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A Comparative Nootropic Activity of *Crinum asiaticum* and *Crinum defixum* in Animal Models

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Abstract:

The current study examined the nootropic activity of ethanolic extracts of *Crinum asiaticum* (EECA) and *Crinum defixum* (EECD). The effects of the extracts on rodents' by using Elevated Plus Maze (EPM), scopolamine-induced amnesia (SIA), diazepam-induced amnesia (DIA), clonidine-induced (NA-mediated) hypothermia (CIH), lithium-induced (5-HT mediated) head twitches (LIH) and haloperidol-induced (DA-mediated) catalepsy (HIC) models. Piracetam was used as the standard drug. A significant increase in inflexion ratio (IR) was recorded in EPM, SIA and DIA models. A significant reversal effect was observed on rectal temperature in CIH model, reduction of head twitches in LIH models. However, no significant reduction in catalepsy scores in HIC models were observed with test extracts and standard piracetam. The results indicate that nootropic activity observed with EECA and EECD could be through improved learning and memory either by augmenting the noradrenaline (NA) transmission or by interfering with 5-hydroxytryptamine (5-HT) release. Further, the extracts neither facilitated nor blocked release of the dopamine (DA). Thus EECA and EECD elicited significant nootropic effect in mice and rats by interacting with cholinergic, GABAergic, adrenergic and serotonergic systems. Phytoconstituents like flavonoids had been reported for their nootropic effect and these are present in both ethanolic extracts of *Crinum asiaticum* (EECA) and *Crinum defixum* (EECD) and these active principles may be responsible for nootropic activity.

Keywords: *Crinum asiaticum*, *Crinum defixum*, Nootropic activity, Alzheimer's disease, piracetam, scopolamine

Introduction

Chronic brain illness, the root cause of millions of mental and behavioural problems worldwide, affects cognitive skills such as memory and comprehension [1]. Cholinergic dysfunction and tau-protein anomalies characterise Alzheimer's disease (AD), a leading cause of dementia. Synthetic medications that alleviate AD symptoms, such as donepezil and rivastigmine, come with side effects and problems with bioavailability [2]. Because of this, people are looking at herbal remedies for memory loss and mental health issues, such as Crinum asiaticum and Crinum defixum. This research assesses the impact on learning and memory of ethanolic extracts of Curcuma asiaticum (EECA) and Curcuma defixum (EECD).

Materials and Methods

Fresh whole plants of Crinum asiaticum and Crinum defixum, collected and authenticated from Salipur, Cuttack, Odisha, were defatted with petroleum ether and extracted with 50% ethanol using Soxhlet apparatus. The concentrated extracts were prepared for pharmacological tests by suspending in 3% Tween-80 saline to obtain doses of 200 and 400 mg/kg, while control groups received Tween-80 (2 mL/kg). Male Swiss albino mice (18–22 g) and rats (150–200 g), housed under standard conditions and approved protocols **(IAEC 1053/PO/Re/S/07/CPSCSEA),** were used for the study, with test samples administered orally. Standard drugs included piracetam, scopolamine, diazepam, clonidine, lithium carbonate, amitriptyline, and haloperidol.

Acute toxicity (LD_{50}) determination

Acute toxicity of Crinum asiaticum (EECA) and Crinum defixum (EECD) extracts was assessed in albino mice (16–25 g) using the OECD 425 up-and-down method. Following a single-dose administration, no mortality was observed within 48 hours, establishing the LD50 as >2000 mg/kg for both extracts [3].

Pharmacological activity study

Study evaluated nootropic effects of ethanolic extracts of Crinum asiaticum (EECA) and Crinum defixum (EECD) using various learning and memory models, including Y-maze, Elevated Plus Maze, Morris Water Maze, and scopolamine- and diazepam-induced amnesia in rats. Behavioural studies were conducted using clonidine-induced hypothermia (NA-mediated), lithium-induced head twitches (5-HT mediated), and haloperidol-induced catalepsy (DA-mediated). Additionally, acetylcholinesterase enzyme levels were estimated to explore the extracts' potential mechanisms [4].

Models used in laboratories to assess memory and learning:

EECA and EECD's effects on rats Y-maze task

In this study, seven groups of six rats each were used to assess memory enhancement using the Y-maze exercise. Each individual was placed into one of seven groups: Normal saline was given to I, which acted as a control; scopolamine 1 mg/kg i.p. was given to II, a negative control; piracetam 200 mg/kg i.p. + scopolamine 1 mg/kg i.p. was given to III, the standard treatment; EECA 200 and 400 mg/kg were given to IV and V, respectively; and EECD 200 and 400 mg/kg were given to VI and VII, respectively. We calculated the percentage of changes using the following formula [5] and utilised the rats' ability to transfer arms as a gauge of spontaneous change:

$$\text{Percentage alterations} = \frac{\text{No. of positive entry}}{\text{Total no. of arm entries} - 2} \text{x } 100$$

Effects of EECA and EECD on elevated plus maze in mice (Exteroceptivebehaviour model)

The effects of EECA and EECD on memory were assessed using the Elevated Plus Maze (EPM) in seven groups of mice, with six animals per group. Piracetam was administered at a dose of 200 mg/kg to Group III, whereas scopolamine was administered at a dose of 1 mg/kg to Group II and normal saline to Group I (the control). The following groups were given EECA and EECD, respectively, at doses of 200 and 400 mg/kg: IV, V, VI, and VII. The Inflexion Ratio (IR) was determined by recording the transfer latency (TL) on day 7, 90 minutes after the final dosage, the formula is IR = (L0 - L1) / L0, where L0 is the initial TL on day 1 and L1 is the TL on day 2 [6-8].

Effects of EECA and EECD on Scopolamine induced amnesia in rats (Interoceptive behaviour model):

The Elevated Plus Maze (EPM) was used to evaluate the effects of EECA and EECD on scopolamine-induced memory impairment in rats. For 14 days, seven groups of six mice each got daily treatments. Except for Group I (control), scopolamine (1 mg/kg) was given 60 minutes after the extract treatments [9]. On the 14th day (acquisition day) and the 15th day (retention day), transfer latency (TL) was measured using the EPM. Comparing the treated groups to the scopolamine-only group, the findings showed significant variations in TL and memory retention [10, 11].

Effects of EECA and EECD on Diazepam-induced amnesia (Interoceptive behaviour model)

This research aimed to examine how EECA and EECD affected the memory impairment that diazepam caused in mice. For seven days, seven groups of six animals each received treatment. Ninety minutes after the treatments, diazepam (5 mg/kg) was given on day 7, and transfer latency (TL) was noted [12]. A day later, memory retention was assessed. The standard approach was used to determine the Inflexion Ratio (IR). In addition to diazepam, the groups received treatment with piracetam (200 mg/kg), EECA (200 and 400 mg/kg), or EECD (200 and 400 mg/kg) [13].

Effects of EECA and EECD on Morris Water Maze in mice

To assess spatial memory, 42 male albino mice were trained in the Morris Water Maze and split into seven groups, each consisting of six animals [14]. The mice were taught to find a visible platform during the acquisition phase. The platform was immersed under the water's surface, and the water was rendered opaque during the retention phase. Normal saline was administered to Group I (control), scopolamine (1 mg/kg) to Group II, piracetam (400 mg/kg) to Group III, and EECA (200 mg/kg and 400 mg/kg) or EECD (200 mg/kg and 400 mg/kg) to Groups IV–VII. The distance travelled and the amount of time needed to locate the platform were noted [15].

Effects of EECA and EECD on rats' hypothermia caused by clonidine (NA-mediated)

The effects of EECA and EECD on clonidine-induced hypothermia were tested in seven groups of rats (6 animals/group, weighing 150–200 g). Distilled water (10 ml/kg) was given to Group I, clonidine (1 mg/kg) to Group II, piracetam (400 mg/kg) to Group III, and clonidine in combination with either EECA (200 mg/kg and 400 mg/kg) or EECD (200 mg/kg and 400 mg/kg) to Groups IV–VII. To determine if the therapies may reverse hypothermia, rectal temperatures were taken at 30-minute intervals after clonidine delivery [16, 17].

Impact of 5-HT-mediated lithium-induced rat head twitches

Rats weighing 150–200 g were divided into eight groups, and the effects of EECA and EECD on lithium-induced head twitches were assessed. Distilled water was administered to Group I, 190 mg/kg of lithium carbonate to Group II, 400 mg/kg of piracetam and lithium carbonate to Group III, and 200 and 400 mg/kg of EECA or EECD, followed by lithium carbonate, to Groups IV–VII. The number of head twitches was recorded 60 minutes after lithium was administered [18, 19].

Impact of DA-mediated (haloperidol-induced) catalepsy in rats

Eight rats (6 animals per group) were used to study the effects of EECA and EECD on catalepsy caused by haloperidol. Distilled water was given to Group I as a control, haloperidol (1 mg/kg) was given to Group II, piracetam (200 and 400 mg/kg) was given to Groups III-IV, and varying dosages of EECA (200 and 400 mg/kg) and EECD (200 and 400 mg/kg) were given to Groups V–VIII. The Bar Test measured the length of time the rats kept an enforced posture to evaluate catalepsy at various time intervals (0, 30, 60, 90, 120, 150, and 180 minutes) [21,19].

Rats' acetylcholinesterase levels in relation to EECA and EECD

This research measured acetylcholinesterase activity in rats given extracts, saline, scopolamine, or piracetam. After being dissected, the brains were mixed together in a phosphate buffer. The change in absorbance at 412 nm after the addition of acetylthiocholine iodide was used to measure the acetylcholinesterase activity. The following formula was used to determine the enzyme activity [22–24]:

$$R = (\delta \text{ OD volume of assay} / E) \times \text{mg of protein}$$

where R=enzyme activity in μM/L/min/g tissue, and δOD=change in absorbance per minute.

Statistical analysis

The data were subjected to statistical analysis by One- way Analysis of Variance(ANOVA) followed by Dunnet's 't' test and $P < 0.05, 0.01$ and 0.001 were considered as significant [25-28].

Results

EECA and EECD's effects on Y-maze mice

The Y-maze paradigm proved to be sensitive when assessing memory for spatial recognition. The study's main goal was to calculate the proportion of behaviour changes. The behavioural alternation was reduced in the negative control group.However, as compared to the negative control group II, the standard treatment group, which was treated with EECA and EECD at a dose of 400 mg/kg,p.o., showed a substantial increase in behavioural alternation. The results are given in Table 1.

Effect of EECA and EECD in mice with elevated plus maze model (Exteroceptivebehaviour model):

Elevated Plus Maze (EPM) paradigm was used to assess the impact of EECA and EECD on the Inflexion Ratio (IR) in mice. An elevated IR was the outcome of treatment with piracetam (200 mg/kg), EECA (100 and 200 mg/kg), and EECD (200 and 400 mg/kg). With the exception of the EECA (100 mg/kg) group, the groups treated with piracetam, EECA (400 mg/kg), and EECD (200 and 400 mg/kg) showed a statistically significant reduction in Transfer Latency (TL) (Table 1).

Impact of EECA and EECD on Modified Conduct and Transfer Latency in Rats with Amnesia Induced by Scopolamine

Rats with scopolamine-induced amnesia showed poor memory retention and increased transfer latency in the Elevated Plus Maze (EPM) test. On both acquisition and retention days, however, piracetam (200 mg/kg), EECA, and EECD (400 mg/kg) therapy dramatically decreased transfer latency and improved memory retention. These findings imply that EECD and EECA could improve cognition Result are show in Table 1.

Mice with Diazepam-Induced Amnesia: The Impact of EECA and EECD (Interoceptive Behaviour Model)

In this model, diazepam caused dose-dependent amnesia, which resulted in a lower Inflexion Ratio (IR) than in the normal control group. All dosages of EECA and EECD, together with piracetam treatment, caused the IR to rise. The 400 mg/kg dosage of both extracts significantly improved IR and successfully reversed the amnesia caused by diazepam. Table 2 presents the findings.

Impact of EECA and EECD on Rats in the Morris Water Maze

Albino mice's poor performance in the Morris water maze exercise when compared to the control group demonstrated that scopolamine injection reduced their capacity for learning and memory. However, rats given piracetam at 200 mg/kg (i.p.) and EECA and EECD at 400 mg/kg (p.o.) demonstrated a strong defence against the learning and memory impairments brought on by scopolamine. Table 1 displays the findings.

Rats Hypothermia Caused by Clonidine and the Impact of EECA and EECD (NA-Mediated Mechanism)

Rats rectal temperatures significantly decreased from 97.45 ˚F ± 0.19 to 92.62 ˚F ± 0.38 when exposed to the α2-adrenergic agonist clonidine, as compared to the normal control group. The temperature decreased somewhat from 97.75 ˚F ± 0.19 to 97.43 ˚F ± 0.16 after piracetam treatment. However, as Table 2 demonstrates, clonidine-induced hypothermia was considerably restored by both dosages of EECA (200 and 400 mg/kg) and EECD (200 and 400 mg/kg).

Rats Lithium-Induced Head Twitches and the Impact of EECA and EECD (5-HT Mediated Mechanism)

Over the course of 60 minutes, the group that received lithium had 18.2 ± 2.0 head twitches. Piracetam treatment decreased this to 3.23 ± 0.43. Head twitches were considerably (*$p<0.05$) reduced by EECA and EECD at 200 mg/kg, to 11.32 ± 0.49 and 9.51 ± 0.62, respectively. EECA further decreased head twitches to 5.36 ± 0.41 at the 400 mg/kg dosage, but EECD increased them to 14.36 ± 0.32 (**$p<0.01$). According to Table 3, amitriptyline caused the most decrease, bringing the count down to 1.52 ± 0.37.

Effect of EECA and EECD on haloperidol induced (DA mediated) catalepsy

Haloperidol induced a time-dependent increase in catalepsy, peaking at 150 minutes. Piracetam potentiated this effect at all intervals, while EECA and EECD (200 and 400 mg/kg) showed no impact on haloperidol-

induced catalepsy at any time point, result are show in Table 3.

Effect of EECA and *EECD* on Acetylcholinesterase levels in rats

The study found low AChE levels in the normal control group and high levels in the negative control. Piracetam significantly reduced AChE levels, and EECA and EECD (400 mg/kg) also showed a notable reduction, indicating their potential as anti-cholinesterase agents with nootropic activity, result are show in Table 4.

Discussion

This study explored the nootropic potential of ethanolic extracts of Crinum asiaticum (EECA) and Crinum defixum (EECD) in improving memory and cognitive functions in animal models. The extracts demonstrated significant memory-enhancing effects, as evidenced by improved performance in the Y-maze, elevated plus maze, and water maze tests. Both extracts reversed scopolamine- and diazepam-induced amnesia, suggesting a potential mechanism through cholinergic modulation, notably by inhibiting acetylcholinesterase activity and increasing acetylcholine (Ach) levels. Findings point toward the neuroprotective effects of EECA and EECD, potentially offering therapeutic benefits for neurodegenerative diseases like Alzheimer's. Future research should focus on detailed pharmacological studies to validate the clinical efficacy of these extracts, assess their long-term effects, and explore their mechanisms in greater depth. Clinical trials will be crucial in determining the feasibility of these extracts for human use in memory-related disorders.

Table 1. Effect of EECA and EECD on behavioral performance in Y-maze, Elevated Plus Maze, and Morris Water Maze.

Group	Treatment	Y-Maze Alteration (%)	Y-Maze Acquisition (secs)	Y-Maze Retention (secs)	Elevated Plus Maze Inflexion Ratio	Morris Water Maze Acquisition Phase (secs)	Morris Water Maze Retention Phase (secs)
I	Control	61.25 ± 2.31	43.25 ± 1.96	35.23 ± 1.33	0.685 ± 0.15	6.5 ± 2.6	7.9 ± 1.1
II	Negative control (scopolamine)	33.26 ± 2.96	71.26 ± 3.26	69.45 ± 2.54	0.395 ± 0.21	11.9 ± 1.9	10.5 ± 1.5
III	Standard (piracetam + scopolamine)	81.25 ± 2.56**	26.32 ± 2.62**	22.31 ± 1.52**	2.135 ± 0.26**	4.99 ± 2.1**	6.5 ± 1.3**
IV	EECA 200 mg/kg	60.25 ± 1.85	58.15 ± 2.51	38.31 ± 1.33	0.835 ± 0.03	10.6 ± 1.1	7.8 ± 1.6
V	EECA 400 mg/kg	69.65 ± 1.75**	32.03 ± 2.12**	29.32 ± 1.62**	1.439 ± 0.10*	8.2 ± 1.3*	6.6 ± 1.5*

VI	EECD 200 mg/kg	61.25 ± 2.31*	53.25 ± 1.85*	32.22 ± 2.32*	1.639 ± 0.13**	8.38 ± 1.5	6.8 ± 1.1
VII	EECD 400 mg/kg	73.37 ± 1.31	26.51 ± 1.25	25.32 ± 1.15	2.853 ± 0.13**	5.1 ± 0.8**	5.9 ± 1.05**

Table 2. Effect of EECA and EECD on Diazepam- and Clonidine-induced amnesia in mice.

Group	Treatment	Diazepam Inflexion Ratio	Clonidine Rectal Temperature (°F)
I	Control	0.698 ± 0.065	97.75 ± 0.19 (0 min)
II	Diazepam (5 mg/kg)	0.039 ± 0.06	97.45 ± 0.19 (0 min)
III	Standard (piracetam + diazepam)	1.848 ± 0.93**	97.69 ± 0.19 (0 min)
IV	EECA 200 mg/kg + Diazepam	1.362 ± 0.11*	97.56 ± 0.21 (0 min)
V	EECA 400 mg/kg + Diazepam	1.639 ± 0.14**	97.61 ± 0.23 (0 min)
VI	EECD 200 mg/kg + Diazepam	1.246 ± 0.12*	97.67 ± 0.23 (0 min)
VII	EECD 400 mg/kg + Diazepam	1.742 ± 0.05**	97.34 ± 0.16 (0 min)
VIII	Control	-	97.75 ± 0.19 (0 min)
IX	Clonidine (1 mg/kg)	-	97.45 ± 0.19 (0 min)
X	Standard (piracetam + Clonidine)	-	97.69 ± 0.19 (0 min)

Table 3. Effect of EECA and EECD on Lithium-induced head twitches and haloperidol-induced catalepsy.

Group	Treatment	Lithium-induced Head Twitches (for 60 mins)	Haloperidol-induced Catalepsy Score
I	Control	-	0
II	Lithium (190 mg/kg)	18.2 ± 2.0	1.5 ± 0.12
III	Standard (piracetam + Lithium)	3.23 ± 0.43**	1.0 ± 0.23
IV	Amitriptyline (20 mg/kg + Lithium)	1.52 ± 0.37**	1.3 ± 0.23
V	EECA 200 mg/kg + Lithium	11.32 ± 0.49*	1.3 ± 0.23
VI	EECA 400 mg/kg + Lithium	5.36 ± 0.41**	1.41 ± 0.21
VII	EECD 200 mg/kg + Lithium	9.51 ± 0.62*	1.39 ± 0.32
VIII	EECD 400 mg/kg +	4.36 ± 0.32**	1.32 ± 0.18

	Lithium		

Table 4. Effect of EECA and EECD on Acetylcholinesterase Levels in Rats.

Group	Treatment	O.D. after Treatment	AChE (µM/L/min/mg of protein)
I	Control	565 ± 2.2	13.11 ± 1.2
II	Scopolamine (1 mg/kg)	252.5 ± 1.7	24.5 ± 1.1
III	Standard (piracetam + scopolamine)	515 ± 2.1*	15.5 ± 1.11*
IV	EECA 200 mg/kg + Scopolamine	350 ± 1.11	22.8 ± 1.6
V	EECA 400 mg/kg + Scopolamine	401 ± 1.32*	21.9 ± 1.5*
VI	EECD 200 mg/kg + Scopolamine	360 ± 1.51	19.1 ± 1.1*
VII	EECD 400 mg/kg + Scopolamine	480 ± 0.8*	16.9 ± 1.05**

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

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Conflict of Interest

The authors declare no conflict of interest.

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