EVALUATION OF ANTI-ALZHEIMER AND ANTI-PARKINSON ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANTS OF *CELOSIA CRISTATA LINN.*

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***Abstract*- In the present study, ethanolic extract of *Celosia Cristata.* was assessed for the prevention of alzheimer and parkinsonism in rats. Two doses of the plant extract (200 mg/kg & 400 mg/kg) were selected for treatment. The plant extracts produced significant prevention of Alzheimer and Parkinsonism in rats on a dose dependent manner. Based on the above results, it was concluded that the ethanolic extract of *Celosia Cristata.* has shown good anti-alzheimer and anti-parkinson's activity against in rats. Itmay be due to the presence of active phytoconstituents (anti-oxidants) in the extract. Thus, the exploitation of this plant will help the humankind to get potential API or drugs that can be used for the treatment of neurodegenerative diseases at very cheap and economically affordable prices. Our study will also avoid the synthetic route for the manufacture of some potential API or drugs and may prevent the huge investment and pollution caused during the synthesis of some potential API or drugs that can be used for the treatment of neurodegenerative diseases.**

***Key words*: Anti-Alzheimer, Anti-Parkinson Activity, Ethanolic Extract, Whole Plants, Celosia Cristata Linn.**

INTRODUCTION

Alzheimer's disease (AD) is widely known as the most common cause of dementia, and AD is most frequently observed in older individuals [1,2]. Characteristics of AD include behavioral disturbances, neuronal death, memory loss, cognitive deficit, and cholinergic dysfunction. AD pathogenesis includes complex processes and a deficit of the neural pathways associated with memory function [3]. Early-onset AD has been detected in individuals over 65 years old. Nonetheless, over 90% of diagnosed cases are linked with the late-onset of AD, which is commonly observed in individuals over 65 years old [4]. On the other hand, preselinin 1 (*PSEN1*) mutation (P117L) is linked to familial AD (FAD) and can cause death of as young as 28 years old [5]. It has been reported that early-onset AD development is linked with various genetic mutations, particularly in amyloid precursor protein (*APP*), *PSEN1*, and preselinin 2 (*PSEN2*) genes [6]. Dysregulated expression of these genes might be present in around 5–10% of diagnosed cases of early-onset AD [4,6]. Indeed, apolipoprotein E (APOE) polymorphic alleles play a significant role in the development of early-onset and late-onset AD [7,8]. In addition, the presence of *APOE4* alleles is linked with an elevated risk of cerebral amyloid angiopathy and age-associated cognitive deficit during normal aging [9].

Major neuropathological features of AD include nerve cell death, intracellular neurofibrillary tangles (NFTs), and extracellular amyloid plaques [10,11,12,13,14]. Sequential APP cleavage takes place via two pathways, including the amyloidogenic pathway and the non-amyloidogenic pathway [15]. The amyloid plaques are made of amyloid beta (Aβ), which is generated by the amyloidogenic APP cleavage. In this pathway, APP is cleaved via β-secretase (BACE1) and subsequently via γ-secretase to generate Aβ [14]. It has been revealed that there is a link between FAD mutations and increased ratio of Aβ42/40 [16,17], which indicates that increased Aβ42 levels (as compared to Aβ40) play a crucial role in AD pathogenesis, possibly via providing the core for Aβ assembly into amyloidogenic plaques, fibrils, and oligomers [18,19]. In the elderly, Aβ accumulation might take place due to the change in APP cleavage. It was reported that an excessive level of age-linked acetylation of the α-secretase gene might reduce non-amyloidogenic APP processing [20]. In early AD brain tissue, increased BACE1 action was found to elevate amyloidogenic APP processing [21,22]. Monomers of Aβ progressively aggregate into fibrils, oligomers, and insoluble amyloid plaques [10]. NFTs are composed of hyperphosphorylated tau, and these NFTs are known as the histopathological hallmark of AD [23]. Tau can mediate the stabilization of microtubules under normal conditions. In contrast, when tau is hyperphosphorylated, it can accumulate into tangles made of paired helical filaments [11]. It is suggested by the amyloid cascade hypothesis that Aβ accumulation dysregulates neuronal and synaptic function, which can mediate the intracellular environment for the formation of NFTs, eventually resulting in loss of neurons and further deterioration of neurotransmitter activity [10].

Various synthetic medicines are prescribed for Alzheimer's and Parkinson's disease but they exert side effects. Still there is a challenge to the medical system for Management of Alzheimer's and Parkinson's disease without any side effects.

Consequently, the search for natural drugs from medicinal plants is being increased because of its fewer side effects, willingly availability and low cost. Thus, the scientific validation of medicinal plants traditionally used in the treatment and management of Alzheimer's and Parkinson's disease is demanded.

On the basis of literature and documentation of existing uses of *Celosia Cristata* , an effort has been made to establish the scientific validity to investigate anti-alzheimer and anti-parkinson activity.

MATERIALS & METHODS

SELECTION OF THE PLANT MATERIAL

The isolation of secondary plant metabolites begins with the selection of the plant, themost critical aspect of the project. In order to locate a plant, previously in folklore practices, one should turn to the discipline of ethanobotany. Ethanobotany literally means "people's botany" and is defined as the study of plants important to primitive people. Ethanobotany can include present day people and involves inter disciplinary study surrounding a core of botany with chemistry, pharmacology and anthropology among others.

As the medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. The library search will yield detail of folk medicinal plants utilized in particular area. This will ensure the availability of plants for collection purposes.

So, for the selection of a medicinal plant with an active anti-parkinson and anti- alzheimer activity, includes discussion with a tribal medical practitioner for the traditionaland tribal uses of the plants which have been used for neurological diseases. The complied list of plants must therefore be subjected to a literature survey to confirm that the plant has not been previously investigated for anti-alzheimer and anti-parkinson activity. In addition to the verification that the plant constituents have yet to be identified, note must be taken of which plant parts were utilized and how they were culturally prepared.

Based on the ethnopharmacological survey and literature review, the plant *Celosia Cristata* . was selected for the present work.

COLLECTION AND AUTHENTICATION OF THE PLANT MATERIAL

The fresh plants of *Celosia Cristata* . were collected from the Chitthur district,Andra Pradesh, India, during the month of September 2022. The plant was taxonomically identified and authenticated by the Botanist.

EXTRACTION OF PLANT MATERIAL

The fresh plants were air-dried under shade and then coarsely powdered using a mechanical grinder. The powder was then passed through sieve no.40 and stored in an airtightcontainer for the extraction. About 500gms of powder has been used for the process of extraction.

The cleaned and powdered material of whole plants of *Celosia Cristata* . were used for extraction purpose. About 500gms of powdered material was evenly packed ina Soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as Petroleum ether, Ethanol and Aqueous successively. The solvents used were purifiedbefore use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 Hrs. The aqueous extraction was carried out by cold-maceration process.

METHODS OF EXTRACTION:

- Continuous hot percolation process
- Cold maceration process

SOLVENTS USED:

i. Petroleum ether
ii. Ethanol
iii. Distilled water

PREPARATION OF EXTRACTS:

Petroleum ether extract of whole plants of *Celosia Cristata* :

The shade dried coarsely powdered whole plants of *Celosia Cristata* . (500gm) were extracted with petroleum ether (60-80˚C), for 72 hrs. After completion of extraction, the defatted extracts were filtered while hot through Whatmann filter paper (No.10) to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10. Then the concentrated extract was transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Dark greenishbrown coloured extract was obtained. The concentrated extract was then kept in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container forfurther studies.

Ethanolic extract of whole plants of *Celosia Cristata* :

The main marc left after Pet. ether extraction was dried and then extracted withethanol 95% v/v (75-78˚C), for 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark greenish coloured extract was obtained. The extract was then stored in a desiccator to remove the excessive moisture. The dried extract was then packed in an air tight glass container for further studies.

Aqueous extract of whole plants of *Celosia Cristata* :

The marc left after ethanol extraction was again dried and then macerated withdistilled water in a 2 litre round bottom flask for 72 hrs and 10 ml of chloroform was added toavoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown coloured extract was obtained and it was stored in a desiccator to remove the excessive moisture. The dried extract was packedin air tight glass container for further studies. The percentage yields of the above extractswere expressed in Table no.1.

IDENTIFICATION OF PHYTOCHEMICAL ACTIVE CONSTITUENTS PRELIMINARY PHYTOCHEMICAL STUDIES

The extracts obtained (Petroleum ether, Ethanol and Aqueous) were subjected to thefollowing preliminary phytochemical studies.

PHARMACOLOGICAL SCREENING

ANIMALS

Healthy adult Wistar rats, weighing 180–220 g, were used and acclimatized to laboratory conditions for one week. All animals were housed in well-ventilated polypropylene cages (12hrs light and 12 hrs dark schedule) at 25°C and 55–65% RH.

The rats were provided with a standard diet. Rats were freely allowed to commercial pelleted rats chow and water ad libitum.

ANTI-ALZHEIMER'S ACTIVITY

Object recognition test

The apparatus consisted of plywood (70 × 60 × 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trial. The apparatus was illuminated by a 40 W lamp suspended 50 cm above the box. The objects to be discriminated were also made of plywood in two different shapes of 8 cm height coloured black.

The day before test, rats were allowed to explore the box (without any object) for two min. On the day of the test in the first trial (T_1) two identical objects were placed in two opposite corners of the box and the amount of time taken by each rat to complete 20 sec of object exploration was recorded. Exploration was considered directing the nose at a distance < 2 cm to the object and/or touching it with the nose. During the second trial (T_2, 90 min afterT_1) one of the objects presented in trial T_1 was replaced by new object and the rats were leftin the box for 5 min. The time spent in exploration of familiar (F) and the new object (N)were recorded separately and discrimination index (D) was calculated (N-F/N+F). Care was taken to avoid place preferences and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T_2 and cleaning the apparatus with hydrogen peroxide.

Wistar rats of either sex were selected and divided into four groups of six animals each and treated as follows:

- Group I: Administered propylene glycol (5 ml/kg body weight), served asvehicle group
- Group II: Administered extract at the doses of 200 mg/kg body weightintraperitonially
- Group III: Administered extract at the doses of 400 mg/kg body weightintraperitonially
- Group IV: Received Piracetam (100 mg/kg body weight)

The rats were treated with vehicle, extract (200 and 400 mg/kg, i.p.) and Piracetam (100 mg/kg, i.p.) 30 minutes before the first trial. The second trial was performed 90 min after the first trial. Each group consisted of 6 animals.

Y – Maze Test

Wistar rats of either sex were selected and divided into four groups of six animalseach and treated as follows:

- Group I: Administered propylene glycol (5 ml/kg body weight), served asvehicle group
- Group II: Administered extract at the doses of 200 mg/kg body weightintraperitonially
- Group III: Administered extract at the doses of 400 mg/kg body weightintraperitonially
- Group IV: Received Diazepam (10 mg/kg body weight) as standard drug

The test was performed in Wistar rats at 60 min & 120 min after treatment. The rats were placed individually in symmetrical Y-shaped runway (33 x 38 13cm) for 3 min and the number of times, a rat entered in the arm of the maze with all 4 ft (an entry) were counted.

ANTI-PARKINSON'S ACTIVITY

HALOPERIDOL-INDUCED CATALEPSY IN RATS

All the animals were divided into 5 groups (n = 6)

- Group I: Administered propylene glycol (5 ml/kg body weight), served as vehiclegroup
- Group II: Administered haloperidol (1 mg/kg, i.p.) daily for a period of 7 days, servedas the negative control group.
- Group III: Received Syndopa (10 mg/kg body weight) as standard drug
- Group IV: Administered extract at the doses of 200 mg/kg body weightintraperitonially
- Group V: Administered extract at the doses of 400 mg/kg body weightintraperitonially

Haloperidol was given 30 minutes prior to standard and test drug administration. Bodyweight changes and behavioural assessments were carried out before the start of the treatment. Various parameters like Catalepsy (Bar test), Locomotor activity (Actophotometer test), and Muscle Rigidity (Rotarod test) were measured in all animals.

CHLORPROMAZINE-INDUCED CATALEPSY IN RATS

All the animals were divided into 5 groups (n = 6)

- Group I: Administered propylene glycol (5 ml/kg body weight), served as vehiclegroup
- Group II: Administered chlorpromazine (3 mg/kg, i.p.) daily for a period of 21 days,served as the negative control group.

- Group III: Received Syndopa (10 mg/kg body weight) as standard drug
- Group IV: Administered extract at the doses of 200 mg/kg body weightintraperitonially
- Group V: Administered extract at the doses of 400 mg/kg body weightintraperitonially

Chlorpromazine was given 30 minutes prior to standard and test drug administration. Bodyweight changes and behavioural assessments were carried out before the start of the treatment. Various parameters like Catalepsy (Bar test), Locomotor activity (Actophotometer test), and Muscle Rigidity (Rotarod test) were measured in all animals.

BEHAVIORAL ASSESSMENT

Catalepsy bar test

Catalepsy is a state of activity characterized by muscle rigidity associate with failure to correct an externally induced oblique posture for a protracted amount of time. The standard bar test is used for the assessment of catalepsy. Antipsychotic agents usually increase hypersomnia, thereby providing a measure of the extrapyramidal side-effects observed in humans exposed to chronic antipsychotics. Catalepsy induced by the typical neuroleptic agents in rodents can be used as a model for extrapyramidal effects in PD. Catalepsy is most typically measured by the standard bar technique consists of inserting an animal, after administration of a neuroleptic such as haloperidol/CPZ in a position with its front legs resting on a bar suspended on top of the ground. The intensity of catalepsy is measured by the length of time the subject maintains this externally induced abnormal posture.

Catalepsy was measured by a grading technique given below.

Step I-0 Rat moved normally when placed on the table.Step II-0.5 Rat moved only when touched/pushed.

Step III-0.5 Front paws of the rats were placed alternately on a 3 cm highblock. If the rat failed to correct the posture within 15 sec, a scoreof 0.5 for each paw was added to the score of step 1.

Step IV-1.0 The front paws of the rat were alternately placed on a 9 cm highblock. If the rat failed to correct the posture within 15 sec, a scoreof 1 for each paw was added to the scores of step I and II. Thus, 3.5 is a highest score for an animal.

Rotarod Activity test

Rotarod apparatus has a horizontal grooved rod rotating at a fixed speed. The rats are made to balance on this rod. Dependent upon their motor co-ordination, Central nervous activity, and grip strength the animal either stays on the rotating rod for a specific time and after that falls down on the platform of each compartment. The floor of each compartment has sensors that deactivate the timers and the exact fall off time for each rat is displayed onthe respective display. A cut-off time of 180 seconds was maintained throughout the experiment. The average results were recorded as the fall of time. In free riding, the mouse holds the rotating rod and rotates with it. Hence, free ridings are considered as a sensitive parameter related to grip strength and muscle coordination.

Locomotor activity test

The spontaneous locomotor activity was monitored using a digital actophotometer equipped with infrared-sensitive photocells. The apparatus was placed in a darkened, light and sound attenuated, and ventilated testing room. Each interruptionof a beam generated an electrical impulse that was denoted on a digital counter. Each animal was observed over a period of 1 min following haloperidol and chlorpromazine administration and values were expressed as counts per min.

RESULTS AND DISCUSSION

Plant derived natural products such as flavonoids, phenolic compounds, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant activity. There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by research into their potential benefits to human health. One of their main properties in this regard is their antioxidant activity, which enable them to attenuate the development of neurodegenerative diseases. Antioxidant plays an important role in inhibiting and scavenging free radicals, thus providing protection to degenerative diseases. Realizing the fact, this research was carried outto evaluate the anti-alzheimer activity and anti-parkinson activities of extracts of *Celosia Cristata* .

DETERMINATION OF EXTRACTIVE VALUES OF WHOLE PLANT OF *CELOSIA CRISTATA.*

The shade dried coarsely powdered whole plants of *Celosia Cristata* . were extracted by using different solvents of increasing polarity by continuous hot percolation process using Soxhlet apparatus and aqueous extracts by cold maceration method. Extractive values were presented in table no: 1

TABLE NO:1 EXTRACTIVE VALUES OF WHOLE PLANTS OF *CELOSIA CRISTATA* .

Plant name	Partsused	Method ofextraction	Yield in percentage		
			Petroleum Ether	Ethanol	Aqueous
Celosia Cristata .	Wholeplant	Continuous Hot Percolation and Cold Maceration	5.6	9.3	15.6

PHYTOCHEMICAL EVALUATION:

The phytoconstituents present in the various extracts were identified by performing chemical tests and the results were showed in Table No:4.

Petroleum Ether : Chlorophyll, Starch, Fat, Fixed oil.
Ethanol : Carbohydrates, Glycosides, Tannins, Saponins, Flavonoids andPhenolic compounds.
Aqueous Extract : Carbohydrates, Glycosides, Flavonoids and Phenolic compounds.

From the above stated extracts, ethanolic extract showed the presence of more phytoconstituents. Hence, ethanolic extract (EECC) was selected for the pharmacological evaluation.

TABLE NO. 2: PRELIMINARY PHYTOCHEMICAL STUDIES OF EXTRACTS OFWHOLE PLANT OF *CELOSIA CRISTATA*

S. No	Constituents	Tests	PetroleumEther	Ethanol Extract	Aqueous Extract
1.	ALKALOIDS	Mayer's test	-	-	-
		Dragendorff's test	-	-	-
		Hager's test	-	-	-
		Wager's test	-	-	-
2.	STEROLS	Libermann's Burchard test	-	+	+
		Salkowski's test	-	+	+
3.	CARBOHYDRATES	Molisch reagent	-	+	+
		Fehling's reagent	-	+	+
		Benedict'sreagent	-	+	+
		Anthrone test	-	+	+
4.	FIXED OILS ANDFATS	Spot test	+	-	-
5.	PHENOLIC COMPOUNDS	Fecl3	-	+	+
		Gelatin test	-	+	+
		Lead acetate test	-	+	+
6.	PROTEIN AND AMINO ACIDS	Biuret test	-	-	-
		Ninhydrin test	-	-	-
		Xanthoprotein test	-	-	-

		Millon's reagent	-	-	-
7.	SAPONINS	Foam test	-	+	-
8.	TANNINS	Gelatin test	-	+	-
		$FeCl_3$	-	+	-
9.	GUM AND MUCILAGE	Precipitationwith 95% alcohol	+	-	-
10.	FLAVONOIDS	Shinoda's test	-	+	+
		Conc. H_2SO_4	-	+	+
11.	GLYCOSIDES	Molisch's test	-	+	+

Pharmacological evaluation

ANTI-ALZHEIMER'S ACTIVITY

Object recognition test

In the object recognition test, the animals spent more time to explore the objects in the first trial (T_1 session). In the second trial (T_2 session), when a new object replaced a familiar object, ethanol extract of *Celosia Cristata* . and Piracetam significantly reduced the time to explore the familiar object as compared with the time to explore the new object. Moreover, ethanol extract of *Celosia Cristata* . also showed significant increase in discrimination index (Table 3).

Table no. 3 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON OBJECT RECOGNITION TEST USING RATS

S.No	Treatment	Object recognition time (seconds)		Discriminationindex
		Familiarobject	Novelobject	
1.	Group I (Propylene glycol-5ml/kg)	80.42±1.14	95.20±1.02	0.084±0.12
2.	Group II (EECC – 200mg/kg)	51.71±0.96	85.33±0.88	0.245±0.46
3.	Group III (EECC – 400mg/kg)	45.64±0.82	81.21±1.04	0.280±0.31
4.	Group IV (Piracetam –100mg/kg)	34.20±1.16	76.83±0.98	0.383±0.20

All the values were expressed as mean± SEM and n=6 in each group. All the data wereanalyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Y - Maze test

In the Y-maze test, the animals treated with the extract in tested doses have shown a marked decrease in exploratory behaviour compared with control group (Table no. 4). Thus, ethanol extract of *Celosia Cristata* . showed significant decrease in exploratory behaviour indicating facilitator action on learning and memory.

Table no.4- EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON Y – MAZE TEST USING RATS

S. No	Treatment	Exploratory Time(seconds)	
		60 min	120 min
1.	Group I (Propylene glycol-5ml/kg)	10.30±1.45	11.18±1.45

2.	Group II (EECC – 200mg/kg)	7.41±1.06	7.04±0.96
3.	Group III (EECC – 400mg/kg)	6.13±0.83	5.22±0.71
4.	Group IV (Diazepam – 10mg/kg)	4.34±0.94	4.08±0.89

All the values were expressed as mean± SEM and n=6 in each group.
All the data were analyzed by one-way ANOVA method.
P values <0.05 are considered to be significant.
The Y-maze test and object recognition test is a specific and sensitive test of spatial recognition memory in experimental animals. The animals treated with ethanol extract of *Celosia Cristata* . showed significant cognitive improvement as shown by the decrease in transfer latency in Y-maze test and increase in discrimination index in object recognition test.
Thus, ethanol extract of *Celosia Cristata* . has a neuroprotective effect and hence may have a role in improving cognition. It suggests the Anti-Alzheimer's activity of *Celosia Cristata* . is due to presence of high quantity of anti-oxidants such as flavonoids and polyphenol components.

ANTI-PARKINSON'S ACTIVITY
HALOPERIDOL INDUCED MODEL:
Effect of EECC on Haloperidol-induced Catalepsy in Rats:
All the animals were evaluated using a catalepsy bar test for the assessment ofcatalepsy for a week. The control animals (group-I) shown a catalepsy time of about 1.5-2.5 seconds during their entire observation period. All the groups shown a significant change in the catalepsy time on day 0. On day 7 Group-II animals (haloperidol alone) were found to be more retaining on the bar for a longer duration as compared to group-I. Group-III (200mg/kg) and Group-IV (400mg/kg) (pre-treated with different doses of extract) showed a significant
reduction in the catalepsy time as compared to Group-II. Group-V animals (Syndopa)significantly reduced the catalepsy time as compared to Group-II on day 7.
The values were indicating that EECC treated groups (group-III and group-IV) significantly reduces the catalepsy time on day 7. The results were shown below in table.no.5.

Table no.5 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON HALOPERIDOL INDUCED CATALEPSY IN RATS

S.No	**Treatment**	**Time (seconds)**	
		0th day	**7th day**
1.	Group I (Propylene glycol -5ml/kg)	1.91±0.21	2.34±0.32
2.	Group II (Haloperidol – 1mg/kg)	3.43±0.26	19.20±0.60
3.	Group III (HP + EECC – 200mg/kg)	2.80±0.31	9.61±0.48
4.	Group IV (HP +EECC – 400mg/kg)	2.42±0.19	7.92±0.76
5.	Group V (HP + Syndopa – 10mg/kg)	2.27±0.31	6.52±0.56

All the values were expressed as mean± SEM and n=6 in each group.
All the data were analyzed by one-way ANOVA method.P values <0.05 are considered to be significant.

Effect of EECC on Haloperidol-induced Hypolocomotion in Rats:
All the animals were evaluated for locomotor activity using Actophotometer. The locomotor activity score of group-I was found to be 70-73 counts/min throughout the week. For group-II, the activity score was reduced to 49.51 ± 0.61 on day 7. It showed a decrease in the locomotor activity on group-II (haloperidol) as compared to group-I (vehicle)., Animals pre-treated with EECC (group-III and group-IV) showed a significant increase in the locomotor activity when compared to group-II. Group-V animals showed an increase in the locomotor activity as compared to group-II.

Group-IV animals showed a much significant increase in the activity score similar to that of group-V animals. The results were shown below in table.no.6.

Table no.6 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON HALOPERIDOL INDUCED HYPOLOCOMOTION IN RATS

S.No	Treatment	Locomotor activity (counts/min)	
		0th day	7th day
1.	Group I (Propylene glycol - 5ml/kg)	70.42±0.61	71.80±0.23
2.	Group II (Haloperidol – 1mg/kg)	65.10±0.46	49.51±0.61
3.	Group III (HP + EECC – 200mg/kg)	66.61±0.40	63.84±0.39
4.	Group IV (HP +EECC – 400mg/kg)	67.13±1.04	64.24±0.73
5.	Group V (HP + Syndopa – 10mg/kg)	69.21±1.10	65.72±1.02

All the values were expressed as mean± SEM and n=6 in each group.
All the data were analyzed by one-way ANOVA method.P values <0.05 are considered to be significant.

Effect of EECC on Haloperidol-induced Muscular Rigidity in Rats:
Muscular rigidity was evaluated by using a Rotarod apparatus. The mean fall-off time was considered to be an indicator of muscular rigidity. The mean fall-off time of group-I was found to be 95-100 seconds during the entire weekly observation. All the groups shown a nonsignificant difference in muscular rigidity on day 0 and then showed a significant difference in muscular rigidity on day 7 except group-I. Group-II showed a significantreduction in the mean fall-off time when compared to group-I. Group-III and Group-IV significantly shown the reduction in mean fall-off time compared to group-II. Group-V showed a significant increase in the mean fall-off time as compared to group-II. The results were shown below in table.no.7. The results coincide with the previous reported article.

Table no. 7 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON HALOPERIDOL INDUCED MUSCULAR RIGIDITY IN RATS

S.No	Treatment	Fall off time(counts/min)	
		0th day	7th day
1.	Group I (Propylene glycol - 5ml/kg)	96.31±0.37	95.83±0.81
2.	Group II (Haloperidol – 1mg/kg)	92.22±1.64	76.54±0.96
3.	Group III (HP + EECC – 200mg/kg)	93.42±0.23	88.31±0.72
4.	Group IV (HP +EECC – 400mg/kg)	95.14±0.76	90.71±0.88
5.	Group V (HP + Syndopa – 10mg/kg)	94.20±1.21	95.13±0.91

All the values were expressed as mean± SEM and n=6 in each group.
All the data were analyzed by one-way ANOVA method.P values <0.05 are considered to be significant.

CHLORPROMAZINE INDUCED MODEL:

Effect of EECC on Chlorpromazine-induced Catalepsy in Rats:

Animals were evaluated by using bar test for the assessment of catalepsy for weekly observation for a period of 21 days. All the animals were evaluated on day 0, day 7, day 14, day 21 after treatment. Group-I animals showed catalepsy score between 2.0-2.5 seconds during their entire observation period. Group-II animals showed a significant increase in catalepsy time when compared to group-I on day 7. On day 14, group-II animals still showed a significant increase in the catalepsy time compared to group-I and the time increases on day

21. Group III and Group IV slightly reduce the catalepsy time after a week compared to group-II. On day 14, the reduction increases and on day 21, group-V has shown a much more significant reduction in the catalepsy time. Group-V animals showed a significant reductionin the catalepsy time as compared to group-II on day 7 and the reduction in catalepsy time increases after each week. On day 21, group-V showed a much significant reduction in catalepsy time compared to group-II. The results were shown in table.no.8.

Table no.8 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON CHLORPROMAZINE INDUCED CATALEPSY IN RATS

S.No	Treatment	Time (seconds)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol -5ml/kg)	2.12±0.52	2.31±0.36	2.40±0.48	2.43±0.62
2.	Group II (Chlorpromazine – 3mg/kg)	4.31±0.40	9.81±0.31	16.42±0.47	20.37±0.82
3.	Group III (CPZ + EECC – 200mg/kg)	2.90±0.51	5.24±0.64	7.94±0.88	9.63±0.49
4.	Group IV (CPZ +EECC – 400mg/kg)	2.73±0.46	4.92±0.80	7.14±0.94	7.82±0.76
5.	Group V (CPZ + Syndopa – 10 mg/kg)	2.31±0.52	4.40±0.63	6.76±0.74	7.05±0.96

All the values were expressed as mean± SEM and n=6 in each group. All the data wereanalyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Effect of EECC on Chlorpromazine-induced Hypolocomotion in Rats:

All the animals were evaluated for locomotor activity using Actophotometer. All the animals were evaluated on day 0, day 7, day 14, day 21 after treatment. The locomotor activity score of group-I was found to be 65-75 counts/min for the entire observation period. Group-II animals showed a significant reduction in the locomotor activity score when compared to group-I on day 7 and the reduction in the locomotor activity score increases on day 14, and 21. Group III and Group IV showed gradual increase in the locomotor activity score on day 7, day 14, and day 21 as compared to group-II. Group-V showed an increase in the locomotor activity score on day 7 when compared to group-II and the values were significant. Group-V animals showed much significant increase in the locomotor activity score on day 14 and day 21. The results were shown below in table.no.9.

Table no.9 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON CHLORPROMAZINE INDUCED HYPOLOCOMOTION IN RATS

S.No	Treatment	Locomotor activity(counts/minute)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol - 5ml/kg)	69.23±1.08	71.41±1.14	73.62±0.89	74.34±1.02

2.	Group II (Chlorpromazine – 3mg/kg)	64.25±1.34	58.02±1.05	47.43±0.81	36.81±1.34
3.	Group III (CPZ + EECC – 200mg/kg)	66.31±0.75	64.31±0.66	62.48±0.44	61.06±1.15
4.	Group IV (CPZ +EECC – 400mg/kg)	66.82±1.16	65.21±1.34	64.71±0.87	64.13±1.40
5.	Group V (CPZ + Syndopa – 10 mg/kg)	67.10±1.64	67.52±1.38	68.24±0.94	68.82±0.82

All the values were expressed as mean± SEM and n=6 in each group. All the data wereanalyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Effect of EECC on Chlorpromazine-induced Muscular Rigidity in Rats:

Muscular rigidity was evaluated using the Rotarod apparatus. The mean fall-off time was considered to be an indicator of muscular rigidity. All the groups showed a reduction in muscular rigidity after each week except group-I. The mean fall-off time of group-I wasfound to be 88-92 seconds during the entire observation period. Group-II showed a significant reduction in the mean fall-off time on day 7 when compared to Group-I and the reduction level increases on day 14, and day 21. Group-III and Group-IV slightly showed a significant increase in the fall-off time when compared to group-II on day 7. The fall-off time increases after each week on day 14, and on day 21. The results were shown below in table.no.10. The results coincide with the previous reported article.

Table no.10 - EFFECT OF ETHANOLIC EXTRACT OF *CELOSIA CRISTATA* ON CHLORPROMAZINE INDUCED MUSCLE RIGIDITY IN RATS

S.No	Treatment	Fall off time(seconds)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol - 5ml/kg)	88.20±1.14	88.62±1.20	89.30±0.94	89.71±1.26
2.	Group II (Chlorpromazine – 3mg/kg)	83.83±0.78	69.52±1.40	61.04±1.53	42.82±1.28
3.	Group III (CPZ + EECC – 200mg/kg)	84.22±1.41	83.61±1.37	84.71±1.63	85.43±1.25
4.	Group IV (CPZ +EECC – 400mg/kg)	85.11±0.86	84.30±1.39	83.42±1.08	85.62±0.93
5.	Group V (CPZ + Syndopa – 10mg/kg)	86.61±0.85	85.70±1.46	84.38±1.03	88.37±1.33

All the values were expressed as mean± SEM and n=6 in each group. All the data wereanalyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

DISCUSSION

Anti-Alzheimers activity

The high metabolic activity of nervous tissues attached with lipid present in the brain leads to oxidative damage. Additionally, catecholamines present in brain showed more sensitive for production of free radicals. The catecholamines such as adrenaline, noradrenaline and dopamine can spontaneously break down (auto-oxidize) to free radicals, or can be metabolized to radicals by

the endogenous enzymes such as monoamine oxidase.
The antioxidants such as flavonoids containing substance will protect nervous tissue from damage by oxidative stress. Consequently, clinical studies exhibit that Alzheimer's are accomplished of exciting the generation of free radicals and depletion of antioxidant levels. The reactive oxygen species imparts chief role in the pathogenesis of Alzheimer's diseases. Various researches reported that antioxidant containing plants are neuroprotective and hence may have a role in improving memory in aging and neurodegenerative diseases [65].
The animals treated with ethanol extract of *Celosia Cristata* . showed significant cognitive improvement as shown by the decrease in transfer latency in Y-maze test and increase in discrimination index in object recognition test. Thus, ethanol extract of *Celosia Cristata* . has a neuroprotective effect and hence may have a role in improving cognition.

Anti-Parkinson's activity
Catalepsy is a behaviour or nervous condition of animals characterized by muscular rigidity and fixity of posture for a prolonged period known as akinesia [66,67]. Catalepsy is a well-known motor symptom of Parkinson's disease. Group-III and Group-IV were found to reduce the catalepsy time in animals similar to that of standard drug.
Moreover, Locomotor activity is considered to be an indicative of movement which isimpaired or affected in PD which is known as bradykinesia. It is considered to be a cardinal motor symptom of PD. Hence, the locomotor index can be an indicator of Parkinsonism. Group-III and Group-IV were found to increase the locomotor index comparatively than group-II.
Muscular rigidity is also known as muscle stiffness characterized by the inability of the muscles to relax. It is also regarded as the main motor symptom of Parkinson's disease. Fall-off time from the rod indicates the level of rigidity in animals. Thus, muscular rigidity also can be an index of Parkinsonism. Group-III and Group-IV were found to show prevention of reduction in fall-off time. This indicates that ethanol extract of *Celosia Cristata* . has a neuroprotective effect and hence may have a role in anti-parkinsonism activity.

SUMMARY AND CONCLUSION
In the present study, ethanolic extract of *Celosia Cristata.* was assessed for the prevention of alzheimer and parkinsonism in rats. Two doses of the plant extract (200 mg/kg & 400 mg/kg) were selected for treatment. The plant extracts produced significant prevention of Alzheimer and Parkinsonism in rats on a dose dependent manner.
Based on the above results, it was concluded that the ethanolic extract of *Celosia Cristata.* has shown good anti-alzheimer and anti-parkinson's activity against in rats. Itmay be due to the presence of active phytoconstituents (anti-oxidants) in the extract.
Thus, the exploitation of this plant will help the humankind to get potential API or drugs that can be used for the treatment of neurodegenerative diseases at very cheap and economically affordable prices.
Our study will also avoid the synthetic route for the manufacture of some potential API or drugs and may prevent the huge investment and pollution caused during the synthesis of some potential API or drugs that can be used for the treatment of neurodegenerative diseases.

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