Neuroprotective Potential of *Lawsonia inermis* Against β-Amyloid-Induced Neurotoxicity

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Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, synaptic dysfunction, and neuronal loss, with β-amyloid (Aβ)-induced neurotoxicity playing a central role in disease progression. The accumulation of Aβ peptides initiates a cascade of pathological events, including oxidative stress, mitochondrial dysfunction, neuroinflammation, apoptosis, and impaired cholinergic neurotransmission, ultimately leading to neuronal degeneration. Despite the availability of symptomatic therapies, effective disease-modifying treatments remain limited, prompting the exploration of medicinal plants as alternative neuroprotective agents. *Lawsonia inermis* L. (henna), a medicinal plant widely used in traditional medicine, is rich in biologically active phytochemicals such as lawsone, flavonoids, phenolic acids, coumarins, tannins, and triterpenoids. These constituents possess potent antioxidant, anti-inflammatory, anti-apoptotic, and enzyme-modulating properties that may counteract multiple pathological mechanisms associated with Alzheimer's disease.

The present study aims to investigate the neuroprotective potential of *Lawsonia inermis* against β-amyloid-induced neurotoxicity through a comprehensive evaluation of its phytochemical composition, antioxidant capacity, cholinesterase inhibitory activity, and ability to attenuate Aβ-mediated neuronal damage. The proposed research includes phytochemical characterization of the extract using advanced chromatographic techniques, followed by in vitro assessment in neuronal cell models exposed to Aβ peptides. Cellular viability, intracellular reactive oxygen species generation, mitochondrial membrane potential, apoptotic biomarkers, inflammatory mediators, and acetylcholinesterase activity will be evaluated to elucidate the underlying mechanisms of neuroprotection. In addition, computational approaches, including molecular docking and network pharmacology, may be employed to predict interactions between major phytoconstituents and key Alzheimer's disease targets such as acetylcholinesterase, β-secretase (BACE1), glycogen synthase kinase-3β (GSK-3β), and amyloid precursor protein processing pathways.

It is anticipated that *Lawsonia inermis* will mitigate β-amyloid-induced neuronal injury by reducing oxidative stress, suppressing neuroinflammatory responses, preserving mitochondrial function, inhibiting apoptotic signaling, and improving cholinergic neurotransmission. The findings of this study are expected to provide mechanistic evidence supporting the therapeutic potential of *Lawsonia inermis* as a multi-target neuroprotective agent and establish a scientific foundation for the future development of plant-based interventions or advanced nanoformulations for Alzheimer's disease management.

Keywords: *Lawsonia inermis*; Alzheimer's disease; β-amyloid; Neuroprotection; Oxidative stress; Neuroinflammation; Apoptosis; Acetylcholinesterase inhibition; Phytochemicals; Molecular docking; Medicinal plants.

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i. Introduction:

Henna leaves are used as a prophylactic against skin disease. They have astringent properties and antioxidant properties. They are used externally in the form of paste or decoction against boils, burns, bruises, and skin inflammations. The leaves decoction is used as gargle for relaxed sore throat. The essential oil obtained from the flowers finds use in perfumery due to its β-ionone content. Henna was also reported to have tuberculostatic activity. The roots of this plant are useful in burning sensation, leprosy, stranguary and premature graying of hair.[1]

Lawsonia (family Lythraceae) is a monotypic genus represented by Lawsonia inermis linn (syn.L.alba Lam). It is a native of the North Africa and south west Asia widely cultivated as ornamental dye plant. A glabrous, much branched shrub or small tree with grayish brown bark. Leaves opposite, sub-sessile, elliptic or broadly lanceolate, entire, acute or obtuse, often mucronulate; flowers numerous, small, white or rose-colored, fragrant, in large terminal pyramidal panicled cymes; capsule globose, about the size of a pea,with numerous, pyramidal, smooth seeds.[2]

The principal colouring matter of henna is

lawsone, 2-hydroxy-1:4 napthaquinone ($C_{10}H_6O_3$, m.p.190º) besides lawsone other constituents present are gallic acid, glucose, mannitol, fats, resin (2 %), mucilage and traces of an alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol. Flowers yield an essential oil (0.01-0.02 %) with brown or dark brown colour, strong fragrance and consist mainly of α- and β- ionones; a nitrogenous compound and resin. Seeds contain proteins (5.0 %), carbohydrates (33.62 %), fibers (33.5 %), fatty oils (10- 11 %) composed of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid and linoleic acid. The unsaponified matter contains waxes and colouring matter. The root contains a red colouring matter. The leaves have been reported to contain various compounds like coumarins, flavonoids, gallic acid, naphthoquinones, tannins, naphthalene derivatives, triterpenoids, aliphatic constituents, phenolic glycosides and xanthones .

Lawsonia inermis leaves possess antibacterial, antifungal, antiviral, anti-parasitic, antidermatophytic, tuberculostatic, antioxidant, analgesic, anti- inflammatory activity, cytotoxic activity and enzyme inhibitory activities.

ii. Drugs and Chemicals

All the drug solutions were freshly prepared prior to the experiment. Donepezil was obtained from Cipla Pharmaceutical Company, India Normal pallete diet for rodents and D- galactose and L-methionine were purchased respectively from Ashirwad Industries, Chandigarh and SISCA Laboratory, Delhi, India.

iii. Methodology

Plant collection and extraction method

The fresh *L. inermis* leaves were collected from local area of bhopal. The fresh leaves were thoroughly washed with running tap water, followed by triple- distilled water to remove the dust. The leaves were dried at room temperature (RT) for two hours (hrs) without being exposed to sunlight. After 2 hrs, it was cut into coarse and fine pieces using a blender. These cut leaves were used to make various solvent extracts ranging from non-polar to polar solvents.

200 g of cut leaves of *L. inermis* were taken in a 1000 mL round bottom flask and extracted with increasing order of non-polar to polar solvents such as n-hexane, chloroform, ethyl acetate, methanol, and triple-distilled water (aqueous) was added one by one. Each solvent was individually refluxed at 80 °C for 1 hr using the double-valve reflux condenser and the extract obtained was filtered using Whatman No. 1 filter paper. All the filtrate was concentrated under reduced pressure by rotary evaporator to obtain the residues. These residues were collected and stored separately at 4 °C in the refrigerator for further studies.[3]

iv. Characteristics of extract

Organoleptic characteristic

An organoleptic characteristic such as color and nature was characterized, and results are recorded.[4]

Melting point determination

The melting point was determined using melting point apparatus. Extract was filled in a glass capillary whose one end was sealed on flame. The capillary containing Extract was dipped in liquid paraffin bath inside the melting point apparatus. Liquid paraffin was heated till Extract melted. The temperature at which Extract melted was recorded.[5]

UV Spectroscopy (Determination of λmax)

Extract stock solution (100 µg/ml) was made in water. This solution was properly diluted using water separately, to achieve a concentration of 10 µg/ml. The UV visible spectrophotometer recorded the UV spectrum in the 200–400 nm range.Different dilutions are prepared for calibration curve. [6]

Preliminary Phytochemical Screening

The residues of aqueous fraction were subjected to a preliminary qualitative phytochemical investigation according to the standard procedures.[7]

Quantitative estimation of flavonoids and phenolics

Total phenolic content

The total phenol content was estimated by the standard Folin–Ciocalteu method .0.1 mL of 0.5 N Folin-Ciocalteau reagents were mixed with 0.5 mL of different concentrations (10, 20, 40, 60, 80, and 100 µg/mL) of test samples respectively. The reaction was carried out to incubate in a dark place at room temperature for 15 min. Then 2.5 ml of 2 M sodium carbonate was added, and the mixture was again incubated in the dark at RT for 30 min. After 30 min, the absorption of the reaction mixture was measured at 765 nm using UV-vis spectra. The average absorption values obtained at different concentrations of std. gallic acid were used to plot the calibration curve. The total phenolic content was calculated as std. Gallic acid equivalent. [8]

Estimation of total flavonoid content

The prepared extract was estimated for its total flavonoid content by the aluminium trichloride colorimetric method. For the study, Rutin was used as the standard. The stock solution of Rutin was prepared by dissolving 5.0 mg Rutin in 1.0 mL ethanol. The stock solution was further diluted with methanol to prepare a solution having concentrations 10-100 µg/ml. One ml of these dilutions was mixed with 1 mL of 10 % ethanolic aluminium trichloride and incubated for 30 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 430 nm using UV- Visible spectrophotometer. Similarly extract was mixed with 10 % ethanolic aluminium trichloride solution, and absorbance was measured. The concentration of total flavonoid content in the

extract was measured using the calibration plot for Rutin and reported as mg Rutin equivalent (QE)/g of dried plant material.[9]

v. Assessment of *in vitro* antioxidant activity for *L. Inermis* leaf:

DPPH Radical Scavenging Activity:

DPPH was used to test extract of *L. Inermis* leaf's ability to scavenge free radicals. In a brief, 0.6 mM DPPH was dissolved in ethanol and 0.5 ml ml of this solution was combined with 0.5 ml of MELIS at varying concentration (2-10 g/ml). With ethanol, the volume of this reaction mixture was increased to 5 mL. Readings were taken at 517 nm using a UV-Vis spectrophotometer using ethanol as a blank. Ascorbic acid was utilised to compare the results of extract of leaf of *L. Inermis*. The following calculation was used to compute the % inhibition of DPPH by seed extract. Assay was carried out for three times and the readings were averaged.[10]

Hydrogen Peroxide Scavenging Property:

H_2O_2 scavenging activity of the extract of leaf of *L. Inermis* was tested in a 50 mM phosphate buffer with 10 mM H_2O_2. Different concentrations of extract of leaf of *L. Inermis* (2-10 g/ml) was combined with 2 ml of H_2O_2solution and kept at room temperature for 30 minutes. A UV-Vis spectrophotometer was used to read un-reacted H_2O_2 at 230 nm, whereas phosphate buffer was used as a blank. The DPPH assay formula was used to calculate the % of scavenge for H_2O_2 at each concentration. The percent H_2O_2 scavenging of extract of leaf of *L. Inermis* was compared to Ascorbic acid, a standard scavenger, and values were taken as an average from three replicates.[11]

Fe3+ Reduction Capacity:

The Fe3+ Reduction ability of extract of leaf of *L. Inermis* was performed by taking different concentration ranging from 2-10 g/ml extract and 0.05 ml of 2 mM $FeCl_3$ solution into a test tube. Addition of 0.2 ml of 5 mM ferrozine to the above content was started the reaction. Further, the reaction mixture was incubated for 10 minutes at RT. In a UV-Vis spectrophotometer, all of the measurements for varying concentrations of the extract were taken at 562 nm. The same assay was performed at triplicates, and the results were averaged. The Optical Density values of all of these solvent extracts were compared to that of Ascorbic acid, a known antioxidant.[12]

Molybdenum Reduction Capacity:

The ability of the extract of leaf of *L. Inermis* to reduce molybdenum was tested at varied concentrations (2-10 g/ml). In a test tubes 0.5 ml of varying doses of extract of leaf of *L. Inermis* (2-10 g/ml) was added to 4.5 ml of molybdneum reagent solution. The blank was made in the same way, where instead of extract, ethanol was used. Now, incubated for 90 minutes, refrigerated, and the absorbance at 695 nm was measured against a blank in a UVVis spectrophotometer. As a reference, Ascorbic acid was used. The amount of extract with the highest Optical Density value was used to evaluate which had the most antioxidant activity.[13]

vi. In vitro acetylcholinesterase inhibitory activity

The spectrophotometric method previously reported by Ellman et al 1961 was used to evaluate the enzyme inhibition activities for acetylcholinesterase (AChE). 1500 µL phosphate buffer (pH 8), 200 µL AChE solution (0.3 U/mL), 200 µL test sample (2 mg/L) and 1000 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (3 mM) were mixed in a 1 cm path length glass cell and incubated at 37°C for 15 minutes. The reaction mixture was then supplemented with 200 µL ATCI (15 mM). The samples were incubated for another 10 min at room temperature before being treated with 200 mL galantamine hydrobromide (1 mg/mL) to stop the reactivity. The absorbance of the produced yellow 5-thio-2-nitrobenzoate anion was measured at a wavelength of 412 nm, spectrophotometrically (Shimadzu, USA). A control mixture was created without the addition of extract. The average of triplicates was used to calculate the results. The enzyme inhibition (%) was calculated from the rate of absorbance change with time. All of the measurements were made three times and the averages were plotted on a graph.[14]

vii. Anti-inflammatory activity determination of extract

In vitro anti-inflammatory activity using protein denaturation assay

The in vitro anti-inflammatory activity of henna leaf extract (*Lawsonia inermis*) was evaluated using the protein denaturation method with Bovine Serum Albumin. A 1% aqueous solution of BSA was prepared, and 0.5 mL of this solution was mixed with 0.5 mL of henna leaf extract at different concentrations. The pH of the reaction mixture was adjusted to about 6.3 using dilute hydrochloric acid. The samples were incubated at 37°C for 15 minutes and then heated at 70°C for 5 minutes to induce denaturation. After cooling to room temperature, the turbidity of the samples was measured spectrophotometrically at 660 nm. A control was prepared by replacing the extract with distilled water, while a standard was prepared using Diclofenac sodium. The percentage inhibition of protein denaturation was calculated by comparing the absorbance of the test samples with that of the control, and the anti-inflammatory activity of the extract was determined accordingly.[15,16]

viii. In Vivo Studies of extract (Scopolamine-induced model of AD)

The study was performed using adult female

Wistar rats weighing 250-300 g, aged 6- 7 months. The animals were housed in polypropylene cages (12-h light/dark cycles) under standard laboratory conditions. The room was maintained at 25 ± 2 °C and relative humidity of 60 ± 5%. Animals had free access to a commercial pellet diet and water ad lithium. Pre-training was conducted on animals and those with failure to learn were excluded. A total of 48 rats were selected and divided into 8 groups of 6 rat each: First group received i.n. saline (control group), second group received scopolamine + intra nasal saline (scopolamine group), third group received rivastigmine patch followed by scopolamine (rivastigmine group), three treatment groups received intranasal at dose of 100 mg/kg , 300 mg/kg and 500 mg/kg followed by scopolamine. The rats were pretreated with either intra nasal saline (10 µL) or rivastigmine patch (Exelon Patch, Novartis 13.3 mg/ 24h) for 14 days, however i.n. extract (10 µL) was administered once on day to day 9. From day 9 onwards for next 5 days the animals (except control group) were daily injected with intraperitoneal scopolamine (1 mg/kg) to induce experimental AD. The scopolamine injection was administered 30 minutes post- treatment. The animals were then subjected to the cognitive behavioral test including MWM and PSA.[17]

Morris water maze (MWM) test

The MWM test consists of a pool (diameter 150 cm and height 50cm) filled with water (26 ± 2°C) up to the depth of 30 cm. The pool was hypothetically divided into four quadrants. The test was conducted in two phases, training and experimental . Training trial (day 9 onwards) was conducted every day for 5 consecutive days immediately after administration of scopolamine. During training phase, an escape platform (diameter 10 cm) was kept 1 cm above the water level. Rat is placed into the pool at a random location near the edge of the pool and is allowed to locate the escape platform. Each rat was given a maximum time of 120 s to locate and climb the platform. In case the rat was unable to find the platform, it was manually guided or placed on the platform and allowed to stay there for 60 s. Total of four trials were conducted each day. The inter-trial time was two minutes and the same animal was placed into the pool at different location. Over all time required by the rat to locate and climb the platform was determined as escape latency. The experimental phase was conducted immediately after training phase and also after 24-h after the last training session. In experimental phase, the escape platform was removed from the water maze and each rat was given a maximum of 120 s to memorize the platform. The parameters such as escape latency, dwell time (time spent in the target quadrant) and path length were evaluated.[18,19]

Passive Avoidance

A fear-based PSA test is to assess the ability of rats to learn from an aversive event, was conducted in two phases. Assembly used in the test consists of a well-illuminated compartment and a dark compartment interconnected with each other through an automatic sliding door. The dark chamber was equipped with an electrified grid floor. For initial 5 min, the animals were familiarized with the assembly followed by training phase after a gap of 15 min. The rat was kept in the illuminated chamber and the sliding door to the dark chamber was opened after 30 s. Step-through latency was measured as time taken by rat to enter the dark chamber. If the rat failed to enter the darkroom within 300 seconds, it was excluded from the test. Once the rat entered the dark chamber, the sliding door was closed and an inescapable electric foot-shock of 0.5 mA for 3 seconds was given through the electrified grid floor. At the end of the training trial, the rat was placed back into the cage. The assembly was cleaned thoroughly between training sessions to remove olfactory cues. An experimental trial was performed, 24-h post training phase. The time taken by the rat to completely (four paws in) enter the darkroom was recorded. During the experimental phase no foot shock was given. If the rat took more than 300 seconds to enter the darkroom then it was returned to the cage and step-through latency was noted as 300 seconds.[20]

ix. Biochemical Estimation Tissue Preparation

Immediately after behavioral tests, the rats were sacrificed and the brain was removed from the skull following the heart perfusion technique to remove any blood clots that might obscure the results. The brain homogenate was prepared by washing the brain with chilled isotonic saline and homogenizing using pH 7.4 phosphate buffer. The homogenized tissue sample was cooled centrifuged at 10,000 g for 15 min and supernatant was collected. The brain homogenate so obtained was stored at cool conditions till further used.[21]

SOD (Superoxide Dismutase) Estimation

This enzyme functions by eliminating the damage causing superoxide radical to hydrogen peroxide and oxygen. It owns a free radical scavenging property reducing damaging effects just like antioxidants. The estimation is based on inhibiting the auto oxidation of Pyrogallol by the SOD enzyme. For measurement of Superoxide Dismutase enzyme (SOD) activity, the brain homogenate (20µl) was mixed with tris-HCL buffer solution prepared in ethylenediaminetetraacetic acid (pH=8.2, 940 µl). To the mixture, pyrogallol (40 µl, 13mM) was added and absorbance was taken at 420 nm.[22]

GSH (Glutathione) Estimation

Glutathione is a peptide with low molecular weight that functions as an antioxidant. It basically breaks down hydroxyl free radicals and reduces oxidative

stress. The estimation method is derived from a method. Glutathione (GSH) level was estimated in brain homogenate by colorimetry. Briefly, 10% trichloroacetic acid was mixed with an equivalent quantity of brain homogenate. To the mixture, 5.5-dithiobisnitro benzoic acid (0.5 mL) was added, immediately after the absorbance was recorded at 412 nm.[23,24]

x. Result

Preliminary study of extract

Table 1: Preliminary study of extract

Sr. No.	Parameters of extract	Observation
1.	Colour	Brownish green
2.	Appearance	Amorphous, non crstalline
3.	Melting point	195 oC
4.	pH	5.7

Phytochemical screening results of *lawsonia inermis* leaf extract

This describes the solubility and preliminary qualitative phytochemical screening results of n-hexane, chloroform, ethyl acetate, methanol, and aqueous fraction residues from *L. inermis* leaf.

Solubility Test

The n-hexane, chloroform, ethyl acetate, methanol, and aqueous fraction of the *L. inermis* extract was concentrated to obtain the residues having weights 0.33 g, 0.843 g, 0.567 g, 2.412 g, and 5.211 g respectively. All the fractions were tested in the solubility in dimethyl sulphoxide (DMSO) and water.

Table 2: Solubility of the *L. inermis* leaf extracted fractions

S.No.	Fraction	Solvents	
		DMSO	Water
1.	n – Hexane	Soluble	Insoluble
2.	Chloroform	Soluble	Insoluble
3.	Ethyl acetate	Soluble	Insoluble
4.	Methanol	Soluble	Partially soluble
5.	Aqueous	Soluble	Soluble

Phytochemical screening of aqueous fraction of extract

Table 3: Phytochemical screening of aqueous fraction of extract

Phytochemical screening of Aqueous Fraction	
Phytochemicals	Result
Isoflavonoids	Present
Alkaloids	Absent
Flavonoids	Present
Steroids	Absent
Terpenoids	Absent
Saponins	Present
Phenols	Present
Carbohydrates	Present
Amino acids	Present
Glycosides	Present
Anthraquinone	Absent
Tannins	Present

From the results of a preliminary phytochemical a screening of all five fractions of *L.inermis* leaf, most of the pharmaceutically valuable phytochemical compounds are found to be present in the methanol and Aqouos fraction. Based on the eco-friendly concept, the Aqouos fraction was used for further studies.

Quantitative estimation of phytoconstituents

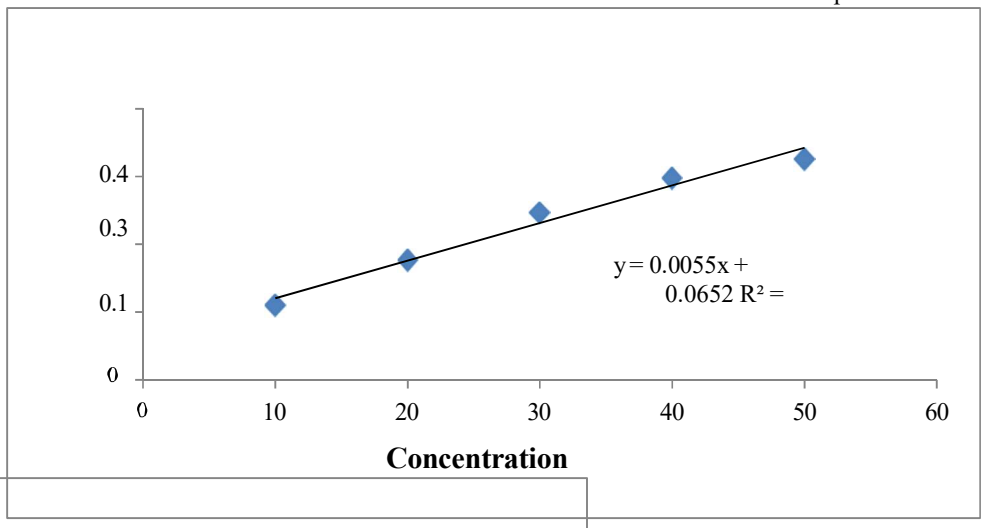
Quantitative estimation of total phenolic

Table 4: Standard curve (Concentration Vs Absorbance)

1.

S. No.	Conc. (µg/ml)	Absorbance (^max= 760 nm)
1.	10	0.110
2.	20	0.176
3.	30	0.247

4.	40	0.298
5.	50	0.326



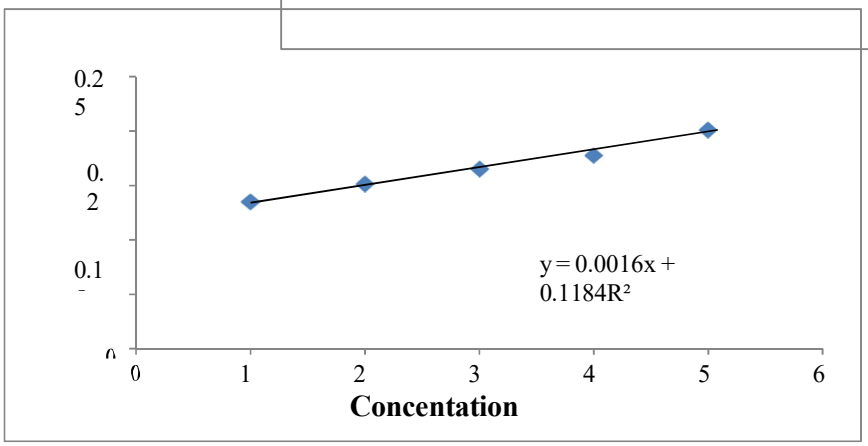


Figure 1 : Calibration graph for gallic acid standard

Quantitative estimation of total flavonoid

Table5: Standard curve (Concentration Vs Absorbance)

S. No.	Conc. (µg/ml)	Absorbance(^max= 510nm)
		Rutin
1.	10	0.135
2.	20	0.151
3.	30	0.165
4.	40	0.177
5.	50	0.201

Figure 2 : Calibration graph for rutin standard

The standard curve for gallic acid using folin-ciocalteu method was found linear with regression coefficient R^2 = 0.9761. Total phenolic content in leaf extracts was found to be 134.4±0.917GAE/g, . The standard curve for rutin using aluminium trichloride colourimetry method was found linear with regression coefficient R^2 = 0.9856. Total flavonoid content in leaf extracts was found to be 157.67±3.512Rutin/g .

Determination of Antioxidant activity of leaf extract:

Table 6: Antioxidant activity assay of leaf extract

Conc. (µg/mL)	DPPH	Hydroxyl radical	Ferrous reduced	Molybdenum

					uction		**eduction**	
	Extract	**Gallic acid**	**Extract**	**Gallic acid**	**Extract**	**Gallic acid**	**Extract**	**Gallic acid**
2	38.62	48.5	48.331	47.3	25.28	29.8	22.23	25.54
4	43.12	62.5	56.53	56.63	39.52	42.61	36.67	36.14
6	54.87	79.5	65.32	63.526	58.25	54.81	48.53	42.73
8	77.37	83+663	78.32	76.423	70.95	67.18	55.42	54.34
10	91.62	98.37	89.01	82.417	81.14	87.96	60.68	64.65

The oxidation induced by ROS can result in membrane protein damage, cell membrane disintegration and DNA mutation, which can induced or stimulates propagate the development of many diseases of high risk such as cancer, liver injury, cardiovascular disease and neurodegenerative disorders . Although the body itself has an in built nature defense mechanisms, as enzymes and antioxidant nutrients, they protect the body from damaging properties of ROS . Phytochemical as antioxidants are potential free radical scavengers which help to reduced ROS related diseases in animals and plants. It was well established that generally, hydrogen peroxide, a major source of hydroxyl radical is release into cellular environment during respiration. Nevertheless, its presence in large quantities in cells reacting with metals and finally produce highly toxic hydroxyl radicals intern which induce cellular damage.

In-vitro anti-inflammatory activity of leaf extract

Table 7: Percent Protein denaturation inhibition

Concentration (µg/mL)	**% Inhibition**
Control	0.0
10	20.73
20	34.15
30	58.78
40	73.63
50	82.31
Diclofenac (100 µg/mL)	83.39

AchE inhibition activities of leaf extracts

Table 8: AchE inhibition activities of leaf extracts

Conc. (µg/ml)	**AchE inhibition activities of leaf extracts** Mean ± SD (n = 3)	
	Leaf Extract	**Galantamine (Standard)**
10	5.43±0.12	7.94 ± 0.04
25	17.13±0.12	16.08 ± 0.08

40	32.72 ± 0.12	38.61 ± 0.12
50	49.03 ± 0.14	48.69 ± 0.12
75	61.91 ± 0.16	60.33 ± 0.08
100	84.52 ± 0.25	84.02 ± 0.08

In vivo studies of leaf extract (*Lawsonia inermis*)

Morris Water Maze Test

The results of the MWM test during training and experimental phase are shown in Figure. The escape latency in the control group (89.4 ± 4.61 s) was found to be significantly lower ($p < 0.001$) than scopolamine group (129.8 ± 4.60 s), however the values were similar to rivastigmine group (89.6 ± 6.30 s). Intranasal leaf extract (*Lawsonia inermis*) exhibited dose-dependent decrease in escape latency was significant from scopolamine group ($p < 0.001$) but not significantly different ($p > 0.05$) from control and rivastigmine group . Similar observations were recorded for path length, where values for control, rivastigmine, and test groups were insignificantly different ($p > 0.05$). However, the values were significantly ($p < 0.001$) lower than scopolamine group. Dwell time results showed that scopolamine group animals spend significantly less time in the target quadrant ($p < 0.001$) depicting impairment of memory. However, Intranasal leaf extract (*Lawsonia inermis*) treated rats showed significant improvement ($p < 0.001$) in dwell time compared to scopolamine group and the values were similar to control group. Further, the results for testing and experimental phase corroborated with each other.

Table 9: Data of Morris Water Maze Parameters

Study	Morris Water Maze Parameters		
	Escape Latency	Path Length	Dwell Time
Group	Mean time (sec) (n=5)	Mean (mm) (n=5)	Mean time (sec) (n=3)
Control Group	89.4 ± 4.61	1601.5 ± 69.85	33.66 ± 1.52
Scopolamine Group	129.8 ± 4.60	3560.8 ±12.48	13.66 ± 2.08
Rivastigmine Group	89.6 ± 6.30	1575.1 ± 61.29	33.66 ± 2.51
Extract(100mg/kg)	98.4 ± 3.20	2013.4 ± 12.33	29.33 ± 2.51
Fxtract(300mg/kg)	88.4 ± 2.88	1610.4 ± 46.59	33.33 ± 1.52
Extract(500mg/kg)	85.4 ± 3.50	1543.6 ± 34.16	35.66 ± 2.08

Passive Avoidance

The results of the PSA test is shown in Figure, As expected, the retention latency of the scopolamine group was significantly lower (68.0 ± 2.64) than control group (190.0 ± 5.0). Further, intranasal leaf extract (*Lawsonia inermis*) resulted in dose-dependent increase in retention latency and was not significantly different ($p > 0.05$) from control and rivastigmine group. Notably the results of the experimental phase and training phase were similar for both MWM and PSA test.

Table 10: Data of Passive Avoidance

Study	Passive Avoidance
Group	Mean time(sec)
Control Group	190 ± 5.00
Scopolamine Group	68 ± 2.64
Rivastigmine Group	200 ± 5.29
Extract (100mg/kg)	149 ± 3.60
Extract (300mg/kg)	188 ± 6.00
Extract (500mg/kg)	198 ± 3.5

Biochemical Estimation

Superoxide Dismutase (SOD) and Glutathione (GSH) Estimation

The results of the biochemical estimation of the brain homogenate shown that the SOD levels of the control group (10.33 ± 0.57 μMmg^{-1}) was significantly higher ($p < 0.001$) than that observed for scopolamine group (3.76 ± 0.15 μMmg^{-1}) but was not significantly different ($p > 0.05$) from rivastigmine group (10.40 ± 0.30 μMmg^{-1}), intranasal leaf extract (*Lawsonia inermis*) also showed increase in SOD levels but the values were significantly less ($p < 0.001$) than control group. Similar results were also observed for GSH level where scopolamine group showed significantly lower ($p < 0.001$) GSH level (1.96 ± 0.35 μMmg^{-1}) compared to control (7.06 ± 0.30 μMmg^{-1}). Nevertheless, the GSH levels of rivastigmine group (7.30 ± 0.45 μMmg^{-1}) and intranasal leaf extract (*Lawsonia inermis*) were not significantly different ($p > 0.05$) from control.

Table 13: Superoxide Dismutase (SOD) and Glutathione (GSH) Estimation

Study	SOD	GSH
Group	µM/mg protein (n=3)	µM/mg protein (n=3)
Control Group	10.33 ± 0.57	7.06 ± 0.30
Scopolamine Group	3.76 ± 0.15	1.96 ± 0.35
Rivastigmine Group	10.40 ±	7.30 ±

	0.30	0.45
ALE(100mg/kg)	6.80 ± 0.20	5.46 ± 0.21
ALE(300mg/kg)	9.86 ± 0.5	6.93 ± 0.63
ALE(500mg/kg)	10.66 ± 1.2	7.46 ± 0.38

The link between oxidative stress and AD is well established, therefore, targeting this event might be beneficial. Several antioxidants including vitamins, curcumin, berberine, quercetin, resveratrol and melatonin exhibited promising results in AD models . Recently, leaf extract (*Lawsonia inermis*) have emerged as a regenerative antioxidants and their therapeutic potential in the neurodegenerative disorders including AD has been explored. Several in vitro studies demonstrated that leaf extract (*Lawsonia inermis*) decreases protein accumulation, alleviate Aβ-induced cell death and trigger signal transduction pathway of neuronal survival . Current evidence suggest that intranasal delivery of drugs or particles allows their direct brain targeting and it hypothesized that delivery of leaf extract (*Lawsonia inermis*) directly into the brain would prevent the behavioral changes in experimental AD model. Intranasal delivery could also result in reduction of dose of leaf extract (*Lawsonia inermis*). In the present study, for the very first time we reported the efficacy of intranasal leaf extract (*Lawsonia inermis*) in the management of cognitive effects in experimental scopolamine-induced AD.

Conclusion

Since the connection between oxidative stress and AD is well established, this event might offer a therapeutic target. Different antioxidants were proved to be effective in AD models, including vitamins, curcumin, berberine, quercetin, resveratrol and melatonin. Lately leaf extract (Lawsonia inermis) have gotten a regenerative antioxidants and their healing potential within the neurodegenerative issues which includes AD have been examined. Multiple in vitro studies revealed that leaf extract (Lawsonia inermis) reduces protein aggregation, mitigates Aβ-induced cell death and stimulates neuroprotective signaling. It has been postulated that completely by-pass the blood brain barrier in a controlled manner, drugs or particles delivered through the intranasal route can go to their target in the brain directly and current evidence from other laboratories also indicate thatIN delivery of herbal preparations like leaf extract (Lawsonia inermis) can deliver herbs directly to their targets in the CNS and gonads to prevent behavioral changes induced my experimental cumbersome AD model. By intranasal delivery, this may even be able to reduce the dose of leaf extract (Lawsonia inermis). In the current study, for the first time we demonstrated potential effect of intranasal leaf extract (Lawsonia inermis) on cognitive function in an animal model of scopolamine-induced AD.

An AD patient is characterized by cognitive and behavioral decline, which gradually decreases due to the loss of neurons and synapses in the hippocampus and related areas Therefore, MWM test, PSA test as well as biochemical estimation of brain was carried out. The MWM test assesses both spatial memory deficits that can be affected by the age and AD-like, while the PSA test evaluates long-term contextual memory. In this study, we found that intraperitoneal injection of scopolamine in the rats led to a significant impairment in cognition or memory function. The escape latency, path length, and dwell time and retention latency values of scopolamine group were significantly ($p < 0.001$) different than the control group. Furthermore, the levels of antioxidant enzymes such as SOD and GSH in the brain were also significantly reduced in the scopolamine-treated group. For this study, rivastigmine transdermal patch was used as a reference drug for comparison and also titration of the i.n. leaf extract (Lawsonia inermis). Since leaf extract (Lawsonia inermis) is known to undergo redox & regenerative properties, thus it was assumed that a single dose may result in long term effects. MWM and PST test results indicated that both i.n. leaf extract (Lawsonia inermis) modulate cognitive functions with a dose-dependent response.

The results for the maximum wound closure percentage (MWM) and ratio of wound healing (RHarmonic) per day were nearly equal in i.n. leaf extract (Lawsonia inermis), control and standard treatment. The observations depicting retention latency showed the similar type of effects with increasing dose of i.n. leaf extract (Lawsonia inermis) and were matched to control and rivastigmine group. Leaf extract (Lawsonia inermis) for protection against behavioral changes during progression of AD, however further studies with more number of animals are warranted to derive a conclusion. In addition, results of biochemical analysis revealed that SOD and GSH levels are maintained after a single dose i.n. pre-treatment with (Lawsonia inermis) as leaf extract. Conclusion: intranasal administration of leaf extract (Lawsonia inermis) significantly attenuated impairment of cognitive functions (spatial and non-spatial memory) associated with scopolamine induced AD model. Consequently, the intranasal leaf extract i.e., Lawsonia inermis, can be an effective treatment for reducing symptoms caused by AD since it has potent and restorative antioxidant activity

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