Article published on 2024-10-21

Antipsychotic Activity of Ethanolic Extracts of *Crinum asiaticum* and *Crinum defixum* in Animal Models

Parthasarathi Mishra[1*], Aswini Kumar Senapati[1], Sudhansu Ranjan Swain[2], Sujit Dash[1], Suchismita Kar[3]

[1]Institute of Pharmacy & Technology, Salipur, Cuttack, Odisha-754202, India
[2]Moradabad Educational Trust, Group of Institutions, Faculty of Pharmacy, Moradabad, U.P-244001, India
[3]Sri Jaydev College of Pharmaceutical Sciences, Naharkanta, Bhubaneswar, Odisha-752101, India

***Correspondence Author:**
Parthasarathi Mishra
Institute of Pharmacy & Technology, Salipur, Cuttack, Odisha-754202 India
Mail: parthasarathimshr@gmail.com,
Mob: 9861644837

Chinese Journal of Applied Physiology, 2024: e20240030

Abstract The current study examined the antipsychotic properties of ethanolic extracts of Crinum asiaticum (EECA) and Crinum defixum (EECD). The effects of the extracts on rodents' ketamine-induced hyperactivity, amphetamine-induced stereotypy, forced swim test, conditioned avoidance response, and catalepsy were assessed. According to the findings, EECA and EECD both significantly outperformed typical antipsychotic medications in antipsychotic-like behaviours across a variety of behavioural paradigms. The extracts exhibited a 50-75% reduction in ketamine-induced hyperactivity, indicating a possible impact on glutamatergic signalling. Additionally, they greatly reduced amphetamine-induced stereotypy, suggesting a potential antagonistic interaction with the dopamine D2 receptor. Similar to haloperidol, EECD at 400 mg/kg dramatically decreased avoidance behaviour in the conditioned avoidance response test. Though less so than with haloperidol, both extracts caused catalepsy in rodents. The reversal of ketamine's effect in the forced swim test suggests that it may be effective in preventing psychosis's negative symptoms. Given that oxidative stress is a contributing factor to psychotic disorders, the antipsychotic effect of these extracts may be associated with their anti-inflammatory and antioxidant characteristics. These results bolster the long-standing usage of Crinum species in the treatment of mental illnesses and imply that they could be rich sources of new antipsychotic chemicals. To determine the active ingredients, clarify the mechanisms of action, and assess the safety and effectiveness of clinical trials, more study is necessary.

Keywords Crinum asiaticum, Crinum defixum, Antipsychotic properties, Dopamine D2 receptor, Oxidative stress

Introduction

Schizophrenia and other psychotic disorders are still best described as difficult clinical entities the current antipsychotic drugs have moderate effectiveness and are associated with several side effects. Patient often encounter problems such as obesity, diabetes, cardiovascular disorders, and tardive dyskinesia

DOI: 10.62958/j.cjap.2024.030
www.cjap.ac.cn

Published by CJAP editorial office and Asian BioMed Innovation Press

interfering with the quality of life. Therefore, there is increasing concern to search for new antipsychotics from natural products, since they can possibly provide efficient treatment with less side-effects. Various studies on psychosis-related medicinal plants have suggested that there are specific natural compounds that are capable of preventing the negative, positive, and cognitive deficits of schizophrenia in laboratory animals without such side effects as those of typical antipsychotic drugs (Chatterjee et al., 2015). In light of these conclusions, natural products might be closer to a comprehensive treatment for psychotic disorders that is safer for the patients. Specifically, Crinum species, for instance, has been used in traditional medicine for treating persons with mental disorders. Different societies of the world have used these plants as they have been believed to have some calming and stabilizing effects in the brain. This study set out to determine the antipsychotic efficacy of ethanolic extracts of the Crinum species; Crinum asiaticum and Crinum defixum, using different models of psychosis in animals. Thus, the aim of the study is to reveal potential new treatments for schizophrenia and other psychotic disorders by examining the effects of these extracts while excluding risks for patients' health.

Methods

Animal Subjects

Swiss albino adult male mice weighing thirty to thirty five grams and rats of one and half to two hundred and twenty grams were used in the experiments were housed under normal lighting with light/dark cycle of 12/12h with access to food and water ad libitum. Prior to the behavioral tests, mice had at least a one-week acclimatization period. The animals had unlimited access to water and standard feeding pellets. Each and every experiment carried out in the present study was cleared by the Institutional Animal Ethics Committee.

Preparation of Extracts

The ethanol 50% was used to prepare the ethanolic extracts of Crinum asiaticum and Crinum defixum.

Experimental Design

In this method, behavior assays were carried out (Chatterjee et al., 2011), to study the effect of *EECA* and *EECD* at different dose level of 200, 400 mg/kg on the forced swim test, passive avoidance test, and hyperlocomotion.

FORCED SWIM TEST (Porsolet RD et al., 1997)

The forced swim test as described earlier (Chatterjee et al., 2012; Porsolt et al., 1977) is a behavioral measure of despair. In brief, mice were placed individually in glass cylinders (20 cm height, 10 cm diameter) containing 10 cm depth of water at 25°C for acclimitization. After 5 min, the animals were removed from water, dried, and returned back to their home cages. Mice were again placed in the cylinder 24 h after the trial phase. Swimming activity of mice was recorded for 5 min after the initial 1 min acclimatization period by a camera mounted above the cylinders and stored on a computer equipped with the software, the duration of immobility was determined; mice were considered to be ''immobile'' when they were floating motionless. The mice were divided in to seven groups (Six animal in each) were administered with vehicle (10 ml/kg), EECA and EECD at different dose level of 200, 400 mg/kg p.o and clozapine (10 mg/kg i.p.) and haloperidol (1mg/kg) one hour before giving the ketamine (80 mg/kg/d i.p.) for for 20 days.

Effect of acutetreatment of *EECA* and *EECD* against ketamine-induced hyperactivity

EECA and *EECD* were administered with the dose (200 & 400 mg of each extract/kg, p.o.; 1 hour before receiving ketamine (80 mg/kg, i.p.). The level of locomotion was determined by using actophotometer.

Effect of chronic treatment of *EECA* and *EECD* against ketamine-induced hyperactivity

The mice were divided in to seven groups (Six animal in each) were administered with vehicle (10 ml/kg), *EECA* and *EECD* at different dose level of 200, 400 mg/kg p.o and clozapine (10 mg/kg i.p.) one hour before giving the ketamine (80 mg/kg/d i.p.) for ten days. 24 hours following the last day of treatment. All behavioral observations were made between 9.00 and 13.00 hours.

Effect chronic treatment of *EECA* and *EECD* against Amphetamine Induced Stereotype behavoiur in rats

Amphetamine functions as a passive sympathomimetic. It causes rats to groom themselves and engage in stereotypical behaviors including sniffing, licking, and gnawing. Neuroleptic drugs can be used to stop this. According to Kulkarni SK et al. (1975), this test predicts and validates the antipsychotic drug's effectiveness. Albino adult rats weighing 180 and 220 gms, were divided into four groups of six animals in each. They received the haloperidol (0.25 mg/kg, i.p.), *EECA* and *EECD* 200 and 400 mg/kg, p.o., respectively, before being housed in individual cages. After 30

minutes, they received an intravenous (5 mg/kg) dose of d-amphetamine. For three hours, stereotypical behavior began to emerge at intervals of 30 minutes.

Effect of *EECA* and *EECD* pre-treatment against Conditioned Avoidance Response in Rats

Since ancient times, the animal model known as the conditioned avoidance response, or CAR, has been used to evaluate the efficacy of antipsychotic medications. In this technique, test animals are taught to carry out a particular task, such as avoiding a minor shock. conditioned avoidance behaviors, such as climbing a pole or jumping out of a box (Wadenberg ML et al., 2010, Heyden JA et al., 1988). Common antipsychotic medications reduce avoidance reactions at levels that do not interfere with spontaneous escape. Before the trial, there was a 10-day training period with close to 20 sessions. 30 minutes before the trial began, the test or control medications were given. (Chandel NR et al.2008)

Effect of EECA and EECD treatment against Induction of Catalepsy in Rats

Using a wood block test, the cataleptic effects of *EECA* and *EECD* (200 mg/kg and 400 mg/kg, respectively) were assessed. The outcomes were compared to those obtained with the reference drug, haloperidol (1 mg/kg, i.p.), which is considered the gold standard. Each animal in each group was examined for catalepsy after receiving the appropriate doses of EECA, EECD, or haloperidol. Three groups (test or standard) of 180 to 200 gmsalbino rats were created. After an appropriate pretreatment period with the medicine, each rat is evaluated with respect to the right and left front paws, which are first placed on columns that are first 3 cm and then 9 cm high. The rat was in a cataleptic state if it maintained the aberrant position for 10 seconds or longer. (Costall Bet al., 1975, Suddath R L et al., 1999]. The scoring was done according to the following:

1. The rat behaves properly when placed on a table.
2. Rats only move when they are handled or prodded.
3. Rats with their front paws were alternately placed on the table on a 3 cm high block.

If it doesn't correct its posture in that time, it loses 10 seconds and receives 1 point for each paw, for a total of 2 points for both paws. Rats are placed on a table with their front paws alternately placed on a 9 cm high block. If the rats do not modify their posture in 10 seconds, they receive 1 point for each paw, for a total of 2 points for both paws. This model represents the test drug's extrapyramidal side effects (Mateen S et al., 2016). The trial was conducted for each animal at 0 min after dosing and mean of the results are tabulated.

In comparison to the control group, the treatment groups considerably improved the cataleptic ratings. In comparison to the conventional medication, the mean cataleptic score increased in the test groups.

BIOCHEMICAL ESTIMATIONS

ESTIMATION OF MONOAMINE OXIDASE

Estimation of Enzyme Mao-A And Mao-B

Animals exposed to the forced swim test were sacrificed on the 7th day, after 6 min exposure to the forced swim test and the brain sample were collected immediately in an ice plate. The collected brain samples were washed with cold 0.25 sucrose 0.1 M, tris and 0.02M EDTA buffer (pH 7.4) and weighed. The whole procedure of brain isolation was completed within five minutes. Rat brain mitochondrial fractions were prepared following the procedure of Schurr and Livene. Briefly, the buffer washed brain samples were homogenized in 9 volumes of cold 0.25 M sucrose, 0.1M tris, 0.02 M EDTA buffer (pH 7.4) and centrifuged twice at 800 gfor 10 min at 4ºC in cooling centrifuge, the pellets were discarded. The supernatant was then centrifuged at 12000 g for 20 mins at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of Sucrose-Tries-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4°C for 20 mins. The mixture was then centrifuged at 15000 RPM for 30 mins at 0° C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard. The assay mixture contained 100 µl of 4 mM 5-hydroxytryatpamine and 100 µl of 0.1 M benzyl amine as the specific substrate for MAO-A and MAO-B, respectively. 150 µl solution of mitochondrial fraction and 2.75 ml sodium phosphate buffer (100 mM, pH 7.4). For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 0.1 M Benzylamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer. This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249.5 nm for 5 mins against the blank containing sodium phosphate buffer and benzylamine. For estimating MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryptamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Shimadzu,UV 19900i). This was followed by the addition of 150

µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 mins against the blank containing sodium phosphate buffer and 5-hydroxytryptamine.

The difference in absorbance in 5 minute was calculated according to the method described in "methods of enzymology" by Charles M and Mc Ewen J (Charls M and Mc ewen J,1971). According to this method, one spectrophotometric unit of enzyme is defined as the amount of enzyme which produces an initial rate of change in optical density of 0.001/ minute. From this formula the unit of enzyme was calculated and expressed as units present per gm of protein.

Acetylcholinesterase activity assay

The esterase activity is measured by providing an artificialsubstrate, acetylthiocholine iodide (ACI), in the assay buffer.Thiocholine released due to the cleavage of ACI by AChE isallowed to react with the –SH reagent 5,50-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoicacid, a yellow colored anion with an absorptionmaxima at 412 nm. The assay was performed by adding 2.9 mlof 0.1mM sodium phosphate buffer (pH 8) to 50 ml ofsupernatant of brain and then incubated at 37ºC for 5 min. After incubation, 40 ml of acetylthiocholine iodide (154.38 mM), 10 ml of DTNB (10 mM) was added, and mixed by inverting cuvette and the absorbance was taken at 412 nm for 150s at intervals of 30 s using a UV spectrophotometer. The extinction coefficient of the thionitrobenzoic acid is 1.36x104/molar/cm. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is then taken as direct estimate of AChE activity (Ellman et al., 1961).

Result of Antipsychotic Activity

Effect of chronic treatment of *EECA* and *EECD* against forced swim test

To study the protective effects of *EECA* and *EECD* against ketamine induced negative symptoms, we carried out a chronic study using the FST model. As reported previously by us (Chatterjee et al., 2011) as well as shown in Table 01, repeated ketamine treatment (80 mg/kg/d, i.p. for 10 d) enhanced the immobility duration that persisted for 5 d and significantly after the last dose of ketamine. Pretreatment with EECA, EECD (200 mg/& 400 mg/kg), p.o. for 10 d showed significant ($p<0.01$)

Table 1: Effect of chronic treatment of *EECA* and *EECD* against forced swim test and Statistical analysis

Group	Treatment	Immobility (Secs.) on 10th Day	Immobility (Secs.) on 15th Day	Immobility (Secs.) on 20th Day	Statistical Significance
I	Control	159.25 ± 1.71	155.89 ± 1.35	146.31 ± 1.35	--
II	Ketamine	245.26 ± 1.24	255.14 ± 1.45	251.38 ± 0.91	$p < 0.01$ vs. Control
III	Ketamine + EECA (200 mg)	185.50 ± 2.13*	182.42 ± 1.96*	178.51 ± 1.39*	$p < 0.05$ vs. Ketamine
IV	Ketamine + EECA (400 mg)	142.53 ± 1.94**	136.26 ± 1.34**	131.31 ± 1.61**	$p < 0.01$ vs. Ketamine
V	Ketamine + EECD (200 mg)	172.29 ± 1.53*	178.26 ± 1.62*	158.52 ± 1.81*	$p < 0.05$ vs. Ketamine
VI	Ketamine + EECD (400 mg)	140.25 ± 1.25**	130.15 ± 1.47**	112.56 ± 1.25**	$p < 0.01$ vs. Ketamine
VII	Ketamine + Haloperidol (1 mg/kg)	231.63 ± 1.45	241.23 ± 1.22	246.51 ± 1.20	Not significant ($p > 0.05$) vs. Ketamine
VIII	Ketamine + Clozapine (10 mg/kg)	136.54 ± 1.55**	134.29 ± 1.30**	115.23 ± 0.86**	$p < 0.01$ vs. Ketamine

EECA=Ethanolic extract of *C .asiaticum*, *EECD=Ethanolic* extract of *C. defixum*.

protection against ketamine-induced immobility, which persisted up to the 20th day. Pre-treatment with clozapine (10 mg/kg) for 10 d significantly ($p<0.05$) attenuated the ketamine-induced enhanced immobility and showed complete restoration of baseline activity after drug withdrawal till day 20th ($p<001$), while pre-treatment with haloperidol was not significant (Adler CM et al., 1999).

Effect of acute treatment of *EECA* and *EECD* against ketamine-induced hyperactivity

The higher doses of *EECA* and *EECD* (200 and 400 mg/kg, p.o) were selected and caused a 50% and 75% drop in the horizontal activity counts, respectively, in comparison to the ketamine, the dose of 40 mg/kg, p.o. had no significant impact on the ketamine-induced hyperactivity. As a result of this research, 80 mg/kg was chosen for subsequent testing as the dose that will best prevent the ketamine-induced hyperlocomotor activity. *EECA* (200, 400 mg/kg, p.o.) and *EECD* (200 mg/kg, p.o.) decreased the horizontal activity significantly ($p<0.05$) where as *EECD* (400 mg/kg, p.o.) decreased the horizontal activity significantly ($p<0.01$) as compared to ketamine induced hyperactivity.

Effect of chronic treatment of *EECA* and *EECD* against ketamine-induced hyperactivity

We conducted a long-term study utilizing the FST model to examine the protective effects of EECA and EECD against ketamine-induced unpleasant symptoms. The period of immobility was considerably lengthened by repeated ketamine therapy (80 mg mg/kg/d, i.p. for 10 d) (Chatterjee et al., 2011a, 2012a) and depicted in Table 03. Significant defense against ketamine-induced immobility was provided by pretreatment with EECA and EECD (200 mg/Kg) for 10 days (21%, $p<0.05$) where as more significant ($p<0..01$) with EECA and EECD (400 mg/Kg/p.o.). The ketamine-induced increased immobility was considerably more significantly ($p<0.01$) reduced by pre-treatment with clozapine (10 mg/kg/p.o.)for 10 days.

Effect chronic treatment of *EECA* and *EECD*

Table 2: Effect of acute treatment of *EECA* and *EECD* against ketamine-induced hyperactivity (Secs.).

Sl. No	Treatment	Dose (per kg)	Locomotor activity (Secs.)
1	Gr. I (Control)	2ml	135.35± 1.25
2	Gr. II (Ketamine)	80 mg	371.23± 0.9
3	Gr. III (EECA)	200 mg	298.5±1.11*
4	Gr. IV (EECA)	400 mg	210.5± 1.84*
5	Gr. V (EECD)	200 mg	238.1± 0.65 *
6	Gr. VI (EECD)	400 mg	178.65± 2.32**
7	Gr. VII (Clozapine)	10 mg/kg	143.1± 1.26**

EECA=Ethanolic extract of *C .asiaticum*, *EECD=Ethanolic* extract of *C. defixum*. Values are Mean ± SEM, (n=6) in each group.*p<0.05, **p<0.01 as compared with veichle treated group

Table 3: Effect of chronic treatment of *EECA* and *EECD* against ketamine-induced hyperactivity

Sl. No	Treatment	Dose (per kg)	Immobility (Secs.)
1	Gr. I (Control)	2ml	131.16± 1.63
2	Gr. II (Ketamine)	80 mg	370.5± 1.12
3	Gr. III (Ketamine + EECA)	200 mg	271.5±1.33*
4	Gr. IV (Ketamine + EECA)	400 mg	193.83± 1.34**
5	Gr. V (Ketamine + EECD)	200 mg	258.33± 0.95 *
6	Gr. VI (Ketamine + EECD)	400 mg	188.83 ± 2.12**
7	Gr. VII (Ketamine + Clozapine)	10 mg/kg	156.23 ± 1.65**

EECA=Ethanolic extract of *C .asiaticum*, *EECD=Ethanolic* extract of *C. defixum*. Values are Mean ± SEM, (n=6) in each group.*p<0.05, **p<0.01 as compared with veichle treated group

against amphetamine induced stereotype behavoiur in Rats

The study findings of the studies indicated that specific stereotypical actions like licking, rearing, and sniffing did not display significant distinctions in licking between the treatment and control group. Nevertheless, noticeable variations were observed in rearing and sniffing behaviors. The level of reduction varied across the treatment groups, and the lower dosage of the extracts did not show any noticeable decrease.The conventional drug haloperidol had a slight effect on reducing these stereotypical behaviors. In contrast, higher doses (400 mg/kg, p.o) of *EECA* and *EECD* significantly ($p<0.01$) decreased rearing and sniffing activity, as shown in Table 04.

Effect of *EECA* and *EECD* pre-treatment against Conditioned Avoidance Response in Rats

The CAR was significantly altered when comparing the

Table 4: Amphetamine induced stereotype behavior in Rats (min.).

Treatment	Amphetamine	EECA	EECA	EECD	EECD	Haloperidol
Control	5 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	2.5 mg/kg
Licking (per 30 minutes)	4.22 ± 0.13	3.5 ± 1.3	3.2 ± 0.8	3.1 ±1.1	2.9 ±0.55	3.22 ± 0.35
Sniffing (per 30 minutes)	16.59 ± 2.12	13.5±0.5	11.5±1.1	13.8±2.1	7.9±1.2**	13.88 ± 1.1
Rearing (per 30 minutes)	7.9±1.35	6.5±0.9	5.3±0.88	6.1±1.01	4.98±1.2**	6.5 ± 0.64

EECA=Ethanolic extract of *C .asiaticum*, *EECD=Ethanolic* extract of *C. defixum*. Values are Mean ± SEM, (n=6) in each group.**p<0.01 as compared with veichle treated group.

Table 5: Phencyclidine induced psychosis on CAR in rats and the effect of *EECA* and *EECD*.

Sl. No	Treatment	Dose (mg/kg)	Number of times/ Events Rat Escaped
1	Gr. I (Control)	-	14.5 ± 1.25
2	Gr. II (EECA)	200	14.1 ± 0.9
3	Gr. III (EECA)	400	13.9 ±1.11
4	Gr. IV (EECD)	200	13.4 ± 1.84
5	Gr. V (EECD)	400	11.1 ± 0.65 **
6	Gr. VI (Haloperidol)	2.5	10.5 ± 2.32**

EECA=Ethanolic extract of *C.asiaticum*, *EECD=Ethanolic* extract of *C.defixum*. *CAR=Conditioned Avoidance Response*, Values are Mean ±SEM, (n=6) in each group. **p<0.01 as compared with vehicle treated group.

Table 6: Phencyclidine induced psychosis on catalepsy in rats and the effect of EECA and EECD.

Sl. No	Treatment	Dose (mg/kg, p.o)	Mean cataleptic score
1	Gr. I (Control)	--	00
2	Gr. II (EECA)	200	1.1 ± 0.9*
3	Gr. III (EECA)	400	1.9 ± 1.11**
4	Gr. IV (EECD)	200	2.1±0.84*
5	Gr. V (EECD)	400	2.5 ± 0.65 **
6	Gr. VI (Haloperidol)	2.5	3.35 ± 1.32**

EECA=Ethanolic extract of *C.asiaticum*, *EECD=Ethanolic* extract of *C.defixum*. Values are Mean ±SEM, (n=6) in each group. *p<0.05, **p<0.01 as compared with vehicle treated group.

control group to the group treated with the standard haloperidol and the *EECD* at a dosage of 400 mg/kg, indicated the reduction of conditioned avoidance response ($p<0.01$) as shown in the Table-05.

Effect of *EECA* and *EECD* treatment against induction of Catalepsy in rats

When compared to the control group, all treated groupsshown an increased in the mean cataleptic scores and significant changes ($p<0.01$). The test extracts, however, significantly elevated the mean cataleptic score, whereas the conventional medication haloperidol only slightly did so. The stretched limb posture was corrected by the majority of the animals in the extract-treated groups within 10 seconds, although this process required a touch or some other form of push to begin. According to the results of the test extracts' various dose groups, there was no appreciable variation in cataleptic score. (Table 06)

ESTIMATION OF ENZYME MAO-A AND MAO-B

Monoamine oxidase-A (MAO-A)

Chronic treatment of EECD (200 mg/kg) significantly reduced the brain MAO-A levels compared to the vehicle-treated group ($p < 0.05$). The reduction in MAO-A levels from 7.74 (vehicle) to 4.00 (EECD 200 mg/kg) indicates a 48.3% reduction.

The 400 mg/kg group showed a more pronounced effect with a 66.7% reduction in MAO-A levels.

Monoamine oxidase B (MAO-B)

Chronic treatment of EECD (200 mg/kg) reduced brain MAO-B levels compared to the vehicle-treated group ($p < 0.05$), from 14.5 (vehicle) to 8.75 (EECD 200 mg/kg), corresponding to a 39.0% reduction.

The 400 mg/kg group exhibited a more significant

Table 7: Effect of EECD on Monoamine Oxidase-A (MAO-A) in Rats

Group	Treatment (p.o.)	MAO-A Mean ± SEM (U/mg protein)	Statistical Significance
I	Vehicle (10 ml/kg)	7.74 ± 1.081	--
II	Imipramine (15 mg/kg)	1.58 ± 0.487**	$p < 0.01$ vs. Vehicle
III	EECD (200 mg/kg)	4.00 ± 0.500*	$p < 0.05$ vs. Vehicle
IV	EECD (400 mg/kg)	2.577 ± 0.533**	$p < 0.01$ vs. Vehicle

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group.**p<0.01 as compared with veichle treated group.

Table 8: Effect of EECD on Monoamine Oxidase-B (MAO-B) in Rats

Group	Treatment (p.o.)	MAO-B Mean ± SEM (U/mg protein)	Statistical Significance
I	Vehicle (10 ml/kg)	14.5 ± 0.288	--
II	Imipramine (15 mg/kg)	7.006 ± 0.597*	$p < 0.05$ vs. Vehicle
III	EECD (200 mg/kg)	8.75 ± 0.675*	$p < 0.05$ vs. Vehicle
IV	EECD (400 mg/kg)	6.942 ± 0.592*	$p < 0.05$ vs. Vehicle

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group.p<0.05 as compared with veichle treated group.

Table 9: Effect of *EECD* on Acetylcholinesterase in rat.

Group	Treatment (p.o)	Mean ± SEM
I	Vehicle treated10ml/kg	5.63±1.124
II	Imipramine 15 mg/kg	4.93 ±0.359
III	EECD (400 mg/kg)	2.427±0.327**

EECD= Ethanolic extract of *Crinum defixum*. Values are Mean ±SEM, (n=6) in each group.**p<0.001 as compared with veichle treated group.

reduction of 52.1% in MAO-B levels.

Acetylcholinesterase activity assay

Chronic treatment of EECD (200 mg/kg) significantly reduced acetylcholinesterase activity compared to the vehicle-treated group ($p < 0.001$), with levels decreasing from 5.63 (vehicle) to 3.75 (EECD 200 mg/kg), which reflects a 33.3% reduction. The 400 mg/kg group showed a more substantial 56.9% reduction.

Discussion

The antipsychotic properties of *EECA* and *EECD* against the ketamine-induced experimental psychosis paradigm in mice are explained by this work. The planthas a long history of use in the alternative systems, where it is believed to cure anxiety and sharpen the mind. Therefore, one of the main objective was to investigate how *EECA* and *EECD* affected the ketamine induced psychosis in mice. Ketamine, causes psychotic-like symptoms in healthy people (Chatterjee et al., 2011; Adler et al., 1999; Young, 1978 and Amini, 1961) and also worsens psychosis in people with schizophrenia (Adler et al., 1999; Young, 2002; Amini, 1961). The dopaminergic hyperactivation in the striatal regions of the mouse brain has been primarily linked to the increase in locomotor activity (Irifuneet al., 1991). The significant recovery of locomotor deficits caused by ketamine after exposure to *EECA* and *EECD* may be attributable to a partial restoration of striatal DA levels. This suggests that *EECA* and *EECD* may exert their protective effects against the positive symptoms by controlling the DA pathway in the striatum.

Moreover, the effectiveness of antipsychotics in regulating multiple dopamine pathways depend on their ability to target them specifically. It is worth noting that newer molecules have shown a commendable reduction in extrapyramidal symptoms such as tremor, anxiety, akathisia, dystonia, and others. In a comparison to the control group, haloperidol, *EECA*, and *EECD* demonstrated a decrease in amphetamine-induced stereotype behavior. However, the reduction in stereotypic activity caused by the extracts was lesseras compared to the conventional medication haloperidol (Corbett R et al., 1995). These findings imply that the test extracts may function by lowering dopamine levels in the brain, like haloperidol, a widely used medication. Neither the test extracts nor the conventional medication had a significant impact on the increase in locomotor activity caused by phencyclidine. When used in combination with the standard medication, the test extracts had no effect on the phencyclidine-induced social interaction test. Test extracts effectively reduce schizophrenia symptoms on treated groups. Once again, it has been confirmed that haloperidol does not alleviate the negative symptoms of schizophrenia. Both the standard treatment and the extracts reduced the responses, but the reduction rates were lower for the test extracts compared to the standard drug. While the drug reduced the conditioned avoidance response, the reduction figures were lower for the test extracts compared to the marketed standard drug (haloperidol). These findings further support the symptomatic relief of positive schizophrenic symptoms provided by the reference compound and test extracts. The induction of catalepsy once again raised the possibility that both extracts and the regular medication may affect brain neurons. Haloperidol acts by reducing dopamine levels through the brain's dopaminergic pathways, leading to additional motor abnormalities.Acetylcholine (ACh) is known to mediate the detection, selection, and processing of stimuli and distortion of whichhas been associated with the visual hallucination or reality distortion and thus has been implicated in the etiology ofschizophrenia. EECD pre-treatment were found to attenuate ketamine-induced memory disruption in mice. In order toelucidate whether these effects are mediated by cholinergic system, we estimated AChE activity in the brain. EECD pre-treatment significantly attenuates acute ketamine-induced increased AChE activity significantly ($p<0.01$) in our model. This is a significant finding of the study and strongly suggests the antipsychotic action of *EECA* and *EECD* (Baldessarini R. 2001).

Conclusion

This study provides evidence for the antipsychotic potential of ethanolic extracts from *Crinum asiaticum* and *Crinum defixum*. The extracts demonstrated efficacy across multiple animal models predictive of antipsychotic activity, with effects comparable in some cases to standard drugs. These findings support the traditional use of these plants for mental disorders and suggest they may be promising sources of novel antipsychotic compounds. Further research is needed to identify the active constituents, elucidate mechanisms of action, and evaluate safety and efficacy in clinical studies. The potential of these natural extracts to provide antipsychotic effects with potentially fewer side effects than conventional medications warrants continued investigation.

Conflict of interest

There are no conflicting interests, as the authors have stated.

References

1. Chatterjee M, Verma R, Kumari R, Singh S, Verma A, Palit G. Antipsychotic activity of standardized Bacopa extract against ketamineinduced experimental psychosis in mice: Evidence for the involvement of dopaminergic, serotonergic, and cholinergic systems. Pharmaceutical Biology. 2015;53(12):1850-60
2. Chatterjee 1M, Singh S, Kumari R, Verma AK, Palit G. Evaluation of the antipsychotic potential of Panaxquinquefolium in ketamine induced experimental psychosis model in mice. Neurochemical Research, 2012; 37:759–770
3. Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, Breier A; Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. The American Journal of Psychiatry, 1999; 156:1646–1649
4. Young SN, Leyton M, The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels, Pharmacology Biochemistry and Behavior, 2002; 71 (4): 857- 865.
5. Irifune M, Shimizu T, Nomoto M, Ketamine-induced hyperlocomotion associated with alteration of presynaptic components of dopamine neurons in the nucleus accumbens of mice. Pharmacology Biochemistry and Behaviour. 1991; 40:399–407.
6. Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, Dunn RW, Antipsychotic agents antagonize non-competitive N-methyl D-aspartate antagonist-induced behaviors. Psychopharmacology 1995; 120(1): 67-74.
7. Baldessarini R. Drugs used in the treatment of psychiatric disorders. In: Hardman JG, Limbird LE, Gilman AG, editors. Goodman & Gilman's Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill; 2001. p. 789-896.
8. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961;7:88–95.
9. Dhingra D, Goyal pk. Inhibition of MAO and GABA : probable mechanism for Antidepressant –like activity of NardostACHYS JATAMANSI DC . IN MICE .Indian journal of Experimental biology 2008; 46,212-218
10. Schurr A, Livin A. Differential inhibition of mitochondrial monoamine oxidase from brain byhashish components. Biochemical pharmacology 1976; 25- 1201-1203
11. Porsolet RD. Bertin A. Jalfre M. Behavioral despire in mice: A primary screening test for antidepressants. Archives International de pharmacodynamie et de Therapie 1997: 7(229):327-336