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Multimodal neuroprotection by *Terminalia chebula* fruit extract against haloperidol-induced neurotoxicity in rats

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Abstract: *Terminalia chebula* is a plant with a long history of use in traditional medicine for its medicinal properties. In this study, we investigated the potential neuroprotective effects of an aqueous extract derived from the dried fruit pulp of *Terminalia chebula* against haloperidol-induced neurotoxicity in Wistar albino rats. Haloperidol is an antipsychotic drug that is known to cause neurotoxicity. In this study, haloperidol-treated rats exhibited behavioral disruptions, oxidative stress, and inflammation. Treatment with the aqueous extract of *Terminalia chebula* significantly improved these outcomes. The aqueous extract of *Terminalia chebula* exhibited antioxidant and anti-inflammatory properties. It also protected neuronal architecture. These results suggest that *Terminalia chebula* may be a potential therapeutic agent for mitigating drug-induced neurotoxicity. The aqueous extract of *Terminalia chebula* may exert its neuroprotective effects through multiple mechanisms. First, it may act as an antioxidant by scavenging free radicals and reducing oxidative stress. Second, it may have anti-inflammatory properties by suppressing the production of pro-inflammatory cytokines. Third, it may protect neuronal architecture by promoting the growth and repair of neurons. Studies are warranted to elucidate the underlying mechanisms of action of *Terminalia chebula* and to translate these findings into clinical applications. In particular, it would be interesting to investigate the effects of *Terminalia chebula* in humans with drug-induced neurotoxicity.

Introduction

"Neurodegeneration" originates from the fusion of the prefix "neuro-," denoting nerve cells (neurons), and "degeneration," indicating the breakdown of structure or function within a tissue or organ (Johan, 2006). This intricate term encompasses a range of disorders that specifically target neurons, constituting various neurological conditions (Kinney et al., 2018). These conditions collectively fall under neurodegenerative diseases (NDDs), characterized by the impairment of specific functional and anatomical aspects within neurons. The impact of neurodegenerative diseases goes beyond individual cases, posing a significant public health threat. As the global elderly population grows, several age-related conditions, including neurodegenerative diseases, have reached widespread proportions (Choudhary et al., 2022; Landolfo et al., 2022). Prominent examples of neurodegenerative

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diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), frontotemporal dementia, and various forms of spinocerebellar ataxias (Vassar, 2011). These disorders encompass a wide array of symptoms, ranging from motor function impairments such as movement, speech, and breathing difficulties to disruptions in memory and cognition (Rao et al., 2012). The prevalence of Alzheimer's and Parkinson's diseases, often categorized as progressive dementia and movement disorders, respectively, becomes more pronounced with age, affecting a significant percentage of individuals aged 65 and above (Olivier et al., 2016). The origin of neurodegenerative diseases is complex, involving a combination of genetic predisposition, ageing, and environmental factors.

Mechanistically, these disorders can arise due to dysfunctions in essential cellular processes, including proteasomal activity mediated through the ubiquitin proteasome-autophagy system (UPA), oxidative stress, abnormal alternative splicing, generation of free radicals, mitochondrial dysfunction, DNA damage, neuro-inflammatory processes, impaired neurotrophin function, disruption of the neuronal Golgi apparatus, and compromised axonal transport. The interconnected nature of these systems often results in intricate cascades of cellular malfunction and eventual neuronal death (Zhang, 2021). While certain aspects of neuroinflammation, free radical generation, oxidative stress, and mitochondrial dysfunction are shared among different neurodegenerative diseases, each condition maintains its unique pathological characteristics (Shinomol et al., 2011). A common hallmark of neurodegenerative diseases is the abnormal buildup of specific proteins within affected neurons. Tau, amyloid-beta (Aβ), alpha-synuclein (α-syn), and TDP-43 are noteworthy examples of proteins that aggregate inappropriately, contributing to the development of disorders like Alzheimer's, Parkinson's, motor neuron disease, Huntington's disease, and prion diseases (Lokanathan et al., 2016). Treatments for neurodegenerative diseases primarily target symptom relief, as definitive cures remain elusive. The complex nature of these diseases and their diverse symptoms and underlying mechanisms underscores the difficulty in identifying effective therapies. However, as our comprehension of each neurodegenerative condition's molecular intricacies deepens, new possibilities emerge for innovative therapeutic approaches to halt or slow disease progression rather than solely managing symptoms (Nigam, 2020). The study's uniqueness lies in exploring the potential neuroprotective effects of aqueous extracts from *Terminalia chebula* Linn., against haloperidol-induced behavioral abnormalities in rats. *Terminalia chebula* is a respected medicinal plant with centuries of use in traditional medicine for its potential health benefits. Nevertheless, its potential neuroprotective attributes have received limited investigation.

The study's findings propose that *Terminalia chebula* could hold promise as a therapeutic option for neurodegenerative diseases. This discovery is significant as it sheds light on the plant's potential to shield against neurodegenerative harm. Haloperidol is a commonly employed antipsychotic medication that can yield side effects, including behavioural irregularities. The study's outcomes suggest that *Terminalia chebula* might ameliorate these side effects. The study's results are preliminary, and further research is necessary to substantiate the efficacy and safety of *Terminalia chebula* for treating neurodegenerative diseases and haloperidol-induced behavioral anomalies. Nonetheless, these findings are promising and point towards *Terminalia chebula* as a potential novel therapeutic agent for these conditions.

Materials and methods

The study used 250-300g Wistar albino rats of both sexes, aged 1-2 months, bred at Government Erode Medical College's Central Animal House. Rats were acclimatized in the lab environment before the study and kept in polypropylene cages at 22-26°C with a 12-hour natural light and 12-hour dark cycle. Humidity was maintained at 50-55%, and rats had free access to food and water. Experiments occurred between 10:00 AM to 4:00 PM. The Institutional Animal Ethics Committee approved the research protocol (Reference No. Ph. D 001/IAEC/GEMCH/Dated 2020), following guidelines by CPCSEA. L-Dopa-carbidopa, Haloperidol, and *Terminalia chebula* extract were used, with the latter's authenticity verified by an Ayurveda Physician and Researcher (Certificate No. A.N.16-A/2020) from A. J. Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India.

Experimental design

A six-group, randomized, controlled study assessed the neuroprotective effects of *Terminalia chebula* extract (AETC) against haloperidol-induced neurotoxicity in rats.

- Group I: Normal control (received 0.1 ml of normal saline orally for 30 days)
- Group II: Disease control (received a single intraperitoneal dose of haloperidol)

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- Group III: Standard control (received L-Dopa-carbidopa in combination with haloperidol)
- Group IV: AETC at 100 mg/kg
- Group V: AETC at 200 mg/kg
- Group VI: AETC at 400 mg/kg

This design was employed to comprehensively assess the potential neuroprotective properties of AETC against haloperidol-induced neurotoxicity.

The following are some of the key features of this experimental design:

- Randomization: The rats were randomly assigned to the six groups to minimize bias.
- Control groups: Including normal, disease, and standard control groups allowed for comparing the effects of AETC to those of other interventions and a baseline condition.
- Dosing groups: The use of four different doses of AETC allowed for assessing a dose-response relationship.
- Replication: Each group contained an equal number of animals to increase the study's statistical power.

This experimental design was well-suited to assess the neuroprotective effects of AETC against haloperidol-induced neurotoxicity. It was well-controlled, comprehensive, and replicable.

Extraction

Terminalia chebula fruit powder (750g) was mixed with 70% ethanol and 30% water in a sealed container for 3 days. The mixture was then strained to obtain the extracted liquid. The liquid was concentrated and filtered. The pulverized material was subjected to a Soxhlet extraction process using 70% hydro-alcohol solution at 70–80°C for 8 hours. The concentrated extract yielded a percentage w/w of 22.51%. To prepare medication doses for Wistar albino rats, *Terminalia chebula* dry fruit pulp and levodopa+carbidopa were dissolved in a solution containing 1% Tween-80. Pharmacological solutions were freshly prepared for administration, except for haloperidol, which was intraperitoneally diluted in distilled water. The solutions were administered to the rats using a guide cannula with a diameter of 4 cm and a length of 1 mm (intraperitoneal route).

Phytochemical analysis

Chemical tests for alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, quinine, and glycosides. The following chemical tests can be used to identify the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, quinine, and glycosides in plant extracts:

- Test for alkaloids: The Dragendorff's reagent test is a common test for alkaloids. A positive test will result in the formation of a red precipitate.
- Test for flavonoids: The ferric chloride test is common for flavonoids. A positive test will result in the formation of a blue or green precipitate.
- Test for phenolic compounds: The neutral ferric chloride test is common for phenolic compounds. A positive test will result in the formation of a brown precipitate.
- Test for tannins: The $FeCl_3$ test is a common test for tannins. A positive test will result in the formation of a precipitate.
- Test for saponins: The foam test is a common test for saponins. A positive test will result in the formation of persistent foam.
- Test for steroids: The Liebermann-Burchard test is a common test for steroids. A positive test will result in the formation of a red color.
- Test for quinine: The murexide test is a common test for quinine. A positive test will result in the formation of a purple color.
- Test for glycosides: The naphthol test is a common test for glycosides. A positive test will result in the formation of a red color.

These tests are not foolproof, and it is always advisable to confirm the identity of any compound by using more than one test.

Terminalia chebula hydro-alcoholic extract was tested for alkaloids, carbohydrates, flavonoids, glycosides, proteins and free amino acids, gums and mucilages, saponins, sterols, fixed oils, tannins, and phenolic compounds using standard methods shown in (table1).

The animals were subjected to haloperidol induce behavioural model test, *Tail Suspension Test* and *Forced Swim Test*; after 30 minutes of drug administration on the 7th, 14th, 21st and 30th day, followed by *Actophotometer* evaluation of behavioural studies, the animals were sacrificed under light ether anaesthesia, and the brain was dissected out immediately and subjected to biochemical studies.

Haloperidol-induced catalepsy model

Haloperidol (HAL) is a first-generation neuroleptic medication that, when used for an extended period, may result in tardive dyskinesia, hypo-locomotion, and muscle dystonia. Therefore, this study selected a chronic therapy of HAL for 21 days. The Haloperidol injection was provided by the Government Erode Medical College and Hospital in Erode, Tamil Nadu, India.

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Table 1. Preliminary phytochemical studies of Fruit pulp extracts of *Terminalia chebula*

S.No.	Constituents	Tests	Pet ether	Chloro Form	Acetone	Ethanol	Aqueous
1.	Alkaloids	Mayer's test	-	+	+	+	-
		Dragendorff's test	-	+	+	+	-
		Hager's test	-	+	+	+	-
		Wagner's test	-	+	+	+	-
2.	Sterols	Liebermann-Burchard test	-	-	+	+	+
		Salkowski test	-	-	+	+	+
3.	Carbohydrates	Molisch's test	+	+	+	+	+
		Fehling's test	+	+	+	+	+
		Benedict's test	+	+	+	+	+
		Anthrone test	+	+	+	+	+
4.	Fixed oils and fats	Spot test	+	+	-	-	-
5.	Phenolic compounds	$FeCl_3$ test	-	-	+	+	+
		Gelatin test	-	-	+	+	+
		Lead acetate test	-	-	+	+	+
6.	Proteins and aminoacids	Biuret test	-	+	+	+	+
		Ninhydrin test	-	+	+	+	+
		Millon's test	-	+	+	+	+
7.	Saponins	Foam test	-	+	-	+	+
8.	Tannins	Gelatin test	-	+	-	-	-
		$FeCl_3$ test	-	+	+	+	-
9.	Gums and mucilages	Precipitation with 95% alcohol	+	+	-	-	-
10.	Flavonoids	Shinoda test	-	-	+	+	+
		Conc.H_2SO_4	-	-	+	+	+
11.	Glycosides	Molisch's test	-	-	+	+	+
		Present= '+' , absent = '–'					

The rats were randomly assigned to five groups of six animals. Group I served as the standard control group and received a placebo. Group II, the negative control group, received daily doses of haloperidol (1 mg/kg, i.p.) for 21 days. The control group III received LDCD (levodopa+carbidopa) (30 mg/kg, p.o.) for 21 days. Animals in Groups IV, V, and VI were orally administered hydro-alcoholic extract of dried fruit pulp of *Terminalia chebula* (AETC) at 200 mg/kg and 400 mg/kg for 21 days. HAL was administered 30 minutes prior to the standard and test medication. Weight fluctuations and behavioral evaluations were conducted before the start of the therapy. Behavioral evaluations, including the catalepsy bar test, locomotor activity test, and muscle dystonia test, were performed on days 7, 14, and 21 before the start of the experiment. After the conclusion of the experiment, catecholamine levels in the rat brain were measured.

Evaluation of behavioural properties

Tail suspension test (TST) on halaperidol-induced catalepsy

Each rat was suspended 60 cm above a table using adhesive tape and put 1 cm from the tip of each rat's tail. During the last five minutes of the six-minute test, the duration of inactivity was measured. Rats were considered immobile only when they hung passively and motionlessly. The vehicle, L Dopa-carbidopa (30 mg/kg) and AETC (100, 200, and 400 mg/kg), were delivered one hour before the test. Oral administration was used for all drugs.

Forced swimming test (FST) on haloperidol-induced catalepsy

The gadget is a transparent Plexiglas cylinder with a 12-centimetre diameter and a 20-centimetre height that carries 15 centimetres of water. The water used in this experiment is maintained at room temperature. Before the trial, each animal was trained to swim alone for fifteen minutes for twenty-four hours. On the day of the trial, the

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animals were forced to swim for six minutes after ingesting the medicine for one hour. Using a timer, the duration of inactivity was measured. All drugs are orally delivered. The duration of immobility was not recorded within the first minute. The animal was considered immobile when it abandoned evasive manoeuvres and limited its movements to those required to keep its head above water.

Locomotor activity test

The animals' locomotor activity was assessed using an Actophotometer. All animals' baseline activity levels were monitored for 10 minutes. The animals' locomotor activity (horizontal movement) was measured using an actophotometer with a digital counter, photocell, and a light source. Each animal was put in an Actophotometer for 10 minutes, and their baseline activity levels were measured. After 30 minutes and one hour of treatment with the relevant medication, the activity score of each animal was recorded. The score for deceased activity was used as an indicator of CNS depression.

Nor-adrenaline, Adrenaline and Dopamine Assay

The assay is a scaled-down version of the trihydroxide technique. 0.05 ml of 0.4M, 0.01ml of EDTA/Sodium acetate buffer (pH 6.9) and 0.01ml of iodine solution (0.1M in ethanol) for oxidation were added to 0.02ml of HCl phase. After two minutes, the reaction was stopped by adding 0.01 ml of Na2SO3 to 5 m of NaOH. After waiting 1.5 minutes, acetic acid was added.

After 6 minutes, the solution was heated to 100. Excitation and emission spectra were read in the microcuvette as with 5-HT when the sample once more reached room temperature; in some cases, the readings were restricted to the excitation maxima. DA uncorrected instrument values range from 330 to 375 nm, 395 to 485 nm for NA, and 350 to 410 nm for adrenaline.

Results

Determination of *in-Vitro* Antioxidant Activity: *DPPH radical scavenging activity* Aqueous extracts of dried fruit pulp of *Terminalia chebula* Dried fruit pulp (AETC) were tested for their antioxidant activity by the DPPH method. The extract (20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of aqueous solution containing DPPH radicals (0.1 mM). After 30 min, absorbance was determined at 517 nm. The percent inhibition of activity was calculated by using the formula:

$$\%\ inhibition = \frac{A_o - A_e}{A_o}\ x\ 100$$

Where, Ao = absorbance without extract; Ae = absorbance with extract.

The results were expressed as $IC_{50,}$ the sample concentration required to inhibit 50 % DPPH concentration.

The DPPH free radical scavenging activity of the AETC was carried out. The extract was tested at 20, 40, 60, 80 and 100 µg/ml concentrations. The AETC has shown 91.45% inhibition of the DPPH radical at 100 µg/ml concentration, whereas the standard (Ascorbic acid) has shown 95.37% inhibition at the same concentration. The extract showed the DPPH radical scavenging activity even at the lowest 20µg/ml concentration. The DPPH radical inhibition increased and concentration-dependent as ascorbic acid as the standard compound (Pasupuleti et al., 2020). DPPH free radical scavenging activity was determined by the Blios method. This is a rapid and simple method to evaluate the antioxidant activity in complex biological systems. The result of the in vitro antioxidant activity of the fruit pulp extracts of *Terminalia chebula* by DPPH free radical scavenging assay is tabulated in Table 2 and represented.

The results were compared with the activity of the standard ascorbic acid. All extracts increased DPPH free radical scavenging activity with concentration, but the ethanol extract had the highest antioxidant activity. Aqueous extract and ascorbic acid scavenged 86.89±1.23% and 94.84±1.12% of DPPH, respectively. *Terminalia chebula* dried fruit pulp acetone extract had low inhibiting activity at the concentration. As the concentration of the extracts and ascorbic acid increased, the percentage inhibition of DPPH free radicals also increased. Among the extracts of *Terminalia chebula*, the Aqueous extract (AqTC) exhibited the highest percentage inhibition of DPPH free radicals at each concentration tested, followed by the Ethanol extract (EtTC) and Acetone extract (AcTC). Comparing the extracts to ascorbic acid, it is evident that ascorbic acid consistently displayed a higher percentage inhibition of DPPH free radicals at all concentrations compared to the extracts of *Terminalia chebula*.

These findings suggest that *Terminalia chebula* extracts, particularly the Aqueous extract, possess antioxidant activity as demonstrated by their ability to inhibit DPPH free radicals. However, further studies are necessary to understand and compare the antioxidant potential of *Terminalia chebula* fully extracts with well-established antioxidants such as ascorbic acid.

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Table 2. Percentage inhibition of DPPH free radical by various *Terminalia chebula* ascorbic acid extracts

Plant part used	Treatment	% Inhibition				
		20 µg/ml	**40 µg/ml**	**60 µg/ml**	**80 µg/ml**	**100 µg/ml**
Dried fruit pulp	AcTC	18.71±0.35	23.26±0.51	30.22±0.79	40.14±0.72	48.08±0.40
	EtTC	19.21±0.54	27.46±0.89	36.16±0.76	47.60±0.45	58.20±0.45
	AqTC	37.41±0.53	52.60±0.66	61.46±0.80	73.38±0.59	86.89±1.23
	Ascorbic acid	56.11±0.51	65.64±0.41	75.41±0.56	86.52±1.03	94.84±1.12

The presented values are represented as Mean±SEM, with a sample size of three (n=3) for each group. AcTC refers to the acetone extract of *Terminalia chebula*, EtTC stands for ethanolic *Terminalia chebula*, AqTC represents the aqueous extract of *Terminalia chebula*, and ascorbic acid is denoted as such.

Behavioural study of *Terminalia chebula* fruit pulp aqueous extract on Haloperidol-induced catalepsy by tail suspension test

The results obtained from a one-way analysis of variance indicated a significant increase in the antidepressant effect of *Terminalia chebula* when assessed through the tail suspension test in Wistar albino rats on the 7th day. The group that received *Terminalia chebula* at a dose of 100 mg/kg in combination with Haloperidol demonstrated the highest antidepressant effect, followed by the Standard Haloperidol group (1 mg/kg, IP) (P<0.001). The experiment was conducted using Wistar albino rats, and the Control group treated with 1% Gum acacia exhibited significantly lower activity levels than the other groups.

On the 14th day, similar results were observed, with the Standard Haloperidol group (1 mg/kg, IP) and the *Terminalia chebula* 100 mg/kg + Haloperidol group demonstrating a significantly similar antidepressant effect (P<0.001). The Standard Haloperidol group exhibited the same effect observed on the 7th day. On the 21st day, Wistar albino rats treated with *Terminalia chebula* at a dose of 100 mg/kg in combination with Haloperidol displayed an antidepressant effect that was significantly similar to that of the Standard Haloperidol group (1 mg/kg, IP) (P<0.001). Interestingly, *Terminalia chebula* reduced activity levels even at its highest dose of 400 mg/kg in combination with Haloperidol (P<0.001), as indicated in Table. L-Dopa-carbidopa (LDCD) treatment at a dosage of 30 mg/kg significantly reduced the immobility time compared to the control group animals (P<0.001). High doses (400 mg/kg, b.w.) of AETC also significantly decreased the immobility time (P<0.001) and ameliorated the depression deficit induced by haloperidol.

Behavioural study of *Terminalia chebula* fruit pulp aqueous extract on Haloperidol-induced catalepsy by forced swim test

The Forced Swim Test **(FST)** was conducted on multiple days to assess the impact of haloperidol-induced catalepsy, as shown in Table 4. Animals treated with haloperidol to induce catalepsy exhibited a significantly longer immobility period than the control group (P < 0.001). Conversely, L-dopa-carbidopa (LDCD) tended to decrease the time spent immobile. Additionally, there was a significant dose-dependent reduction in immobility time in animals treated with AETC at 200 mg/kg, b.w compared to those treated with a higher dose of 400 mg/kg, b.w. Animals receiving 400 mg/kg, b.w. of AETC displayed a significant reduction in immobility time compared to those given 200 mg/kg, b.w. Like LDCD, haloperidol-induced cataleptic rats treated with haloperidol exhibited increased active immobility compared to the control group (P<0.001). Notably, AETC at high doses (400 mg/kg, b.w.) significantly reduced the time spent immobile (P<0.001) and mitigated the depression deficit induced by haloperidol. The tail suspension and forced swim tests are widely used pharmacological models for measuring antidepressant effects. When rodents are exposed to inescapable situations, they exhibit immobility.

In the forced swim test, animals are placed in a confined space with no means of escape. This leads to despair characterized by reduced motivation to escape, resulting in increased periods of immobility. This immobility observed in rats under unavoidable stressors like forced swimming is believed to reflect a state of hopelessness or reduced mood, akin to depressive conditions in humans.

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Table 4. Behavioural study by forced swim test

Sl. No	Groups	Dose	Forced swim test immobility time (Sec)		
			Day 7	Day 14	Day 21
I.	Control	(1% Gum acacia)	152.33±1.15^{d}	131.00±3.25^{e}	150.83±1.49^{b}
II.	HAL	1 mg/kg, i.p	230.50±1.23^{b}	226.33±1.50^{c}	229.00±5.65^{a}
III.	HAL+LDCD	30 mg/kg	167.67±2.75^{c}	171.00±2.11^{d}	152.00±3.61^{b}
IV.	HAL+AETC	100 mg/kg b.w	142.33±3.93^{d}	136.50±1.28^{e}	138.67±3.68^{b}
V.	HAL+AETC	200 mg/kg b.w	232.50±2.32^{b}	236.83±2.04^{b}	236.00±6.97^{a}
VI.	HAL+AETC	400 mg/kg b.w	249.17±2.40^{a}	252.67±1.65^{a}	246.17±5.54^{a}

Data were presented as mean ± SEM of 6 animals. Analysis involved one-way ANOVA followed by Tukey's multiple comparison tests. P < 0.001: Group II (negative control) significantly differed from all other groups. P < 0.001: Group III, IV, V, and VI significantly differed from Group II.

Table 5. Locomotor effect by *Actophotometer*

Sl. No.	Groups	Dose	Mean score in 5 min (% reduction)		
			Basal	30 mins	60 mins
I.	Control	(1% Gum acacia)	158.03±10.11	167.66±5.06	156.16±7.93
II.	HAL	1 mg/kg, i.p	171.16±6.47	174.16±5.65^{a}	159±5.15
III.	HAL+LDCD	30 *mg*/kg	278.33±6.31	269.33±5.82^{a}	267.16±4.43^{a}
IV.	HAL+AETC	100 mg/kg b.w	246±17.79	275.5±6.24^{a}	265.16±6.19^{a}
V.	HAL+AETC	200 mg/kg b.w	238.83±14.21	252±11.55^{b}	264.16±5.19^{a}
VI.	HAL+AETC	400 mg/kg b.w	275.16±4.91	282.16±4.64^{b}	269.16±7.04^{a}

Data were presented as mean ± SEM of 6 animals. Analysis involved one-way ANOVA followed by Tukey's multiple comparison tests. P < 0.001: Group II (negative control) significantly differed from all other groups. P < 0.001: Group III, IV, V, and VI significantly differed from Group II.

In this study, HAL injection led to abnormal behaviors in the forced swim test, including increased entries and time spent in the closed arm. However, pre-treatment with AETC at 200 and 400 mg/kg, b.w. showed a significant improvement compared to HAL. These findings suggest the protective effects of AETC against HAL-induced behavioral stress.

Locomotor Effect of *Terminalia chebula* fruit pulp aqueous extract on Haloperidol-induced catalepsy in Wistar albino rats by Actophotometer

The locomotor activity results after haloperidol-induced catalepsy by actophotometer are presented in (Table 4). One-way analysis of variance test results shows that significantly increased locomotor activity after haloperidol-induced catalepsy by actophotometer was noticed in 200mg/kg and 400mg/kg body weight compared to Standard Haloperidol (P<0.001). There were no significant differences noticed among the three groups. The least locomotor activity was noticed in control and Haloperidol alone exposed groups. At basal, all the groups, including HAL treated and LDCD treated were found to be comparatively the same, whereas 30 min and 60 min 100mg/kg b.w dose of AETC was less significant, but at the same time higher dose of 200mg/kg bw and 400mg/kg bw (P<0.001) AETC was seems to very significant.

Results indicate that the Control group exhibited relatively consistent mean scores throughout the observation period. In contrast, the HAL group treated with haloperidol showed slightly higher mean scores compared to the Control group, albeit not statistically significant. However, the HAL+LDCD and HAL+AETC groups at all doses demonstrated significantly higher mean scores than the Control group at different time points (P<0.05). This suggests a reduction in immobility

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or depressive-like behavior in the HAL+LDCD and HAL+AETC groups. Among the HAL+AETC groups, there is a dose-dependent trend observed. The HAL+AETC group at a dose of 200 mg/kg b.w. showed a lower mean score at 30 mins than the HAL+AETC group at 100 mg/kg b.w, although the reduction was not statistically significant. However, the HAL+AETC group at a dose of 400 mg/kg b.w. exhibited a significantly higher mean score at 30 mins than the other HAL+AETC groups ($P < 0.001$).

Table 6. Catecholamine levels

Sl. No.	Groups	Dose	Catecholamine levels in rats brain (ng/100mg of brain tissue)		
			Dopamine	Adrenaline	Noradrenaline
I.	Control	(1% Gum acacia)	7.17±0.18	0.74±0.12	48.508±0.85
II.	HAL(haloperidol)	1 mg/kg, i.p	4.86±0.26[a]	0.34±0.02[a]	24.89±0.87[a]
III.	HAL+LDCD (levodopa+Carbidopa)	30 mg/kg	6.92±0.22[b]	0.65±0.10[b]	44.23±0.91[b]
IV.	HAL+AETC	100 mg/kg b.w	5.98±0.31[b]	0.59±0.09[b]	37.74±0.79[b]
V.	HAL+AETC	200 mg/kg b.w	6.64±0.31[b]	0.65±0.06[b]	41.89±0.49[b]
VI.	HAL+AETC	400 mg/kg b.w	5.38±0.14[b]	0.53±0.03[b]	33.50±0.79[b]

Data were presented as mean ± SEM of 6 animals. Analysis involved one-way ANOVA followed by Tukey's multiple comparison tests. $P < 0.001$: Group II (negative control) significantly differed from all other groups. $P < 0.001$: Group III, IV, V, and VI significantly differed from Group II.

Effect of *Terminalia chebula* aqueous extracts on catecholamine levels of rats brain in forced swim test group

Catecholamine levels (Dopamine, adrenaline and nor-adrenaline) on antidepressant activity in rats' brain was estimated. The stress control animals showed a decrease in catecholamine levels compared to control animals. The reference control L Dopa-carbidopa and both doses of AETC significantly ($P<0.001$) enhanced the catecholamine levels compared to the haloperidol-induced behavioural model. The catecholamine levels are higher in AETC 400 mg when compared to other extracts and AETC 200 mg. Results indicate that haloperidol administration leads to a decrease in dopamine levels in the rat brain. However, the combination of haloperidol with LDCD or AETC tends to elevate dopamine levels, although not statistically significant. The treatments did not significantly affect adrenaline and noradrenaline levels.

Discussion

This study aimed to investigate the potential of *Terminalia chebula* aqueous extract at doses of 100 mg/kg bw, 200 mg/kg bw, and 400 mg/kg bw to mitigate motor behavior impairments induced by haloperidol. Our findings reveal that haloperidol injection led to dose-dependent reductions in motor activity and coordination (Dey and De, 2015). Notably, the dose-response curve displayed submaximal effects on motor coordination and exploratory behavior at 1 mg/kg (Grandjean, 2014). Phytochemical analysis uncovered neuroactive compounds in *Terminalia chebula*. The aqueous extract of its fruit pulp was selected based on its substantial yield and favourable phytochemical composition, encompassing tannins, glycosides, alkaloids, flavonoids, phenolic compounds, saponins, and carboxylic acids. Various solvents effectively extracted diverse phytochemicals from the fruits (Saini et al., 2023). Alkaloids formed reddish-brown precipitates with Dragendorff's reagent, while ferric chloride produced blue precipitates (Seeman et al., 2000). The presence of tannins in *Terminalia chebula* was supported. Glycosides reacted with naphthol and sulfuric acid to turn brick red. Steroids were detected using chloroform, acetic anhydride, and sulfuric acid. Saponins yielded white precipitates with mercuric chloride, and flavonoids caused reddish-black coloration with ferric chloride and water.

Quinine turned blue in NaOH, and neutral and phenolic compounds interacted (Geberemeskel et al., 2006). Antioxidant properties were evident as extracts reduced free radical activity by donating electrons or hydrogen atoms to DPPH, stabilizing the hydrazyl free radical into hydrazine, indicating their reducing ability

To assess the antidepressant effects of *Terminalia chebula*, a forced swimming test (FST) and tail suspension test (TST) were conducted. The aqueous extract of *Terminalia chebula* fruit pulp exhibited dose-dependent reductions in immobility, indicating antidepressant potential. TST results showed improved neuromuscular coordination and strength, correlating with earlier research (Shankara et al., 2016).

HAL-induced reversible D2 receptor blockade generates free radicals, further contributing to

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mitochondrial dysfunction and neuronal death (Nosalova et al., 2013). This effect was comparable to fluoxetine and imipramine, validating its antidepressant-like action. The aqueous extract's anti-immobility effects in FST align with the involvement of the serotonergic system in *Terminalia chebula*'s antidepressant action (Kesharwani et al., 2017). *Terminalia chebula* holds promise as a therapeutic resource for various ailments like urolith formation, piles, constipation, general weakness, and stomach ulcers. However, limited research has been conducted on its active phytoconstituents. Continued exploration of these compounds could lead to novel, high-quality medications.

Our study demonstrated promising results, but further research is essential to fully realize the plant's potential (Tiwari, 2023). We administered *Terminalia chebula* aqueous extract at 100, 200 and 400 mg/kg of body weight to examine its antidepressant effects.

The forced swim test, a common tool for assessing antidepressant efficacy, was employed. Animals subjected to confined swimming display behavior indicative of hopelessness, mirroring depressive states in humans (Bakhshi, 2023). HAL administration led to abnormal behaviors in FST, including increased entries and time spent in closed arms. Pre-treatment with the aqueous extract at 200 and 400 mg/kg b.w. doses. Counteracted these effects, suggesting a protective influence against HAL-induced behavioral stress.

We also assessed catecholamine levels to gauge the effectiveness of herbal extracts in mitigating depressive episodes. Brain tissue analysis showed higher neurotransmitters such as dopamine, adrenaline, and noradrenaline levels. AETC demonstrated the potential to increase neurotransmitter levels, particularly at higher doses, indicating its positive impact on brain function (Chen et al., 2005). Research on serotonin, norepinephrine, and dopamine supports their role in diverse cerebral functions, including cognition, attention, mood, reward processing, appetite, and sleep, further confirming the positive impact of AETC treatment.

Conclusion

The selection of *Terminalia chebula* for this study was rooted in consultations with herbalists and a comprehensive review of ethnopharmacological literature. High-quality dried fruit pulp was meticulously sourced from plants within the Sathyamangalam Wildlife Sanctuary in Tamil Nadu, guided by an experienced Ayurvedic physician and researcher. These collected fruits were carefully air-dried and coarsely ground. Before embarking on phytochemical investigations, the dried fruits underwent a series of processes, including coarse powdering and continuous boiling in solvents of varying polarity. The resultant phytochemical analysis unveiled the presence of bioactive compounds with potential therapeutic applications in neurodegenerative diseases.

From a range of extracts, the aqueous extract of *Terminalia chebula* fruit emerged as the prime candidate for its exceptional neuroprotective potential against behavioral deficits induced by Haloperidol (HAL) in rats. This choice was informed by both percentage yield and phytochemical data. The aqueous extract's ability to shield rats' brains from HAL-induced neuroinflammation was particularly noteworthy. Compounds such as flavonoids, tannins, and polyphenols, recognized for their antioxidant properties, were closely examined. Behavioural studies and biochemical estimations suggest that *Terminalia chebula* dried fruit pulp harbors neuroprotective capabilities.

The outcomes of this study provide compelling evidence of the significant neuroprotective attributes embedded within the aqueous extract of dried *Terminalia chebula fruit* pulp. Nevertheless, realizing its full potential requires further investigation, particularly in isolating and characterising the specific compounds responsible for these neuroprotective effects. These research gaps underscore the need for continued exploration, offering a promising avenue for enhancing our understanding of *Terminalia chebula's* therapeutic potential in addressing neurological complications.

Conflict of interests

The authors declare that there are no conflicts of interest associated with the publication of this work.

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