centrifuged at 5000xg for 5 mins and the supernatant was collected and used for further analysis.The reagents for the ELISA assays were prepared as per E lab science manuals for dopamine [Catalogue No E-EL-0046] and acetylcholine [Catalogue No- E-EL-0081]

The kit and all samples were brought to room temperature before they were used.
A total of 4 plates were used -two dopamine plates for PFC and VTA samples and two acetylcholine plates for PFC and hippocampus samples.

The assay procedure for each plate is as follows-50 microlitre of the standard or sample was added to each well and was followed by immediate addition of 50 microlitre of Biotinylated detection Antibody to each well. Thereafter the plate was incubated at 37degree celsius for 45 minutes .After the contents of the plate were aspirated , the plate was incubated at 37 degree celcius for 45 mins.

After the contents of the plate were aspirated , the plate was washed 3 times with wash buffer solution.
This was followed by the addition of 100 microlitre of HRP conjugate to each well and the plate was incubated at 37degree celsius for a further 30 mins.

After the contents of the plate were aspirated again , the plate was washed 5 times using a wash buffer solution.Thereafter , 90 microlitre of substrate reagent was added to each well and the palte was incubated at 37 degree celsius for 15 mins . Lastly after incubation 50 microlitre of stop solution was added to each well and the plate was read immediately at 450 nm.

The concentrations of dopamine (DA) and acetylcholine (ACh) present in each sample were determined using a standard curve.

Data Analysis

A paired samples T test were used to analyse the findings when comparing between pre and post induction data or when comparing post induction with post treatment findings. On the same group.
The one way ANOVA test was used to analyse findings between all treatment groups during all phases of the experiment.The two way ANOVA test was used to assess the effect and interaction between Ketamine treatment and drug effect.

The Post HOC test Turkey was applied for its most attraction properties.
IBM SPSS version 28.0 for Windows Software and Microsoft Excel (2016) were used for data analysis and for graph presentations, respectively.
Values for $p< 0.05$ were considered statistically significant,..

RESULTS

Effect of Ketamine ,Risperidone and ethanolic extract of leaf of Piper betle Linn on weight gain
During the adaptation /pre -incubation phase, the average weight gained by the rats was 42 g , 39.32 , 38.12 , 48.34, 38.40 , 42.72 for Control[Saline], Control [Antipsychotic Drug] , Control {Piper betle Extract]

Post induction, the weight gain for was 24g, 21.78g and 18.24g on an average respectively for Control[Saline] Control{Antipsychotic Drug} and Control [Piper betle Extract] respectively.

The weight gained by the groups which received ketamine was lower compared to the control groups [$p<0.005$] with the average weight gain for Experimental[Saline] =13.78, Experimental[Antipsychotic Drug] 12.84 and Experimental [Piper betle extract] .

Post treatment , the average weight gain increased for all groups Control [Antipsychotic Drug] 24.74, Control [Saline} 30.02 , Control [Piper betle Extract] 26.24 , Experimental[Saline] 30.12, Experimental [Antipsychotic Drug] 19.28 and Experimental [Piper betle Extract] 28.64. respectively.The overall weight gained by the experimental groups remained lower compared to the control groups ($p=0.005$)

Effect of Piper betle Linn on Sociability--
Based on the findings from the sociability phase of the Crawleys Sociability test , the time spent interacting with the caged animal [Stranger 1] reduced significantly post induction for all animals which reached ketamine .

Time (N) pre -induction for Experimental [Saline] , Experimental [Antipsychotic Drug] and Experimental[Piper betle Extract] groups was 272.22 s, 204.56 s and 204 .08 s respectively.

Post induction , statistical significance was noted between Control[Antipsychotic Drug] vs Experimental [Antipsychotic Drug] for Time [N] in chamber with stranger 1 [p=0.005] Statistical significance was also noted between Control[Antipsychotic Drug] vs Experimental [Antipsychotic Drug] for Time [F] in chamber. With Stranger 1 post induction.[p=0.005]

Post treatment , Time [N] increased significantly for Experimental [Antipsychotic Drug] . and Experimental [Piper betle Extract] as 142.54, 182.68 and 192.56 respectively..

Time (n) for control group also decreased post induction.
Time [N] pre induction for Control [Saline] , Control [Antipsychotic Drug] and Control [Piper betle Extract] was 312.12, 304.24 and 314.12 for Control [Saline], Control[Antipsychotic Drug] and Control[Piper betle extract] respectively.
Control [Piper betle Extract] spent more time on chamber with an empty cage (312.04 s) and a statistical significance was noted between Control[Piper betle Extract] and Experimental[Piper betle Extract] for total time spent in the chamber with an empty cage .

The sociability RI decreased post induction and decreased further post treatment for all control groups , the decrease was particularly noticeable for Control[Piper betle extract].

Pre induction RI for Control[Saline] , Control [Antipsychotic Drug] and Control [Piper betle Extract] was 0.88, 0.84 amnd 0.90 respectively. Post Induction, RI for Control[Saline],Control[Antipsychotic Drug] and Control[Piper betle Extract] was 0.78, 0.80 and 0.64 and post treatment RI for these groups was 0.70, 0.78 and 0.74.

For Experimental groups , was 0.84, 0.82 and 0.78 and 0.74, 0.82 and 0.78 respectively.For Experimental [Antipsychotic Drug] and Experimental[Piper betle Linn] , the RIpost treatment was higher compared to RI post induction.The p values for sociability RI were 0.384,0.312 and 0.040 for pre induction , post induction and post treatment phases., respectively.
Statistical Significance was noted in the post treatment phase.

Statistical significance was also noted between for Time (F) in chamber with Stranger 1 post induction (p=0.007)

Post Treatment , Time (N) increased slightly for Exp and increased significantly for Experimental group.
It was also noted that time for control groups also decreased post induction.However , not as sharply as observed for the experimental groups(N) pre induction this decreased significantly..Post Treatment (N) continued to decrease for all control groups .Post Treatment Time increased slightly for experimental saline group to 142.6 s .Post Treatment Time decreased for all control groups to 190.22s, 178.62 s and 136.46 s for control saline , control APD and control PB respectively control PB spent more time in chamber with an empty cage (310.76) compared to the total time spent on the chamber with STRANGER 1(210.24) AND A STATISTICAL SIGNIFICANCE WAS NOTED BETWEEN control pb and experimental pb (p=0.034) for the time spent in the chamber with an empty cage.Control spent more time on chamber with an empty cage () compared to the total time spent on the chamber with stranger and a statistical significance was noted between control and exponential for total time spent in the chamber with an empty cage.

The sociability RI decreased post induction and decreased further post treatment for all control groups , the decrease was particularly noticeable for control.The p values for sociability RI were for pre induction , post induction and post treatment,post induction and post treatment phases, respectively.Statistical significance was noted between groups in the pre and post induction phases , statistical gignificance was noted between results on sociability phase of the 3 chambered sociability test (Prl-A<B) pre induction (Pol c-D) post induction and post treatment.
After 5 min of habituation, all animals were allowed to explore the 3 chambers of the apparatus (One at
a time) and a randomly selected Stanger 1 (S1) animal (1of n=20of animals which served as strangers and were not part of the test animals to undergo the sociability test was random and the side (left or right) where Stranger 1 was introduced also selected randomly.

The other cage on the other side was left empty (E) during this phase.
Findings are presented for time spent interacting [TN or Time (N)] with the empty cage or cage with SI for the pre and post induction and post treatment phases.Total Time [TT] spent in each chamber with these cages was also noted.Statistical significance was noted in post induction Time (N) in Chamber with stranger for control. And in post treatment total time spent in chamber with empty cage.

No statistical significance interaction was noted for the sociability phase however marginal significance was noted for the time spent interacting with S1 post treatment.The interactional effects for this phase were as follows-

Social Interaction Novelty
The sociability RI decreased post induction and decreased further post treatment for all the control groups., the decrease was particularly significant for experimental PB group. Based on the findings from the Novelty phase of the Crawleys sociability test , all groups preferred interaction with stranger 2 as opposed to Stranger 1.However , the time spent interacting with Stranger 2 decreased post induction.

Pre induction RI for CONTROL SAL, CONTROL APD , CONTROL PB was 0.82, 0.80 and 0.88 respectively.
Post induction RI was 0.74, 0.78 and 0.64 respectively for CONTROL SAL , CONTROL APD , CONTROL PB.
Post induction RI for CONTROL SAL, CONTROL APD, CONTROL PB WAS 0.78, 0.80 , 0.64 AND post treatment was 0.72, 0.66 and 0.60 respectively.

For experimental groups, RI post induction and post treatment was post induction 0.78, 0.82, 0.76 and post treatment 0.76, 0.78 , 0.86Induction RI decreased post induction . Post Treatment , the time spent interacting with stranger 2 for EXP increased significantly .

Novelty phase of 3 chambered sociability test pre Induction , post induction and post treatment .
After 10 mins of sociability test , all animals were allowed to again explore the 3 chambers of the apparatus.(One ata time) and randomly selected Stranger 2[S2] animal (Also 1 of n=20 of animals which served as strangers and were not part of the test animals) was introduced in the cage which was previously empty during the sociability phase of the social interaction test .

During the novelty phase (5 Mins)the test animals had access to explore as interact with either the familiar animal (Stranger 1/31) or the novel animal (Stranger 2/S2) .

The test animals completed all phases of the social interaction test (Habituation,Sociability and Novelity) before another randomly selected test animals was brought into the behavioural testing room to also complete the 3 phases. Findings are presented for time spent interacting (TN) with caged S2 for the pre and post induction and post treatment phases.Total time (TT) spent in each chamber with these cages was also noted.Statistical significance was noted in post induction Time (N) in chamber with Stranger 1 for Post Induction.

Sucrose Preference
The preference for 1% sucrose solution for both control and experimental groups decreased post induction and further decreased post treatment with SP% for experimental groups remaining higher than that of control groups post induction and post treatment .Post induction it decreased .Post treatment it further decreased .

Sucrose Preference Percentage
Anhedonia was measured using preference for normal drinking water over a sucrose solution.All test animals (n=66) were given 2x50 ml bottles {One with normal drinking water and one with 1% sucrose solution) in their home cages for 4 consecutive days.

The sides which these bottles were placed was alternated every day.Liquid consumed was measured and the sucrose preference percentages (SP%) were calculated for each day. The sucrose preference test was done for all 3 phases of the experiment (pre and post induction and post treatment) .The preference for 1% sucrose solution for both control and experimental groups decreased post inductiom and further decreased post treatment. With SP% for experimental groups remaining higher than that of control groups post induction and post treatment.

The SP % pre induction for control and exoerimental groups remaining higher than that of control groups post induction and treatment.

The SP% pre induction for control and experimental groups was 60.34% and 64.26% respectively.Post induction it decreased to 44.24% and 54.26% respectively.Post treatment it further decreased to 22.24% and 30.16%.
Pre induction the SP% for CONTROL SAL CONTROL APD, CONTROL PB, EXP SAL, EXP APD AND EXP PB was 56.82, 64.36, 61.26, 70.24, 64.36 and 56.28 respectively.

Post induction the SP% for CONTROL SAL CONTROL APD, CONTROL PB, EXP SAL EXP APD, EXP PB was 30.26, 47.94, 66.86,56.84, 54.86, 50.72 and 60.84 % respectively.Post treatment SP% for CONTROL SAL, CONTROL APD, CONTROL PB, EXP SAL, EXP APD, EXP PB was 10.12, 34.48, 38.24, 27.28, 22.76, 45.86 respectively.No statistical significance was noted between groups for sucrose preference.Post induction it decreased ..The post treatment is decreased

Biochemical Analysis
The DA concentration in the PFC was higher in both the control and experimental groups which received RIS and ethanol extract of leaf of Piper betle Linn, with a statistical significant difference noted between the Exp [SAL] and Exp PB groups ($p<0.001$).The DA concentration in the PFC was also higher in both the control and experimental groups which received RIS and the Piper betle Linn extract with a statistical significance difference noted between the Exp [SAL] and Exp PB groups.The DA concentration in the VTA was also higher in the control and experimental groups which received RIS and the Piper betle extract.

The ACh concentration in the PFC was higher in the PFC was higher in the PFC was higher in the control groups which received RIS and Piper betle extract .

With a statistical significance noted between Ctrls and Ctrl [p-0.028] .
There was no difference noted for ACh concentration on the PFC in the experimental groups.
There was also no difference noted in the Ach concentration between all treatment groups. However , this concentration was Lower in the Exp group compared to all other groups.

The study suggests that RIS and Piper betle extract increased the levels of DA .The negative and cognitive symptoms of schizophrenia are associated with dopaminergic hypofunction .
The control groups which received RIS and Piper betle extract had higher Ach concentrations compared to the control groups.However no difference in concentration were noted in the experimental groups.
This suggests that RIS and Piper betle extract are capable of increasing Ach release.

DISCUSSION
The modelling of a complete symptomatic spectrum of psychiatric disorders 22,23,24,25s in animals remains a challenge for researchers.

Hence , animal models have been extremely important and used to enhance understanding of human pathophysiology of psychiatric diseases. This is for proper understanding of the mechanism and possible treatment with herbal medicinal plants to reduce the side effects.Geyer and Markao26 in 1995 highlighted about these significances.The lack of of higher mental status and integrity of animals are limitations of animal models with simple brain cortical structure .

In this study sub-chronic administration of ketamine was part of production of a schizophrenia model

The control groups in the study showed decreased weight gained in post induction incidenceswhich acan be explained by use of scruff restraint for peritoneal injection which is categorised asa mild stressor.

The administration of ketamine led to social withdrawal effects on the experimental groups in the current study, indicating a successful model for asociality27,28,29.Control groups had been reported to show social withdrawal symptoms , which were not as significant as those in the experimental groups , which might have been induced by the mentioned mild stress condition caused due to scruff restraint.

Social isolation could be another contributing factor to the change in behaviour observed in the control groups.
Post treatment , the significance is as follows.A major reverse in social withdrawal was noted in the experimental groups which received the RIS or Risperidone and extract of Piper betle Linn .30,31

The control groups did not show improvement in social withdrawal post treatment.
Pre induction all animals showed preference for social novelty.
The time spent interacting with the species called STRANGER 2 significantly decreased for the experimental groups post induction.

The time spent interacting with STRANGER 1 also decreased post induction .
The post treatment explanation is as follows.After treatment , significant increase in the prefernce for social novelty had been observed for the experimental groups which received RIS or Resperidone and extract of Piper betle Linn.
Antipsychotics32 like clozapine have also been reported to reverse deficits of social novelty discrimination in a study in Literature survey.33,34,35,36

Social withdraw from stranger 2 or social novelty improved post treatment for the control grouops which received RIS or Resperidone and extract of Piper betle.37

And the control group which received saline showed no improvement in social novelty.

Post induction , sucrose preference for all groups decreased with the SP% for experimental groups being higher compared to control groups.

This suggests that the groups which received ketamine were less anhedonic compared to the rats which did not receive ketamine .Sucrose preference continued decreasing for all groups post control and experimental groups which received extract of Piper betle had been shown to be higher compared to other groups and the sucrose preference percentage for Resperidone group was only higher in controls.

This indicated that the herbal plant extract viz Ethanolic extract of leaf of Piper betle Linn had a slight but significant potential to decreasing anhedonia which is a novel function.The pathway of chronic stress in animal models simultaneously corresponds to continuous decrease in sucrose preference.This explains the continued decrease in sucrose preference post induction and post treatment all groups , as all animals in present study were exposed to some levels of chronic stress, contributed to by isolation and handling during the induction. By reproducing the disorders to understand their etio pathology and study the effects of new treatments.

CONCLUSION

The study provides evidence that administration of ketamine induces deficits in sociability and social novelty.the current study also provided evidence of the antipsychotic potential of herbal extract of leaf of Piper betle Linn as animals treated with ethanol extract of leaf of Piper beetle Linn reversed social and novelty deficits caused by ketamine .

Thus leaf of Piper betle Linn in ethanol extract form can be considered as a herbal antipsychotic drug in experimental animal model and further studies in clinical scenario is encouraged.

Conflict of interests-The authors declare that there is no conflict of interests..

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