Neuroprotective Effect of *Cassia angustifolia* Leaf Extract against Colchicine: An Experimental Study on Cognitive Dysfunction and Biochemical Alterations in Mice

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ABSTRACT

Objective: To examine the neuroprotective effects of *Cassia angustifolia* using behavioural memory tests and to estimate the biochemical parameters using brain homogenate. To examine the protective role of *Cassia angustifolia* ethanolic leaf extract in colchicine-induced cognitive dysfunction and oxidative damage in Swiss albino mice. Methods: For a total of 28 days (4 weeks), a colchicine-induced model was used for the study. Utilizing the elevated plus maze, passive avoidance paradigm, morris water maze and actophotometer for behavioural studies, acetyl cholinesterase (AChE), total protein, lipid peroxidation (MDA), and glutathione (GSH) levels in brain homogenate were calculated. Eight groups of six Swiss albino mice each were created from the 48 total. Carboxymethylcellulose, 1% w/v, was given to class I. Group II piracetam was administered i.p. (200 milligrams / kg). Group III obtained 1 mg / kg of colchicine i.c.v. Group IV and V were provided with 400 and 800 mg / kg of ethanolic *Cassia angustifolia* leaf extract. Group VI was administered Piracetam (200 mg / kg, i.p.) to mice for 28 consecutive days. Colchicine 1 mg / kg, i.p., at 60 min after 28th day piracetam injection was injected. Group VII and VIII received 400 and 800 mg / kg oral ethanolic *Cassia angustifolia* leaf extract for 28 consecutive days, and colchicine (1 mg / kg) was injected i.c.v at 90 min after administration of extract on 28th day. All the data were analyzed using One-way ANOVA followed by a check by Tukey's test. **Results:** The extract from *Cassia angustifolia* leaves significantly reduced the levels of raised plus maze and Morris water maze transfer latency. It revealed noticeably longer transmission latency for the passive avoidance paradigm. Following administration of ethanolic *Cassia angustifolia* leaf extract at doses of 400 mg/kg and 800 mg/kg, acetylcholinesterase and GSH levels significantly increased (P 0.001), while total protein, NO, and MDA levels significantly decreased (P 0.001). This study demonstrates that the ethanolic leaf extract of *Cassia angustifolia* protects against memory loss brought on by colchicine.

Key words: *Cassia angustifolia*, Neuroprotective, Alzheimer's disease, Colchicine.

INTRODUCTION

Alzheimer's disease (AD) is a chronic and progressive neurological condition that slowly causes loss of memory, behavior change, personality and ability to think. Dementia in older people is primarily caused by Alzheimer's disease. Duration between symptom initiation and death takes about 8.5 years. Approximately 15 million world population suffer from Alzheimer's disease.[1] Alzheimer's disease typically affects people around age 65 years.[2] More free radical generation mostly damages the central nervous system. The excessive formation of free radicals can cause neuronal damage to DNA, membrane lipids and proteins. There is a marked decline of cholinergic neurotransmission in elderly people due to decreased levels of acetylcholine in the brain.[3] The most important cause of dementia is Alzheimer's disease (AD) where neuron loss occurs in distinct areas of the brain.[4] AD is characterized by neurotic plaque formation that includes amyloid β protein. Cholinergic cell failure occurs in the forebrain and acetylcholine that is responsible for the development of dementia.[5] Pollutants, stress and hereditary predilection are the key risk factors for neurodegenerative disorders.[6] The conventional medicine is commonly used for the prevention, diagnosis and treatment of various diseases. The treatment by herbal plants of different diseases depends on observations and previous experiences found in books or taught verbally.[7] Neuroprotection involves the therapeutic strategies which can delay or cure neuronal damage. Nowadays, herbalism is as reliable, healthy and cheaper as it is in trend.[8]

Senna, or *Cassia angustifolia*, is a member of the Leguminosae family. Most often in Eastern and Western nations, senna is used to alleviate constipation.[9] Due to the inclusion of sennoside A and sennoside B, two anthraquinone glycosides, senna has a laxative effect. Aloe-emodin, anthrone diglucoside, rhein, rhein-8-glucoside, sennosides C and D, napthalene glycosides like tinnevellin glycoside and 6-hydroxy musizin glycoside, flavonoid (kaempferol), phytosterols, resin, and calcium oxalate are also present in *C. angustifolia*.[10] According to rumours, the earliest kind of senna was discovered in Egypt and Sudan along the Nile River.[11] It is grown commercially in India's Kutch (Gujarat) and Jodhpur (Rajasthan). For a period of two to three years, it can be grown as a perennial crop.[12]

MATERIALS AND METHODS

Plant material

Cassia angustifolia leaves were collected from the college campus of Shri Ram Murti Smarak (College of Engineering and Technology), Bareilly (Uttar Pradesh) and identified by Prof. A.K. Jaitly, Head, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh (specimen number- RU/PS/2016/415). A voucher specimen of the collected sample has been deposited for future reference in the institutional herbarium.

Preparation of extracts

The leaves of *Cassia angustifolia* were first washed in tap water, then dried in the shade, and lastly ground into powder. The resulting powder was placed inside a Soxhlet column and extracted with petroleum ether at 60 to 80 degrees Celsius for 24 hours. The resulting marc was extracted for 24 hours using ethanol extraction first (68-78^{o}C), then chloroform extraction (50-60^{o}C). The extracted were concentrated in a water bath at 50 degrees Celsius. At room temperature, the dried powder extract was stored. A yield of 9.50 percent (w/w), 7.65 percent (w/w), 8.95 percent (w/w), 8.50 percent (w/w), and 0.30 percent (w/w) was found for petroleum

extract, chloroform extract, methanol extract, ethanol extract, and water extract. The ethanolic leaf extract was employed throughout the experimental investigation.

Drug Treatment

The obtained extract was suspended in double-distilled water containing carboxy methyl cellulose (1 percent w/v CMC) at doses of 400 and 800 mg/kg p.o. for pharmacological studies. Based on past investigations of the ethanolic extract of *Cassia angustifolia* extract, the doses were given to specific mice in groups 4,5, 7, and 8. There has been no medication-related death as of the end of the research period. No abnormalities or death were brought on by the *Cassia angustifolia* medication extract while the patient was receiving treatment.

Animals

Animals were gathered from the pharmacy department's animal house at SRMS CET (Pharmacy), Bareilly, Uttar Pradesh. The Institutional Animal Ethics Committee (IAEC) of the Pharmacy Department, SRMS CET (Pharmacy), Bareilly, U.P. accepted the use of animals in this study. Number of approval (715/PO/Re/S/02/CPCSEA). Young, healthy adult Swiss albino strain mice of both sexes were collected in equal numbers per group (n=6) for the study. At the beginning of the investigation, the weight variations of the used animals were kept nominal and did not surpass 20% of the mean weight of each species. Typically, mice weighed 25-30 g. The experimental animal housing was kept at a constant 22°C (3°C). Relative humidity needs to be in the 50-60% range. Artificial lighting was used, with 12 hours of light and 12 hours of darkness. We used conventional laboratory diets with unlimited access to water. Animals from the same species had been housed in the same cage. The control, standard, and treatment groups were randomly allocated to healthy young adult mice. Before the investigation began, the animals were identified by labelling at the base of the tail and acclimated for at least 5 days.

Drugs and chemicals

Drugs: Sigma Aldrich had purchased piracetam and colchicine.

Chemicals: Ethyl acetate, petroleum ether, methanol, ethanol and chloroform were obtained from Central Drug House Laboratory (CDH).

Vehicle

Mice were given an oral dose of *Cassia angustifolia* extract (CAE), which was suspended in 1% w/v CMC. Colchicine and Piracetam were each dissolved in normal saline and administered intravenously and intraperitoneally, respectively. 1 ml/100 g of mice was used for oral delivery and i.p. injection.

Acute toxicity studies

Cassia angustifolia ethanolic extract has been tested for acute oral toxicity in compliance with revised OECD guidelines No.425. When given by oral route in doses of up to 2000 mg / kg, the extract was devoid of any toxicity in mice. Therefore, the experiment used 400 and 800 mg / kg of ethanolic leaf extract.

Group I: It was a control group. The vehicle was administered orally for 28 consecutive days and the transfer latency was assessed on 28th and again on 29th day after 90 minutes of administration.

Group II: It served as the group's positive control. Young mice were injected with piracetam (200 mg/kg i.p.) for 28 days straight, and transfer latency was assessed on the 28th day after 60 minutes after administration and once more on the 29th day after 24 hours.
Class III: This served as a bad control group. Young mice were given an intravenous injection of colchicine (1 mg/kg), and the transmission latency was measured 45 minutes after the administration and again 24 hours later (i.e., on the 29th).
Class IV and V: The young mice were given CAE (400, 800 mg/kg, p.o.) orally over the course of 28 days. After 90 minutes of administration on the 28th day, and again on the 29th day after 24 hours, TL was recorded.
Group VI: Piracetam was injected intravenously (i.p.) into young mice for 28 days in a row. Following the injection of piracetam on the 28th day, colchicine 1 mg/kg, i.p., was given at 60 minutes. After 45 minutes of colchicine administration and once more on the 29th day after 24 hours, TL was detected.
Group VII, VIII: Young mice were given CAE (400, 800 mg/kg, p.o.) orally over the course of 28 days, and on the 28th day, young mice were given colchicine (1 mg/kg) intraperitoneally (i.p.) 90 minutes after receiving the extract. 45 minutes after the injection and 24 hours later (on the 29th day), TL was detected.

EXTEROCEPTIVE BEHAVIORAL MODELS

Elevated plus maze

The structure is made up of two 50x10 cm open arms, two 50x40 cm closed arms, and a central platform measuring 10x10 cm that is elevated 50 cm off the ground. Mice weighing between 20 and 25 g were used in the study. Two steps were taken to complete the experiment. Each mouse was positioned at the end of an open arm on day 14, the day of the acquisition testing, with its back to the centre. Transfer latency [TL] is a measurement of the amount of time needed to enter any one of the closed arms. Each of the four legs was considered a doorway into the closed arm. The cut-off time was 180 s for each mouse. Animals that failed to reach the closed arms by the cut-off period were not included in the analysis. On the fifteenth day, retention testing was conducted, and transfer latency was reported in the same way as before. Transfer delay reduction indicated memory improvement.[6]

Step through Passive avoidance paradigm

The passive avoidance paradigm was used to assess the long term memory. This device consisted of a tiny room connected to a bigger chamber by a guillotine door. The smaller chamber was also known as the "light chamber" since it used a 7W/12V bulb to illuminate it. Mice had an initial acquisition trial, a retention trial after 24 hours, and then sequential days of retention trials II, III, and IV. Each mouse in the acquisition trial experiment was placed as far away from the guillotine door in the smaller room as possible. We observed how long it took the mouse to enter the darker chamber. The mice that did not arrive at the door within a set time limit (90s) were not employed in the study. After the mouse entered the dim compartment, the door automatically shut, delivering an unavoidable 1 mA for 1 sec foot shock. The mouse was taken out of the dim room in less than ten seconds. With standard, control, and test medications, this technique has been repeated. The gradual increase in delay was regarded as evidence of learning.[13]

Morris water maze

The MWM was used for assessing rodent spatial learning and memory. It consists of a large circular black tank with a diameter of 120 cm, 50 cm height, filled with water at 26±2°C up to a depth of 30 cm. The circular pool was divided into four equal quadrants and an $8cm^2$ platform was submerged 1 cm below the opaque surface of one of the quadrants in the middle. The platform's position was held steady throughout the mission. To hide the location of the submerged platform the water was made colored with non-toxic black dye.

The mice were dropped into the water one at a time, and given 120 seconds to locate the platform. Animals had 2 trials each day for 4 days with an inter-trial interval of 20 minutes and a brief (10 sec) time lag to locate the target. The latencies of mice's escape were measured throughout the session. For each trial session and each mouse, this parameter was averaged. The mouse was permitted to stay on the platform for 10 seconds after finding it. The mouse was placed on the platform for 10 seconds and then taken back into the water if it couldn't find it within 90 seconds. Mice had an initial acquisition experiment, a retention trial 24 hours later, and then a second and third retention trial on subsequent days. Trial 1's daily decrease in escape latency corresponds to long-term memory or reference memory, whereas Trials 1 to 2 and 3 correspond to working memory or short-term memory.[14,15]

Actophotometer

Actophotometer has four optical counters and six built-in photosensors that display the activity of the locomotives. Digitally, it displayed the behaviour. CNS-acting medicines have an impact on the majority of locomotive motions in both humans and animals. An actophotometer, which uses photoelectric cells that are linked to a counter in a circuit and measures the locomotor activity (horizontal activity), may quickly and easily quantify this activity. A count is recorded when the animal interrupts the light beam that is falling on the photo cell. The animal moves in either a circular or a square arena on an actophotometer. The mice were first weighed and numbered, after which the apparatus was turned on and each mouse was separately placed in the activity cage for ten minutes. We recorded the basal activity scores for each animal.[16]

Biochemical Analysis

The biochemical indicators of oxidative stress, such as NO, GSH, MDA, and AChE, were assessed in the mouse brain 28 days after Colchicine administration.

Brain tissue preparation

The mice were put to death while under ether anaesthesia. After the brain was removed and the skull was cut. Using ordinary (chilled) saline solution, the brain was cleaned. With 10 strokes at 2000 rpm and 0.03 M sodium phosphate buffer (pH 7.4), a homogenous 10 percent (w/v) brain sample was produced. It is possible to quantify NO, GSH, MDA, and AChE using a homogenized preparation of brain tissue.

Nitric oxide scavenging activity

The approach described by Marcocci et al., 1994 was used to study the nitric oxide scavenging behaviour. In this procedure, 0.5 mL of phosphate buffer saline (pH 7.4) and 2 mL of 10 mM sodium nitropruside were mixed with 0.5 mL of extracts at various concentrations (50-200 g/mL). The mixture was incubated at 25°C for 150 minutes. Instead, 0.5 mL of the incubated

solution was combined with 1.0 mL of the Griess reagent [(1.0 mL of sulfanilic acid reagent (0.33 percent of 20 percent glacial acetic acid)] and 1 mL of naphthylethylenediamine dichloride (0.1 percent w/v) for 5 minutes at room temperature.

Following a 30-minute incubation period at room temperature, the mixture's absorbance at 546 nm was measured. This equation was used to determine the % inhibition of nitric oxide:
(A0 - A1)/A0 x 100 = % inhibition of NO radical
Where A0 represents the absorbance prior to the reaction and A1 represents the absorbance following the Griess reagent reaction.[17]

Measurement of MDA

MDA is a test for lipid peroxidation. The method developed by Colado et al., 1997 can be used to quantify MDA spectrophotometrically utilising standard 1,1,1,3,3-tetraethoxypropane. Nanomoles of MDA are commonly reported per mg of protein. Brain tissue that had been homogenised was centrifuged at 700 g for 10 minutes. A total of 300 l of 30% trichloroacetic acid (TCA), 150 l of 5N HCl, and 300 l of 2% (w/v) 2-thiobarbituric acid (TBA) were added to 500 l of homogenous brain tissue in phosphate buffer (pH 7.4), respectively.
Aluminium foil was then placed in the test tube's mouth to heat the mixture for 15 minutes at 90°C. After 30 minutes, the tubes were removed and kept in ice-cold water for another 30 minutes. The pink tint of the supernatant was acquired. The mixture was centrifuged at 12,000 g for 10 min, and the supernatant was analysed spectrophotometrically at 532 nm.[18]

Measurement of GSH

When GSH underwent the 5, 5'-dithiobis (2-nitrobenzoic acid) reaction (Ellman, 1959), a yellow chromophore was produced, which was then subjected to spectrophotometric analysis. A protein called GSH is expressed in g/mg. Brain tissue that had been homogenised was centrifuged at 700 g for 10 minutes. 500 l of brain homogenate was mixed with 500 l of 10% trichloroacetic acid, and the mixture was centrifuged at 2000 g for 10 min. at 4°C to separate the proteins. The mixture was shaken onto the vortex after being mixed with 100 l of supernatant, 2 ml of 0.1 M phosphate buffer (pH 7.4), 0.5 ml of 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), and 0.4 ml of double distilled water. The absorbance was measured at 412 nm within 15 minutes.[19]

Acetylcholinesterase (AChE) activity

The chronic depletion of the cholinergic system in the brain is thought to be indicated by acetylcholinesterase activity. Acylcholinesterase levels in the brain were quantitatively evaluated by the Ellman's test. This procedure involved taking 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide, and 0.1 ml of DTNB (Ellman reagent). For two minutes, the absorbance change at 412 nm was measured at 30-second intervals. Results were calculated as acetylcholine hydrolysed/min/mg protein micromoles using the chromophore's molar extinction coefficient (1.36 x 104 M1cm1) as a reference point.[20]

$$R = \frac{\delta \text{ OD} \times \text{Volume of Assay} \times 1000}{E \times \text{mg protein}}$$

Where, R is the rate of enzyme activity in ‘micro’ mole of acetylthiocholine iodide hydrolyzed per minute per mg of protein.
δ OD is the change in absorbance per minute
E is the extinction coefficient (1.36×10^4 $M^{-1}cm^{-1}$).[21, 22]

Protein estimation

Using Lowry's technique and BSA (1 mg/ml) as the standard, protein levels in all brain samples were assessed.[23]

Reagents

1. Alkaline solution
a) 2% (w/v) Sodium carbonate in 0.1 M NaOH.
b) 1% (w/v) Copper Sulphate
c) 2% Sodium Potassium tartrate
Working alkaline solution: 48ml of A + 1ml of B + 1ml of C
2. Stock std. Bovine Serum Albumin (BSA) – 1mg/ml
3. Working standard BSA (1000µg/ml) diluted the stock 20 times.
4. Folin-Phenol reagent (ice-cold) diluted with equal amount of water at the time of use.

Test Method:

5ml of working alkaline reagent, 0.9ml of DDW, and 0.1ml of supernatant were combined. After thoroughly blending, the mixture was left to sit at room temperature for 10 minutes. After that, 0.5 ml of the Folin-Phenol Reagent was added, and it was left to sit for 30 minutes at room temperature. At 750 nm, the absorbance was measured against a blank surface. Then, a standard curve (50-1000 g) and an estimate of the sample's protein content (in mg/ml) were plotted.[23]

STATISTICAL ANALYSIS

All of the findings were presented as Mean ± SEM and evaluated by One-way ANOVA followed by Tukey's multiple post-hoc comparison study. A 'P' value of < 0.05 has been recognized as statistically important. Graph Pad prism software analyzed data.

RESULTS AND DISCUSSION

Elevated Plus Maze Test:

Table 1: Effect of *Cassia angustifolia* leaf extract on Transfer latency in Elevated Plus Maze

Treatment	**Before (Mean ± Sem)**	**After (Mean ± Sem)**
Control	28.15±0.68	19.91±0.65
Piracetam (200mg/kg, i.p.)	24.28±0.57**	14.95±0.66***
Colchicine (1mg/kg, i.c.v.)	38.53±1.02***	41.78±0.69***
Low dose *Cassia angustifolia* , (400mg/kg, p.o.)	20.95±0.67***	16.55±0.75**
High dose *Cassia angustifolia* , (800mg/kg, p.o.)	18.35±0.64***	12.21±0.78***
Piracetam+ Colchicine, (200mg/kg, i.p+1mg/kg, i.c.v.)	24.36±0.71**	14.51±0.52***
Low dose *Cassia angustifolia* + Colchicine,	20.40±0.64***	18.76±0.81

(400mg/kg, p.o.+ 1mg/kg, i.c.v.)		
High dose *Cassia angustifolia* + Colchicine, (800mg/kg, p.o.+ 1mg/kg, i.c.v.)	13.46±0.49***	12.28±0.62***

Findings are illustrated as MEAN ± SEM (n=6), *P<0.05, **P<0.0 1, ***P<0.001when compared to control group by one-way ANOVA and then proceeded by Tukey's test.

Step-through Passive avoidance paradigm:

Table 2: Effect of *Cassia angustifolia* leaf extract on Transfer latency in Passive avoidance paradigm

Treatment	Acquisition (Mean±Sem)	Ist Retention (Mean±Sem)	IInd Retention (Mean±Sem)	IIIrd Retention (Mean±Sem)	IVth Retention (Mean±Sem)
Control	57.43±2.78	159.77±0.92	179.60±0.85	149.93±0.81	183.10±0.96
Piracetam (200mg/kg, i.p)	36.03±0.42***	236.03±0.42***	248.20±0.88***	254.03±0.98***	259.53±0.57***
Colchicine (1mg/kg, i.c.v.)	93.75±1.90***	90.92±0.76***	96.03±0.86***	94.47±0.78***	96.25±0.90***
Low dose *Cassia angustifolia* (400mg/kg, i.p.)	29.68±0.66***	229.51±0.71***	232.51±0.99***	237.35±0.64***	240.41±0.97***
High dose *Cassia angustifolia* (800mg/kg, i.p.)	24.26±0.72***	233.93±0.81***	238.76±0.88***	241.26±0.80***	244.26±0.72***
Piracetam+ Colchicine (200mg/kg i.p.+1mg/kg i.c.v.)	25.76±0.54***	224.98±0.78***	228.6±0.57***	226.1±0.55***	234.76±0.92***
Low dose *Cassia angustifolia* + Colchicine	75.55±1.81***	222.55±0.85***	225.55±0.86***	226.52±0.89***	231.22±0.86***

(400mg/kg i.p.+1mg/kg i.c.v.)					
High dose *Cassia angustifolia* + Colchicine (800mg/kg i.p.+1mg/kg i.c.v.)	23.95±1.00***	224.95±0.96	226.28±0.92***	229.62±0.87***	232.45±0.95***

Transfer latency (TL) of initial (first) day observed learning character of animals whereas, TL of second day reflected information or memory retention ability. The *Cassia angustifolia* introduced for 28 successive days through the mouth, intra-peritoneally, significantly increased TL on first day and also on second days, showing reasonable improvement of learning and memory. Ethanolic extracts of *Cassia angustifolia* (400 and 800 mg/kg) and *Cassia angustifolia* (400 and 800 mg/kg) and Piracetam (200 mg/kg) administered orally for 28 days protected the animals from colchicine-induced impairment in learning and memory (Table 5.17).

Morris water maze:

Table 3: Effect of *Cassia angustifolia* leaf extract on escape latency in Morris water maze

Treatment	**Session 1 (Mean ± Sem)**	**Session 2 (Mean ± Sem)**	**Session 3 (Mean ± Sem)**	**Session 4 (Mean ± Sem)**
Control	36.96±0.83	27.36±0.53	24.86±0.81	36.96±0.83
Piracetam (200mg/kg, i.p)	32.36±0.53***	23.60±0.52***	13.60±0.52***	6.80±0.45***
Colchicine (1mg/kg, i.c.v.)	42.53±0.52***	50.33±0.54***	56.20±0.78***	52.70±0.51***
Low dose *Cassia angustifolia* (400mg/kg, i.p.)	36.11±0.42	30.05±0.55**	22.05±0.54*	11.45±0.42***
High dose *Cassia angustifolia* (800mg/kg, i.p.)	34.10±0.67**	26.11±0.50	20.11±0.75***	10.31±0.65***
Piracetam+ Colchicine (200mg/kg i.p.+1mg/kg i.c.v.)	40.68±0.44	39.48±0.57***	30.98±0.55***	20.51±0.70***
Low dose *Cassia angustifolia* +	37.58±0.58	42.43±0.53***	37.10±0.38***	25.76±0.82***

Colchicine (400mg/kg i.p.+1mg/kg i.c.v.)				
High dose *Cassia angustifolia* + Colchicine (800mg/kg i.p.+1mg/kgicv)	35.36±0.55	41.30±0.28***	34.13±0.40***	23.78±0.43***

Results are represented as MEAN ± SEM (n=6), * P<0.05, * * P<0.0 1, * * * P<0.001 when one way ANOVA followed by Tukey's test is applied to compare with control group. In the leaf extract an increase in escape latency was observed suggesting nootropic behavior. The 800 mg/kg dose of the *Cassia angustifolia* leaf extract showed substantial improvements in the mice's escape latency. This shows the possible nootropic activity of the leaf extract *Cassia angustifolia.*

Table 4: Effect of *Cassia angustifolia* leaf extract on basal activity score in Actophotometer

Treatment	**Day 2 (Mean ± Sem)**	**Day 7 (Mean ± Sem)**	**Day 14 (Mean ± Sem)**	**Day 29 (Mean ± Sem)**
Control	235.40±0.54	232.18±0.70	226.90±0.54	219.31±0.61
Piracetam (200mg/kg, i.p)	242.21±0.74***	239.03±0.48***	240.53±0.66***	214.50±0.59***
Colchicine (1mg/kg, i.c.v.)	232.18±0.70*	233.61±0.66	230.56±0.39**	238.53±0.66***
Low dose *Cassia angustifolia* (400mg/kg, i.p.)	235.70±0.70	235.40±0.54**	231.06±0.64***	216.55±0.66
High dose *Cassia angustifolia* (800mg/kg, i.p.)	238.53±0.66*	236.03±0.54***	227.90±0.58	214.45±0.60*
Piracetam+ Colchicine (200mg/kg i.p.+1mg/k g i.c.v.)	232.00±0.73**	232.00±0.73	230.56±0.39**	213.38±0.69***

Low dose *Cassia angustifolia* + Colchicine (400mg/kg i.p.+1mg/kg i.c.v.)	235.70±0.70	232.00±0.73	228.90±0.43	220.45±0.55**
High dose *Cassia angustifolia* + Colchicine (800mg/kg i.p.+1mg/kg i.c.v.)	238.53±0.66*	236.03±0.54***	233.01±0.51***	218.75±0.69

Results are represented as MEAN ± SEM (n=6), * P<0.05, ** P<0.0 1, *** P<0.001when one way ANOVA followed by Tukey's test is applied to compare with control group. The leaf extract did not show any CNS depressant effect on mice.

Table 5: Effect of *Cassia angustifolia* extract on MDA level

Treatment	**MDA (U/mg Protein)**
Control	1.50±0.19
Standard	2.66±0.28
Colchicine	8.76±0.25***
LD CA	3.46±0.35**
HD CA	3.34±0.57*
Pir+col	3.01±0.47
LdCA+col	4.40±0.18***
HdCA+col	2.82±0.26

The mice were sacrificed on the 29th day, and the brain homogenate was prepared to predict improvements in the amount of MDA. Results are represented as MEAN ± SEM (n=6), * P<0.05,** P<0.01,*** P<0.001when one way ANOVA followed by Tukey 's tests to compare with control group. A substantial decrease in the amount of MDA was found in the leaf extract and thus nootropic activity was confirmed.

Table 6: Effect of *Cassia angustifolia* extract on GSH level

Treatment	**GSH (U/mg Protein)**
Control	2.31±0.092
Standard	20.028±0.20***
Colchicine	37.20±0.31***
LD CA	21.99±0.70***

HDCA	62.90±0.11***
Pir+col	11.19±0.09***
LdCA+col	33.96±0.11***
HdCA+col	11.95±0.12***

The mice were sacrificed on the 29th day and the brain homogenate was prepared to measure GSH level changes. Results are represented as MEAN ± SEM (n=6), * P<0.05,** P<0.01,*** P<0.001when one way ANOVA followed by Tukey 's tests compared with control group. The leaf extract showed a large increase in GSH level, thus proving nootropic activity.

Table 7: Effect of *Cassia angustifolia* extract on Catalase level

Treatment	**Catalase (U/mg Protein)**
Control	27.06±1.88
Standard	12.24±1.66***
Colchicine	5.50±1.04***
LD CA	8.50±0.58***
HDCA	10.58±1.14***
Pir+col	15.93±0.78***
LdCA+col	16.55±0.77***
HdCA+col	18.03±0.83***

The findings are illustrated as mean ± sem, (n=6), evaluated using one-way ANOVA and then proceeded by Tukey's test. *P<0.05, **P<0.01, ***P<0.001, when treated groups are put under comparison with control category.

Table 8: Effect of *Cassia angustifolia* extract on SOD level

Treatment	**SOD (U/mg Protein)**
Control	64.20±1.38
Standard	73.90±1.94**
Colchicine	23.80±1.95***
LD CA	65.39±1.89
HD CA	69.42±1.88
Pir+col	51.64±1.37***
LdCA+col	64.46±1.66
HdCA+col	66.28±1.65

The findings are illustrated as mean ± sem, (n=6), evaluated using one-way ANOVA and then proceeded by Tukey's test. *P<0.05, **P<0.01, ***P<0.001, when treated groups are put under comparison with control category.

Table 9: Effect of *Cassia angustifolia* extract on Nitric Oxide level

Treatment	NO (µg/mg protein)
Control	8.06±1.01
Standard	15.95±2.12
Colchicine	31.02±3.61***
LD CA	23.36±1.15***
HDCA	16.63±2.27
Pir+col	14.34±1.88
LdCA+col	18.45±2.02*
HdCA+col	15.57±2.27

The mice were sacrificed on the 29th day, and the brain homogenate was prepared to predict NO level changes. Results are represented as MEAN ± SEM (n=6), * P<0.05,** P<0.01,*** P<0.001when one way ANOVA followed by Tukey 's tests compared with control group. In the leaf extract a large decrease in the amount of NO was observed, thus proving nootropic activity.

Table 10: Effect of *Cassia angustifolia* extract on Acetylcholinesterase level

Treatment	AChE(µM/min/mg protein)
Control	2.40±0.10
Standard	9.61±0.20***
Colchicine	3.59±0.17***
LDCA	4.33±0.20***
HDCA	4.24±0.14***
Pir+col	4.60±0.17***
LdCA+col	3.75±0.10***
HdCA+col	3.92±0.08***

The mice were sacrificed on the 29th day, and brain homogenate was prepared to estimate improvements in the amount of AChE. Results are expressed as MEAN ± SEM (n=6), * P<0.05, * * P<0.01, * * * P<0.001when one way ANOVA followed by Tukey's tests compared with control group. In the leaf extract an increase in AChE level was observed suggesting nootropic activity.

Alzheimer's disease is a neurological disorder with a slow start. In the allopathic medical system, no effective medication has yet been created for the full cure of Alzheimer's disease. We should now look forward to using herbal medications to treat this illness. In the current study, mice's learning behaviour improved after receiving *Cassia angustifolia* extract orally for 28 days. The higher dose of *Cassia angustifolia* extract (400 mg/kg) significantly improved mice's memory throughout this study, as evidenced by a decrease in Transfer Latency during elevated plus maze testing and an increase in Transfer Latency during passive avoidance testing in comparison to the control group. In contrast to the control group, the Escape Latency decreased in the Morris Water Maze Study. The operation of the locomotive revealed no signs of CNS disturbance. Animals pretreated with *Cassia angustifolia* extract for 28 days were shielded from memory losses brought on by colchicine. These results point to a potential function for *Cassia angustifolia* extract in neuroprotection.

Age-related declines in cognitive function, which may play a role in the development of Alzheimer's disease in senior persons, are mostly brought on by reactive oxygen species (ROS). Antioxidant properties are seen in *Cassia angustifolia*. The antioxidant characteristics of *Cassia angustifolia* extract are related to its neuroprotective activity because they submit the vulnerable neurons to less oxidative stress, which reduces neuronal damage and improves neuronal function. Degeneration of neural circuits, reduction in brain acetylcholine levels, and impairment in neuronal transmission are all signs of dementia. According to the results of the current investigation, an 800 mg/kg dose of the ethanolic extract of *Cassia angustifolia* exhibits nootropic activity that is similar to that of the drug Piracetam. In the raised plus maze, transfer latency was significantly reduced by pretreatment with *Cassia angustifolia*. In the passive avoidance investigation, transfer latency was improved. The level of GSH and AChE was elevated and NO and MDA were lowered by the ethanolic leaf extract of *Cassia angustifolia*. As a result, the ethanolic leaf extract of *Cassia angustifolia* exhibits substantial nootropic action.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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