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Original Research Article

Lavandula stoechas L alleviates dementia by preventing oxidative damage of cholinergic neurons in mice brain

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Abstract

Purpose: *To ascertain if there is any scientific evidence for the folkloric use of Lavandula stoechas as a nootropic substance for memory-related disorders.*
Methods: *Aqueous and n-hexane fractions of methanol extract of aerial parts of L. stoechas were used to explore their anti-amnesic activity. Male albino mice weighing 25 - 30 g were organized in ten groups (G-I to G-X) and treated for seven days. On 7th and 8th day, behavioral studies were conducted using elevated plus maze, hole-board and light-dark paradigms. Furthermore, biochemical studies were performed on brain homogenates of mice.*
Results: *Half-maximal inhibitory concentration (IC_{50}) values for aqueous and n-hexane fractions of L. stoechas were recorded as 35.14 and 357.42 µg/mL respectively, as compared to the standard, ascorbic acid (51.39 µg/mL). Behavioral test results indicate that the aqueous fraction showed significant ($p < 0.001$) build-up of memory in mice, compared to n-hexane fraction. Similarly, the aqueous fraction significantly ($p < 0.001$) reduced the level of acetylcholinesterase and malondialdehyde as well as elevation of natural antioxidants, viz, superoxide dismutase, catalase and glutathione in brain tissues.*
Conclusion: *The aqueous fraction of L. stoechas is a memory-enhancing agent. Its antioxidant activity prevents the loss of memory by providing defense against neurodegeneration.*

Keywords: *Acetylcholinesterase, Elevated plus maze, Behavioral studies, Lavandula stoechas, Malondialdehyde*

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INTRODUCTION

Alzheimer's disease (AD) refer to a memory disorder caused by progressive neuronal decay in brain hippocampus and amygdale. This is the leading cause of dementia, especially in old age and affects not only the behavior but also the social compliance of the patient [1]. AD is developed in the latter half of the life either due to deposition of fats, beta amyloid proteins and nucleic acids in neuronal cell bodies predisposing to inflammatory cell damage [2]. Septohippocampal cholinergic neuronal innervations are impaired in most of the patients of AD and leading to the progression of irreversible dementias [3]. Furthermore, toxic free radicals and oxidants produced within the body

affects memory and cognition by damaging the cholinergic pathways of hippocampus of brain [4].

Patients suffering from AD are given symptomatic treatment like piracetam, donepezil and galantamine which merely improve the patient's conditions by increasing the level of acetylcholine without preventing the neuronal decay [5]. Alzheimer's patients feel themselves more miserable because no proper therapy is available for the management of the disease and many of the drugs used in its treatment are coupled with untoward effects. Thus, the trend is shifting towards the use of herbal medicines which are considered more reliable, gentle, harmless and free of toxic effects [6]. All over the World, almost 150 herbal products have been tested so far for the management of AD which not only improve the symptoms of the patients but also prevent the spread of neurodegeneration [7]. This is really a valuable approach to investigate the herbal bio ingredients which are actually responsible to provide defense against neurodegeneration. This will endorse the importance of customary herbal medicines in the area of neuropsychopharmacology [8].

Lavandula stoechas (L) has been in common traditional practice of conventional healers, who used this herb for the prevention and treatment of migraine headache, epilepsy and all sorts of dementias. Owing to its powerful neuroprotective activity, it is called broom of the brain [9]. The focus of this study is to provide pharmacological basis of neuroprotective efficiencies by which *L. stoechas* enhances memory as claimed by local herbalists.

EXPERIMENTAL

Chemicals

Scopolamine and piracetam were gifted by *Merck Pharmaceutical Pvt. Ltd. Pakistan* and *Jiangxi Yuehua Pharmaceutical, China, respectively for research purpose.* Aluminium chloride, acetylthiocholine iodide*2,2-diphenyl-1-*picrylhydrazyl (DPPH),carboxy methyl cellulose (CMC), 5,5-dithiobis-*2*-nitrobenzoic acid (*DTNB*), *Folin-Ciocalteu* reagent (*FCR),* nitro blue tetrazolium (NBT), hydrogen peroxide (H_2O_2), potassium dichromate, rutin, sodium dodecyl sulfate, trichloracetic acid (TCA), superoxide dismutase (SOD), thiobarbituric acid (TBA), potassium acetate, phenyl methanesulfonate (PMS), reduced nicotinamide adenine dinucleotide (NADH) and tanic acid, were purchased from Sigma Aldrich (USA). Acetic acid, chloroform, ascorbic acid, ethanol, methanol, gallic acid, n-butanol and sodium carbonate were obtained from (Merck Germany) Ms Traders, Lahore Pakistan. All chemicals used in this research were of analytical standard.

Preparation of plant extract

Dried aerial parts of *L. stoechas* were procured from Akbari mandi Lahore, Pakistan. It was authenticated by Dr Zaheer-ud-din Khan (Assistant Professor) Department of Botany, GC-U, Lahore and was allotted herbarium voucher no GC.Herb.Bot.3386. The methanol extract was prepared by standard maceration technique by soaking 1 kg plant material into 5 L of methanol. Methanolic crude extract was used for liquid-liquid extraction to obtain different fractions by subsequently passing *n*-hexane, chloroform, ethyl acetate and *n*-butanol from methanolic extract suspended in water. Then solvents were evaporated in rotary evaporator to get different fractions of *L. stoechas* extract and percentage yield of each was found. Finally, *n*-hexane (nhL.s) and aqueous fractions (aqL.s) were investigated for pharmacological actions.

Phytochemical investigations

Phytochemical studies were performed on both fractions for detection of alkaloids, carbohydrates, proteins, flavonoids, glycosides, saponins, terpenoids, steroids, phenols, tannins, terpenes, quinines, fixed oils and phytosterols [10]. Then phenolic contents present in both fractions were quantitatively analyzed using FCR method [11]. Flavonoids present in *n*-hexane and aqueous fractions of *L. stoechas* were found by aluminium chloride method [11] and modified Folin Ciocalteu [12] method was used to assess quantity of tannins per g of plant extract.

In vitro antioxidant activity

In vitro antioxidant activity of both fractions was determined by DPPH free radical scavenging assay. Stock solution of reagent was prepared by dissolving 1 mmol/L of DPPH in methanol and was covered with aluminium foil. Standard and sample solutions were made by dissolving different concentrations (20–200 µg/mL) of ascorbic acid and plant fractions, respectively in methanol. Reagent solution (2 mL) was added in each test tube containing either sample or standard solution and incubated for half an hour in dark room. Absorbance of sample and standard solution was measured at 527 nm against blank and evaluated as in Eq 1 [13].

% scavenging = Ab–As/Ab x 100 ……. (1)

where Ab and As represent absorbance of the blank and standard solutions, respectively.

Animals

Swiss male albino mice of weight 25 - 30 g were kept in animal house of PUCP, Punjab University Lahore. The animals were used in this research after obtaining approval from institutional animal ethics committee (ref no. AEC/PUCP/1072) ruled under the guidelines of Institute of Lab Animal Resources, Commission on Life Sciences, University National Research Council (1996). Standard living conditions were provided in animal house by adjusting temperature at 25 ± 2 $^{\circ}$C, humidity at 50 ± 5% and light/dark period of 12 h. Animals were given feed and water *ad libitum* and were trained for behavioral studies after acclimatization with lab environment for one week.

Study design

Sixty male albino mice were equally divided into ten groups (G-I to G-X). Group-I was administered with normal saline (10 mL/kg p.o), G-II was given 5% CMC (10 mL/kg p.o), G-III and IV were given piracetam (200 mg/kg p.o), G-V to VII were orally administered with aqueous fraction of *L. stoechas* in 200, 400 and 800 mg/kg respectively, and G-VIII to G-X were given *n*-hexane fraction of *L. stoechas* in 200, 400 and 800 mg/kg, p.o., for seven days. On seventh day, after forty five minutes of last dose administration, scopolamine (10 mg/kg, p.o) was administered to all groups except G-I and G-III to induce amnesia.

Behavioral studies

After 45 min of administration of scopolamine, behavioral studies were conducted in sound proof room on same day (7th day) and after 24 h of scopolamine dose (8th day) by using elevated plus maze, hole-board apparatus and light-dark paradigms [14].

Elevated plus maze

Elevated plus maze (EPM) apparatus (made up of poly acrylic sheets) was used for behavioral study. It consisted of two closed arms of dimensions 16 x 5 x 12 cm and two open arms (16 x 5 cm), joined altogether at central point of area 25 cm^2. Apparatus was fixed at wooden stand 25 cm high from ground level. Mouse was put at the end of opened arm with its back facing the central point and time (sec) taken by it to enter with full four feet into any of the closed arm was noted. Maximum of 90 sec were given to each mouse to get enter into closed arm and animal failed to find closed arm was dragged to closed arm from its tail and then returned to home cage by assigning it 90 sec as latency time [14]. Transfer latency recorded on 7th day was initial transfer latency (ITL) while on day 8th was retention transfer latency (RTL). Inflexion ratio (IR) was obtained as in Eq 2.

IR= ITL– RTL/ITL ……. (2)

Hole-board paradigm

This apparatus was made of transparent poly acrylic sheets fixed in the shape of rectangle box having dimensions 35 x 45 x 45 cm. Dark colored sheet having 16 holes (each with 2 cm diameter and equally spaced) was fixed five cm above the level of floor of box. Mouse from each group was individually placed in the middle of the sheet and number of times it poked the holes of sheet in 5 min was recorded on day 7th and 8th consecutively [14].

Light-dark test

This apparatus consisted of a rectangle box having large light compartment (30 x 30 x 35 cm) made up of transparent acrylic sheet and a smaller dark compartment (20 x 30 x 35 cm) made up of same black sheet. An opening (5 x 5 cm) in middle separating wall was made for the entrance of animal between two chambers. On day 7th and 8th, each mouse from all groups was placed individually in light compartment and was examined for 5 min and time spent by it in each compartment was recorded [14].

Biochemical studies of brain markers

Animals were anesthetized by using isoflurane and their brains were isolated after cervical dislocation. Then 20 mg of each brain was taken after rinsing it with ice cold saline and homogenized in tissue homogenizer with phosphate buffer (1 ml). The mixture was 1st centrifuged at 800 rpm and supernatant was again centrifuged at 10,000 rpm at 4 $^{\circ}$C and biochemical tests were performed then on fine separated supernatant [15].

Estimation of acetylcholinesterase

The level of acetylcholinesterase in brain homogenates was assessed by Ellman's method by mixing brain homogenate (0.4 mL) with phosphate buffer (2.6 mL, pH; 8) and DTNB (100 µL). The absorbance of the mixture was read at 412 nm using UV-visible spectrophotometer.

After that reaction mixture was added up acetylthiocholine iodide (20 µL) and absorbance was recorded at interval of 2 min to find the change in absorbance per min. Values were put in Eq 3 to find the level of AChE.

$R = 5.74 \times 10^{-4} \times A/CO$ …….. (3)

R represents rate of hydrolysis of substrate/min/g of brain tissue, A is change in absorbance per min and CO is original concentration (20 mg/mL) of tissue [15].

Assessment of malondialdehyde (MDA)

The level of MDA in brain was determined by mixing brain homogenate (100 µL) with sodium dodecyl sulfate (200 µL), acetic acid (1.5 mL) and thiobarbituric acid (1.5 mL). Then the mixture was first heated (at 95 °C for 1 h) and then cooled at room temperature. Later on, it was mixed with *n*-butanol (5 mL) and solution was centrifuged for ten min at 3000 rpm to separate the organic layer. Absorbance of the organic layer was taken at 532 nm and MDA level was determined by using as Eq 4 [15].

MDA (µM) = [A (sample) x DF /l x ε] ……. (4)

where, l = Light path = 1cm, ε = *Molar absorptivity* = 1.56 X 10^5 M^{-1} cm^{-1} and DF = Dilution factor = 21.

Estimation of superoxide dismutase (SOD)

Brain homogenate (0.5 mL) was diluted with 1 mL distilled water and then mixed with chloroform (1.5 ml) and chilled ethanol (2.5 mL) and centrifuged. Supernatant was added up sodium pyrophosphate buffer (1.2 mL, pH; 8.4), NBT (0.3 mL), PMS (0.1 mL), NADH (0.2 mL) and distilled water (3mL).

After incubation at room temperature 1 ml of glacial acetic acid was mixed in it, agitated vigorously and added up with few ml of *n*-butanol. Butanol layer was removed to read the absorbance at 560 nm against butanol blank and SOD level was expressed as unit/mg of protein [15].

Estimation of catalase (CAT) Level

Tissue homogenate (0.1 mL) was mixed with phosphate buffer (1 mL, pH; 7.0) and hydrogen peroxide (0.4 mL) and mixture was then poured in 2 mL of dichromate acetic acid reagent. Absorbance was read at 620 nm and CAT activity was expressed as µM of H_2O_2 decomposed/min/mg of protein [15].

Estimation of glutathione (GSH) activity

Brain homogenate (0.4 mL) was mixed with 0.4 mL of TCA and mixture was centrifuged at 10,000 rpm for half an hour. Supernatant was then mixed with 2 mL of DTNB and final volume was made 3 mL by addition of phosphate buffer. Absorbance was read at 412 nm against blank and GSH was used in different concentrations (10-50 µM) after dissolving it in 0.4 mL of TCA. The absorbance of which was taken to draw calibration curve and GSH in brain was expressed as µM/mg of tissue protein [15].

Statistical analysis

The data were expressed as mean ± SEM. Student's t-test analysis was applied on data with paired comparisons and multiple comparisons were made by ANOVA followed by Dunnett's test by using GraphPad Prism software (version 7). $P < 0.05$ was considered significant.

RESULTS

Yield

The yield was 15.21 and 0.91 %, respectively, for aqueous and *n*-hexane fractions of *L. stoechas*.

Phytochemical profile

The result of qualitative analysis of phytochemicals present in aqueous and *n*-hexane fractions of *L. stoechas* is shown in Table 1 while quantitative analysis of phenols, flavonoids and tannins is given in Table 2.

***In vitro* antioxidant activity**

The results of DPPH assay indicated that aqueous and *n*-hexane fractions of *L. stoechas,* respectively showed IC_{50} values as 35.14 and 357.42 µg/mL as compared to ascorbic acid which had IC_{50} value as 51.39 µg/mL as shown in Figure 1.

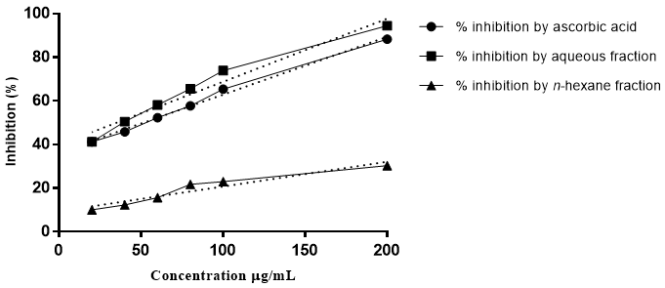

Figure 1: *In vitro* DPPH antioxidant activity of aqueous and *n*-hexane fraction fractions of *Lavandula stoechas*

Table 1: Phytochemical profile of aqueous and *n*-hexane fractions of *Lavandula stoechas*

Phytochemical constituent	Test	Aqueous fraction	*n*-Hexane fraction
Alkaloids	Hagers's test	++	+
	Wagner's test	++	+
	Dragendroff's test	++	++
	Mayer's test	++	+
Carbohydrates	Molish Test	++	+
Fixed oils	Spot test	-	-
Flavonoids	Alkaline reagent test	+	+
Glycosides	Killer Kiliani test	+	+
Phenols	FC method	+++	+
Phytostyerol	Libermann Burchard test	++	+
Proteins	Ninhydrin test	+++	+
Quinones		-	-
Saponins	Foam test	+	-
Steroids	Ring test	+	+
Tannins	Ferric chloride test	+++	++
Terpenes	Salkowski test	++	+
Terpenoids		++	+

+++ = *Highly present,* ++ = *moderately present,* + = *merely present,* - = *absent*

Table 2: Total phenolic, flavonoid and tannin contents in aqueous and *n*-hexane fractions of *Lavandula stoechas*

Extract	Phenols (mg of GAE/g)	Flavonoids (mg of RE/g)	Tannins (mg of TAE/g)
Aqueous fraction	168.39 ± 1.81	65.57 ± 0.72	116.43 ± 1.67
n-Hexane fraction	13.40 ± 0.87	15.39 ± 0.83	68.85 ± 0.71

Behavioral characteristics

Results of EPM indicated significant *(p< 0.001)* reduction in ITL and RTL values of G-IV to G-VII when compared to amnesic control animals. Similarly, increase in inflexion ratio was observed in animals treated with either standard drug or different doses of aqueous fraction which is indication of improvement in memory. Animals treated with *n*-hexane fraction produced non-significant results (Table 3).

Results of hole-board paradigm indicated that aqueous fraction (800 mg/kg p.o) significantly *(p < 0.001)* increased number of hole-pokings on both day 1st and 2nd (Table 4) which indicated memory improvement while *n*-hexane fraction produced non-significant results. Similarly, results of light-dark paradigm indicated that aqueous fraction in all doses significantly *(p < 0.001)* increased the time spent by animals in dark area as compared to amnesic control and *n*-hexane fraction treated animals (Table 5).

Table 3: Effect of aqueous and *n*-hexane fractions of *Lavandula stoechas* on transfer latency (TL) in EPM paradigm

Group	Initial transfer latency (s)	Retention transfer latency (s)	Inflexion ratio (IR)
G-I	21.83 ± 1.01	18.16 ± 1.30	0.16 ± 0.04
G-II	71.66 ± 2.73^{a}	86.33 ± 3.08^{a}	-0.20 ± 0.03^{a}
G-III	18.83 ± 1.16^{b}	16.33 ± 0.76^{b}	0.19 ± 0.02^{b}
G-IV	45.17 ± 1.81^{b}	25.17 ± 1.81^{b}	0.44 ± 0.04^{b}
G-V	58.00 ± 1.89^{b}	51.17 ± 2.79^{b}	0.11 ± 0.04^{b}
G-VI	47.33 ± 1.66^{b}	39.16 ± 2.05^{b}	0.16 ± 0.03^{b}
G-VII	38.33 ± 1.89^{b}	26.16 ± 1.66^{b}	0.30 ± 0.06^{b}
G-VIII	70.66 ± 2.45^{ns}	76.66 ± 4.24^{ns}	-0.08 ± 0.05^{ns}
G-IX	68.16 ± 3.28^{ns}	75.00 ± 3.33^{ns}	-0.12 ± 0.03^{ns}
G-X	69.17 ± 2.02^{ns}	78.16 ± 3.34^{ns}	-0.13 ± 0.04^{ns}

Values are expressed as mean ± SEM with n=6 per group. One way ANOVA followed by Dunnett's test was applied on data set and Group-II was compared with Group-I ($^{a}p \leq 0.001$) while other groups (Group-III to Group-X) were compared with Group-II ($^{b}p \leq 0.001$, $^{c}p \leq 0.01$, $^{d}p \leq 0.05$ or $^{ns}p \geq 0.05$)

Table 4: Effect of aqueous and *n*-hexane fractions of *Lavandula stoechas* on number of hole-pokings in hole-board paradigm

Group	Day 1st	Day 2nd
	No of hole-pokings/5 min	No of hole-pokings/5 min
G-I	46.83 ± 1.49	41.33 ± 1.28
G-II	22.66 ± 1.76^{a}	25.83 ± 1.55^{a}
G-III	51.16 ± 2.34^{b}	44.66 ± 1.77^{b}
G-IV	41.83 ± 1.47^{b}	40.33 ± 1.72^{b}
G-V	32.16 ± 2.15^{d}	31.33 ± 2.07^{ns}
G-VI	34.66 ± 2.07^{c}	32.83 ± 2.30^{ns}
G-VII	40.16 ± 1.92^{b}	38.16 ± 2.27^{b}
G-VIII	20.33 ± 0.84^{ns}	22.66 ± 0.91^{ns}
G-IX	19.66 ± 1.05^{ns}	23.16 ± 1.01^{ns}
G-X	19.83 ± 1.24^{ns}	21.66 ± 1.45^{ns}

Values are expressed as mean ± SEM with n=6 per group. One way ANOVA followed by Dunnett's test was applied on data set and Group-II was compared with Group-I ($^{a}p \leq 0.001$) while other groups (Group-III to Group-X) were compared with Group-II ($^{b}p \leq 0.001$, $^{c}p \leq 0.01$, $^{d}p \leq 0.05$ or $^{ns}p \geq 0.05$)

Table 5: Effect of aqueous and *n*-hexane fractions of *Lavandula stoechas* on time spent in light and dark compartments

Group	Day 1		Day 2	
	Time spent in light compartment (s)	Time spent in dark compartment (s)	Time spent in light compartment (s)	Time spent in dark Compartment (s)
G-I	51.50 ± 2.63	248.50 ± 2.83	46.67 ± 1.64	253.33 ± 1.39
G-II	186.16 ± 5.80[a]	113.83 ± 5.51[a]	205.16 ± 3.50[a]	94.83 ± 3.39[a]
G-III	44.16 ± 5.39[b]	255.84 ± 4.24[b]	34.66 ± 2.45[b]	265.34 ± 2.45[b]
G-IV	66.17 ± 2.80[b]	233.83 ± 4.41[b]	53.67 ± 2.45[b]	246.33 ± 2.67[b]
G-V	122.50 ± 3.03[b]	177.50 ± 3.03[b]	101.34 ± 3.15[b]	198.66 ± 3.15[b]
G-VI	100.16 ± 3.27[b]	199.84 ± 3.27[b]	81.50 ± 2.93[b]	218.50 ± 2.93[b]
G-VII	63.83 ± 1.60[b]	236.17 ± 1.60[d]	53.66 ± 2.39[b]	246.34 ± 2.39[b]
G-VIII	186.83 ± 6.38[ns]	113.17 ± 6.38[ns]	207.84 ± 7.89[ns]	92.16 ± 7.89[ns]
G-IX	191.33 ± 8.88[ns]	108.67 ± 8.88[ns]	190.50 ± 6.76[ns]	109.50 ± 6.76[ns]
G-X	184.83 ± 6.63[ns]	115.17 ± 6.63[ns]	197.50 ± 6.68[ns]	102.50 ± 6.68[ns]

Values are expressed as mean ± SEM (n = 6); one-way ANOVA followed by Dunnett's test was applied on data set and Group-II was compared with Group-I ([a]p ≤ 0.001) while other groups (Group-III to Group-X) were compared with Group-II ([b]p ≤ 0.001, [c]p ≤ 0.01, [d]p ≤ 0.05 or [ns]p ≥ 0.05)

Table 6: Effect of aqueous and *n*-hexane fractions of *Lavandula stoechas* on concentration of AChE, MDA, SOD, CAT and GSH in mice brain

Group	AChE µmol/min/mg	MDA nmol/h/g	SOD U/mg of homogenate	Catalase U/mg of homogenate	GSH µmol/mg
G-I	4.01 ± 0.21	1.43 ± 0.12	25.15± 0.58	1.97 ± 0.05	44.11 ± 1.50
G-II	8.94 ± 0.26[a]	8.96 ± 0.18[a]	7.94 ± 0.31[a]	0.56 ± 0.04[a]	18.90 ± 0.90[a]
G-III	3.53 ± 0.22[b]	1.19 ± 0.08[b]	26.16 ± 0.52[b]	2.10 ± 0.04[b]	46.84 ± 1.17[b]
G-IV	4.49 ± 0.19[b]	2.47 ± 0.13[b]	21.69 ± 0.31[b]	1.44 ± 0.06[b]	39.52 ± 1.49[b]
G-V	6.40 ± 0.26[b]	5.46 ± 0.13[b]	17.12 ± 0.62[b]	0.82 ± 0.06[ns]	36.60 ± 0.89[b]
G-VI	5.81 ± 0.18[b]	3.39 ± 0.18[b]	18.03 ± 0.58[b]	0.95 ± 0.06[c]	38.33 ± 0.94[b]
G-VII	5.04 ± 0.12[b]	2.62 ± 0.15[b]	20.96 ± 0.51[b]	1.04 ± 0.06[b]	38.52 ± 0.51[b]
G-VIII	8.39 ± 0.39[ns]	8.66 ± 0.36[ns]	8.07 ± 0.12[ns]	0.88 ± 0.05[b]	21.10 ± 0.71[ns]
G-IX	8.11 ± 0.34[ns]	7.95 ± 0.07[d]	8.17 ± 0.09[ns]	0.89 ± 0.04[b]	20.49 ± 1.08[ns]
G-X	7.78 ± 0.20[ns]	7.73 ± 0.12[c]	8.33 ± 0.11[ns]	1.03 ± 0.03[b]	20.24 ± 1.05[ns]

Values are expressed as mean ± SEM with n=6 per group. One way ANOVA followed by Dunnett's test was applied on data set and Group-II was compared with Group-I ([a]p ≤ 0.001) while other groups (Group-III to Group-X) were compared with Group-II ([b]p ≤ 0.001, [c]p ≤ 0.01, [d]p ≤ 0.05 or [ns]p ≥ 0.05)

Biochemical characteristics

Significant *(p < 0.001)* reductions in the level of AChE and MDA were observed in G-III to G-VII as compared to amnesic control and *n*-hexane fraction treated animals, which indicated anti-amnesic activity of aqueous fraction of *L. stoechas.* Moreover, it was observed that the levels of SOD, CAT and GSH were significantly *(p < 0.001)* improved by aqueous fraction as compared to amnesic control group which clearly indicated *in vivo* antioxidant activity of aqueous fraction of *L. stoechas*.

DISCUSSION

Dementia due to Alzheimer's disease (AD) is developed slowly with the progression of decay in cholinergic neurotransmission in frontal lobe of cerebral cortex, hippocampus and cingulate gyrus of the brain. Initially, AD is characterized by loss of short term memories which then worsens to altered behavior, speech disorientation, low labile mood, social anxiety disorder, cognitive impairment and finally death. It has been well observed that severe oxidative stress in later half of the life is one of the leading causes of dementia. The use of certain drugs like scopolamine is associated with loss of both short term and working memory due to blockade of muscarinic neurotransmission in brain. Thus, scopolamine is widely used as an amnesic agent to induce amnesia in rodents for the evaluation of memory enhancing potential of different substances. In contrast to scopolamine, an agent preventing the brain from oxidative damage of free radicals and those which potentiate the cholinergic transmission in brain can be employed for managing AD [4].

The results of the current study indicated the effectiveness of aqueous fraction of *L. stoechas* in memory enhancement as compared to standard drug piracetam. The leading cause of selection of natural plants as a primary source of medicine for the management of AD by

researchers is the safe therapeutic profile of herbal constituents along with their sustained neuroprotection [16]. The reason of selection of this plant as a memory enhancer was its widespread use in the management of all memory related brain disorders by traditional healers in India and Pakistan. They have used this plant by boiling its aerial parts in water and extracted solution was administered to the patients by oral route [17]. Current research work provides the pharmacological basis of action of aqueous fraction of *L. stoechas* in built up of memory.

The results of behavioral studies found by elevated plus maze, one of most famous model of assessment of memory, indicated that aqueous fraction significantly, *(P < 0.001)* reduced both initial and retention transfer latencies in mice which is indication of improvement of memory. Similarly, inflexion ratio was observed to be enhanced in extract treated animals which indicates improved learned activities by rodents. Comparison of treated and amnesic animals indicated that pretreatment of mice with different doses of aqueous extract protected the mice brain from harmful effects of scopolamine and learned tasks were retained in treated animals as compared to diseased mice.

The findings of light-dark paradigm indicated that animals treated with aqueous fraction spent maximum of time in dark box as they learned to go to dark area and retained their memory as compared to animals only treated with scopolamine that lost their learned tasks. This is the evidence of usefulness of this model [18] in assessment of enhancement of memory along with the efficacy of plant extract.

Hole-board paradigm was based on the concept that increased number of hole-pokings by mice indicated their natural explorative behavior. However animals treated only with scopolamine lost their memory of exploration and presented decreased number of hole- pokings [18]. The results indicated the significant *(p< 0.001)* increase in number of hole- pokings on both the days by animals treated with aqueous fraction of *L. stoechas*. Results of behavioral studies were further supported by assessment of level of AChE, MDA, SOD, CAT and GSH in brain homogenates of mice.

Findings suggested that aqueous fraction of *L. stoechas* significantly *(p< 0.001)* reduced the level of AChE in mice as compared to scopolamine treated animals. The degradation of acetylcholine is took place by AChE at the level of synapses [19] and agent promoting AChE levels in brain contributes to dementia. Hence, it is clearly evident that aqueous fraction and piracetam are responsible for reduction of AChE levels in mice brain and hence both are potent anti-amnesic agents. Phytochemical investigations indicated that plant is rich in alkaloids and flavonoids (Table 1) which are hallmark of anti-cholinesterase activity [19, 20]. Moreover, the brain is a very sensitive organ and highly labile to the action of free radicals and toxic products if level of GSH is not sufficient in brain. Ɣ-secretase and β-secretase produced by the action of H_2O_2 cause the breakdown of metal ions into toxic free radicals and subsequently affecting neurons in brain [21]. Antioxidant studies of aqueous fraction of *L. stoechas* indicated that it has potential to scavenge free radicals and toxic species and prevents the brain from oxidative stress of oxidizing agents. Biochemical findings suggested significant *(p< 0.001)* elevation in levels of SOD, CAT and glutathiones by the use of aqueous fraction, which are beneficial in prevention of loss of memory by oxidative neuronal damage [22]. The level of MDA was significantly *(P < 0.001)* reduced by aqueous fraction of *L. stoechas* and hence it prevented the brain from oxidative stress of lipid peroxidation. High level of MDA is associated with lipid peroxidation and with the decaying of neurons. Phytochemical investigations suggested the presence of phenols which prevent the oxidative stress in brain by scavenging nascent oxygen, H_2O_2 and superoxide species [23].

It has been investigated that scopolamine damages the memory of rodents by reducing natural protective agents like CAT, GSH and SOD. High levels of SOD attenuate superoxide species and CAT is responsible to prevent the damage of hydrogen peroxide [24]. But on other hand GSH is responsible for scavenging of free radicals in brain [25]. Both *in vitro* and *in vivo* antioxidant activity strongly suggested that supplementation of aqueous fraction of *L. stoechas* not only prevents the neuronal decay in brain but also improves cognition and memory by preventing cholinergic breakdowns in brain.

CONCLUSION

The findings of this study show that the constituents of aqueous fraction of *Lavandula stoechas* prevent dementia by slowing down cholinergic decay as well as providing protection for brain neurons against oxidizing agents. Thus, the study supports the claimed benefits of *Lavandula stoechas* in managing memory-related disorders.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The project was designed and supervised by Mobasher Ahmad and Rukhsana Anwar. Practical work was done by Aamir Mushtaq. All the authors read and revised the manuscript.

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