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Anti-Alzheimer Effect of Ethanolic Extract of *Barleria Prionitis* Leaf in Lipopolysaccharide Induced Neurodegeneration

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ABSTRACT: This study was designed to investigate the effect of ethanolic extract of *Barleria prionitis* leaf in Lipopolysaccharide induced neurodegeneration. Neurodenegeration was induced by administering Lipopolysaccharide (LPS) (1 mg/kg/i.p.) in a Sprague Dawley rat model. Ethanolic extract of *Barleria prionitis* leaf (EEBPL) at a dose of 200 and 400 mg/kg was given orally to desired group of animals for a period of 14 days. After 14 days of drug treatment, parameters such as in vitro anticholinesterase activity, DPPH Radical Scavenging activity, behavioural test for memory and learning (Elevated plus maze test, Open field test), Acetylcholine (ACh) content, Acetylcholinesterase (AChE) activity, Superoxide dismutase (SOD) activity in brain homogenate were analysed. EEBPL at both doses, i.e., 200 and 400mg/kg, decreased the Acetylcholinesterase level in neurodegenerated rats. The EEBPL (400 mg/kg/p.o) had more pronounced effect on memory and learning which is supported by the results of improvement in the body weight and decreased transfer latency time of neurodegenerated animals in elevated plus maze test. Acetylcholine was found to be decreased in untreated neurodegenerated rats due to neuronal inflammation. The EEBPL (400 mg/kg) doses significantly (P<0.001) increased the SOD activity in neurodegenerated rats. Modulation of acetylcholine level by the EEBPL is related with its potential anti-oxidant and cholinesterase inhibitory activity. Further studies are needed to carry out the isolation of active constituents responsible for the activity.

KEYWORDS: Acetylcholinesterase; *Barleria prionitis*; superoxide dismutase (SOD); neurodegeneration; Alzheimer's disease.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological condition in elderly, which continues to be a major challenge for neuroscientists. Multiple neurotransmitter systems are dysfunctional in AD in addition to conventional cholinergic impairment, which is primarily to blame for reduced cognitive abilities [1]. It is the primary contributor of dementia, which affects millions of older people worldwide [2]. Around 36 million individuals globally were affected by AD in 2010, yet the number of cases is rising by 7.7 million each year. According to estimates, there will be 144 million AD sufferers globally by the year 2050 [3].

Alzheimer's disease mostly affects the brain. It causes cognitive function to deteriorate. Neurons in Alzheimer's disease eventually become damaged. Furthermore, the neurons in charge of performing essential biological tasks are destroyed [4]. This disease has a complex aetiology. Many other hypotheses have been proposed to explain the multifaceted character of this disease, including as the cholinergic theory, the Aβ hypothesis, the tau hypothesis, the oxidative stress hypothesis, and the inflammatory hypothesis [5]. To understand the genes linked to the disease, scientists at the Mount Sinai Icahn School of Medicine in New York City conducted research. With the help of their investigations, they were able to identify the gene ATP6VA1 as the master regulator of this neural network and show that altering its expression genetically or

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pharmacologically enhanced neuronal function in flies and cultured cells. Another study conducted at the Icahn School of Medicine found that the VGF gene and protein play a critical part in defending the brain from Alzheimer's [6]. Only five medications are currently licenced by the US Food and Drug Administration (FDA) to treat the cognitive aspects of Alzheimer's disease. Fruits and vegetables that are consumed worldwide have excellent sources of bioactive compounds and having capacity reducing the risk of developing Alzheimer's disease.

The *Barleria prionitisis* is most commonly used as a medicinal purpose in India. *B. prionitis* phytochemistry revealed the presence of glycosides, steroids, tannins, and flavonoids [7]. Aerial portions have yielded iridoid glycosides, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetyl barlerin, 7-methoxydiderroside, and lupulinoside [8]. Several studies have found that the plant has antifungal, antiviral, anti-fertility, antioxidant, antidiabetic, anti-inflammatory, cytoprotective, antiarthritic, diuretic, hepatoprotective, and gastroprotective properties [9].

Antioxidants minimize the harmful effects of free radicals and oxidative damage on biological metabolism. By reducing lipid peroxidation, antioxidants contribute to the preservation of lipids, protein structures, and cell integrity. Antioxidants have the ability to prevent several compounds from oxidising. A number of chronic diseases can be delayed or stopped in their tracks thanks to antioxidants [10]. Antioxidant activity has an important role in the treatment of neurodegenerative disorders like AD. As per the previous literature, no scientific evidance has been proved for *B. Prionitis* against Alzheimer's disease. Hence, this motivated us to study the anti-Alzheimer impact of ethanolic extract *B. prionitis* leaf in a lipopolysaccharide-induced Alzheimer's disease model of rats to demonstrate its utility in the treatment of the disease.

2. RESULTS

2.1. Percentage yield of *B. Prionitis*

The percentage yield of *B. Prionitis* was determined for its leaf using 90% ethanol as the solvent. The resulting extract is pasty in consistency and brownish green in color. The yield is 7.95% as shown in **Table 1**.

Table 1. Percentage yield of *B. Prionitis*

Plant part	Solvent used	Consistency	Colour	Percentage Yield
B. prionitis leaf	90% v/v ethanol	Pasty	Brownish green	7.95%

2.2. Percentage inhibition of EEBPL on *in vitro* AChE enzyme activity

Both EEBPL and Donepezil hydrocholoride produced a dose depend increase in *in vitro* AChE inhibitory activity (**Table 2**). EEBPL has shown 35.93 ± 0.45% Inhibition at 100 µg/mL while Donepezil hydrocholoride has shown 88.23 ± 0.30% Inhibition at 100 µg/mL.

Table 2. *In vitro* AChE inhibitory effect of EEBPL and Donepezil hydrochloride

Concentration (µg/mL)	% Inhibition of AChE by *B.prionitis*	% Inhibition of AChE by Donepezil hydrocholoride
25 µg/mL	19.96 ± 1.23	83.49 ± 0.463
50 µg/mL	32.82 ± 1.12	87.30 ± 0.228
100 µg/mL	35.93 ± 0.45	88.23 ± 0.300
200 µg/mL	38.34 ± 0.55	89.87 ± 0.202
400 µg/mL	43.39 ± 0.31	90.37 ± 0.188

2.3. Percentage inhibition of EEBPL on *in vitro* DPPH radical scavenging activity

Both *B.prionitis* and ascorbic acid produced a dose depend increase on DPPH Free radical scavenging effect. EEBPL has shown 34.43 ± 0.42% inhibition at 100 µg/mL while Ascorbic acid has shown 29.55 ± 0.285% inhibition at 100 µg/mL. Data are shown on **Table 3**.

Table 3. *In vitro* DPPH radical scavenging activity of EEBPL

Concentration (µg/mL)	DPPH Free radical scavenging activity by *B. prionitis* (%)	DPPH Free radical scavenging activity by Ascorbic acid (%)
25 µg/mL	7.32 ± 0.49	8.74 ± 0.602
50 µg/mL	16.11 ± 0.40	20.17 ± 0.366
100 µg/mL	34.43 ± 0.42	29.55 ± 0.285
200 µg/mL	40.20 ± 0.20	33.83 ± 0.546
400 µg/mL	43.08 ± 0.18	35.29 ± 0.130

2.4. Effect of EEBPL on body weight

EEBPL 200 mg and EEBPL 400 mg treated group animals have shown decrease in body weight when compared with disease group animals at the end of week 1 (P<0.05) and week 2 (P<0.001). The data has been tabulated in **Table 4**.

Table 4. Effect of EEBPL on body weight

Group (n=6)	Treatment	Initial weight (g)	End of week 1 (g)	End of week 2 (g)
I	Distilled water	183.33 ± 2.108	185.00 ± 0. 856	192.33 ± 1.498
II	LPS (1 mg/kg/i.p)	183.67 ± 1.978	183.5 ± 1.996^{ns}	187.66 ± 1.892^{ns}
III	LPS (1 mg/kg/i.p)+Donepezil hydrochloride (3 mg/kg/p.o)	185.33 ± 2.044	188 ± 1.687^{ns}	191.33 ± 1.909^{ns}
IV	LPS (1 mg/kg/i.p)+ EEBPL (200 mg/kg/p.o)	184.33 ± 1.820	176.4 ± 0.954*	168.33 ± 0.954***
V	LPS (1 mg/kg/i.p)+ EEBPL (400 mg/kg/p.o)	183.33 ± 2.860	175.6 ± 2.390*	170.33 ± 2.216***

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Analysis of Body weight at the end of week one
Values are presented as mean ± SEM (n=6).
values are analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test
*P<0.05; **P<0.01;***P<0.001, ns - P>0.05
Normal group is compared against disease group
All the treated groups are compared against the disease group

Analysis of Body weight at the end of week two
Values are presented as mean ± SEM (n=6).
Values are analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test
*P<0.05; **P<0.01;***P<0.001, ns - P>0.05
Normal group is compared against disease group
All the treated groups are compared against the disease group

2.5. Effect of EEBPL on learning and memory in Elevated plus maze test

LPS only treated animal showed highly significant increase in transfer latency time on both 14th and 15th day when comparing to normal control group (P<0.001). **Table 5** shows that Donepezil hydrochloride (3 mg/kg/p.o) and EEBPL (400 mg/kg/p.o) treated animal exhibited highly significant decrease in transfer latency time on both 14th and 15th day when comparing to disease control group (P<0.001).

Table 5. Effect of EEBPL on learning and memory (Elevated plus maze test)

Groups	Treatment	Transfer latency on Day 14 (secs)	Transfer latency on Day 15 (sec)
I	Distilled water	27 ± 0.516	20.167 ± 0.401
II	LPS (1 mg/kg/i.p)	41.33 ± 0.494^{a}***	34 ± 2.955^{c}***

III	LPS (1 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	18.667 ± 0.210[b] ***	9.33 ± 0.421[d]***
IV	LPS (1 mg/kg/i.p) + EEBPL (200 mg/kg/p.o)	38.83 ± 1.91[bns]	16.667 ± 1.014 [d]***
V	LPS (1 mg/kg/i.p) + EEBPL (400 mg/kg/p.o)	23.83 ± 0.601[b] ***	15.167 ± 0.307 [d] ***

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Values are expressed as mean ± SEM (n=6)
Values are analysed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.
*P<0.05; **P<0.01; ***P<0.001, ns - not significant
a. Normal group is compared against disease group on day 14
b. All the treated groups are compared against the disease group on day 14
c. Normal group is compared against disease group on day 15
d. All the treated groups are compared against the disease group on day 15.

2.6. Effect of EEBPL on learning and memory in Open field test

Table 6 presents the effects of different treatments on mice behavior, measured by the number of lines crossed and rearing time. Group I showed normal activity with 31 lines crossed and 78.8 seconds of rearing time. LPS only treated group showed a decrease in rearing time when compared to control animals (P<0.001). An improvement in movement activity was seen in LPS-treated animals administered with EEBPL at different doses (200 mg/kg and 400 mg/kg) and Donepezil hydrochloride (3 mg/kg/p.o) (P<0.001).

LPS only treated group showed a decrease in number of lines crossed when compared to control animals (P<0.001). An improvement in movement activity was seen in LPS-treated animals administered with EEBPL at different doses (200 mg/kg and 400 mg/kg) and Donepezil hydrochloride (3 mg/kg/p.o) (P<0.001) shown in **Table 6**.

Table 6. Effect of EEBPL on learning and memory (open field test)

Group	Treatment	Number of line crossed	Rearing time (sec)
I	Distilled water	31.00 ± 0.966	78.8 ± 16.56
II	LPS (1 mg/kg/i.p)	05.00 ± 0.577***	1.20 ± 0.32***
III	LPS (1 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	23.50 ± 0.763***	68.8 ± 16.56 ***
IV	LPS (1 mg/kg/i.p) + EEBPL(200 mg/kg/p.o)	18.00 ± 155 ***	47.20 ± 11.76***
V	LPS (1 mg/kg/i.p) + EEBPL (400 mg/kg/p.o)	23.33 ± 1.145***	64.00 ± 9.60***

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Values are expressed as mean ± SEM (n=6)
Values are analysed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.
*P<0; *P<0.005; **P<0.01; ***P<0.001, ns - not significant
Disease group is compared against normal group
All the thetreated groups are compared against disease group

2.7. Effect of EEBPL on Acetylcholine (ACh) level

LPS only treated group showed significant decrease in acetylcholine level (P<0.05) when compared to normal group. Donepezil (3 mg/kg/p.o) and *B.prionitis* (400 mg/kg/p.o) treated group showed significant increase in acetylcholine level (P<0.05) when compared to LPS only treated group (P<0.001) as shown in **Table 7**.

Table 7. Effect of EEBPL on Acetylcholine (ACh) level

Group	Treatment	Acetylcholine(ACh) level (µ moles of Acetylcholine/g wet wt of tissue)
I	Distilled water	6.00 ± 0.63
II	LPS (1 mg/kg/i.p)	3.67 ± 0.33
III	LPS (1mg/kg/i.p)+ Donepezil hydrochloride (3 mg/kg/p.o)	6.16 ± 0.47
IV	LPS (1 mg/kg/i.p)+ EEBPL (200 mg/kg/p.o)	4.67 ± 0.49^{ns}
V	LPS (1 mg/kg/i.p)+EEBPL (400 mg/kg/p.o)	6.33 ± 0.67***

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Values are expressed as mean ± SEM.
Values are analysed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.
*P<0.05; **P<0.01;***P<0.001, ns - not significant
Disease group is compared against normal group
All the treated groups are compared against disease group

2.8. Effect of EEBPL on Acetylcholinesterase (AChE) level

LPS only treated group showed significant increase in acetylcholinesterase level (P<0.01) when compared to normal group. Donepezil (3 mg/kg/p.o) and *B.prionitis (*400 mg/kg/p.o) treated group showed significant decrease in acetylcholinesterase level when comparing to LPS only treated group (P<0.001) as shown in **Table 8**.

Table 8. Effect of EEBPL on Acetylcholinesterase (AChE)

Group	Treatment	Acetylcholinesterase (AChE) level (M/min/g protein)
I	Distilled water	0.18 ± 0.00
II	LPS (1 mg/kg/i.p)	0.23 ± 0.09
III	LPS (1 mg/kg/i.p)+ Donepezil hydrochloride (3 mg/kg/p.o)	0.16 ± 0.06
IV	LPS (1 mg/kg/i.p)+ EEBPL (200 mg/kg/p.o)	0.20 ± 0.0n^{s}
V	LPS (1 mg/kg/i.p)+EEBPL (400 mg/kg/p.o)	0.17 ± 0.006***

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Values are expressedas mean ± SEM.
Values are analysed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.
*P<0.05; **P<0.01; ***P<0.001, ns - not significant
Disease group is compared against normal group
All the treated group are compared against disease group.

2.9. Effect of EEBPL on Superoxide Dismutase (SOD) activity

LPS only treated group showed decrease in SOD activity when comparing to normal group. Donepezil (3 mg/kg/p.o) and *B.prionitis (*400 mg/kg/p.o) treated group showed increase in SOD activity when comparing to LPS only treated group (**Table 9**).

Table 9. Effect of EEBPL on Superoxide Dismutase (SOD) activity

Group	Treatment	Superoxide dismutase (SOD) activity (u/mg protein)
I	Distilled water	2.71 ± 1.780
II	LPS (1 mg/kg/i.p)	1.95 ± 0.042
III	LPS (1 mg/kg/i.p)+ Donepezil hydrochloride (3 mg/kg/p.o)	2.516 ± 0.477

IV	LPS (1 mg/kg/i.p)+ EEBPL (200 mg/kg/p.o)	2.30 ± 0.036 ns
V	LPS (1 mg/kg/i.p)+EEBPL (400 mg/kg/p.o)	2.50 ± 0.051

p.o – Oral route of administration
i.p – Intraperitoneal route of administration

Values are expressed as mean ± SEM (n=6).
Values are analysed by One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.
*P<0.05; **P<0.01; ***P<0.001, ns - not significant
Disease group is compared against normal group
All the treatment groups are compared against diseased group

3. DISCUSSION

3.1. Effect of EEBPL on body weight

Our findings showed that EEBPL at 200 mg/kg/p.o and 400 mg/kg/p.o reduced body weight (P<0.001) at week 2, which is consistent with Pathak S et al., 2017's discovery that Barleria prionitis Linn has anti-obesity activity in an animal model of High Fat Diet caused obesity [11].

3.2. Effect of lipopolysaccharide on memory and learning

Lipopolysaccharide-only treated rats had lower levels of acetylcholine (P<0.05) and higher levels of acetylcholinesterase (P<0.01), lower transfer latency (P<0.001), and lower number of line crossings and rearing time in the open field test (P<0.001). Our findings are consistent with those of Jiayi Z *et al.*, 2019, who discovered that LPS injection causes increased neuroinflammation as well as disruption to the Blood Brain Barrier (BBB), resulting in amyloid generation and memory deficits [12].

3.3. Effect of EEBPL on free radical scavenging activity

Reactive oxygen spieces (ROS) attack DNA and causes mutation leading to pathological conditions such as neurodegeneration, ageing process and dementia. For the treatment of diseases caused by ROS, antioxidant is used. Phenolic compounds such as flavonoids have been shown to possess significant antioxidant activity [13].

LPS only treated group showed highly decrease in superoxide dismutase level when comparing to normal group (P<0.001). Donepezil (3 mg/kg/p.o) and EEBPL (400 mg/kg/p.o) treated group showed increase in superoxide dismutase level when comparing to LPS only treated group (**Table 9**) and the EEBPL produced potent DPPH scavenging activity when compared with the standard drug ascorbic acid suggest that the EEBPL is a potent scavenger of free radicals (**Table 3**).

3.4. Effect of EEBPL on memory and learning

One of the characteristic changes that occur in AD is increase in acetyl cholinesterase (AChE) activity, the enzyme responsible for acetylcholine hydrolysis, from both Cholinergic and non-cholinergic neurons of the brain [14].

Both EEBPL and Donepezil hydrocholoride produced a dose depend increase in *in vitro* AChE inhibitory activity (**Table 2**). LPS only treated group showed significant increase in *in vivo* acetylcholinesterase level when comparing to normal group (P<0.01). The *in vitro* acetylcholinesterase inhibitory effect of EEBPL correlate with the *in vivo* acetylcholinesterase inhibitory effect of EEBPL (400 mg/kg/p.o) (P<0.001).

Phytochemical analysis of *B. prionitis* shows the presence of sterols, saponins, tannins, and flavonoids [15]. It is suggested that anti-Alzheimer's effect elucidated by the EEBPL may be related to the presence phytoconstituents such as flavonoids and phenolic compounds.

Flavonoids have been shown to be highly effective scavengers of various free radicals and significantly effective in cholinesterase inhibition implicated in Alzheimer Disease. Phenolic compounds were widely found to be responsible for the antioxidant activity of plant materials and hence many of the natural polyphenols possess therapeutic potential for Alzheimer Disease. The terpenoids are abundant chemical constituents found in nature. Pharmacologically, terpenoids have been shown to have neuroprotective effects [16].

When compared to the literature findings for EEBPL, the present results suggested that phenolics, flavonoids, and terpenoids alone or in combination may be the major contributors for the antioxidant, enzyme inhibitory, and neuroprotective activities.

These findings from the elevated plus maze, open field test and increased acetylcholine level suggests that EEBPL has potent learning and memory enhancing effect.

4. CONCLUSION

Alzheimer's disease is a neurodegenerative disorder presently without an effective treatment. Impairment of memory is the original and most significant symptom of disease. AD is associated with a decline in cognitive capacities. This study reveals the anti-alzheimer effect of ethanolic extract of leaves of *B. prionitis* Linn, which is a well- known condiment in Ayurveda.

Results from AChE enzyme inhibition exertion indicates that EEBPL showed veritably potent inhibition of AChE when compared to donepezil. EEBPL may have several active chemicals which retain potent antioxidant and also has anticholinesterase property. This indicates that his plant have wide periphery of medicinal value and also has capabilities for the product of new medicines for the treatment of Alzheimer's complaint.

Still, further studies are needed to isolate the active constituents of EEBPL and to interpret their medium of action at the cellular and molecular level for memory enhancing effect of EEBPL which would grease the use of *B. prionitis* in Alzheimer disorder.

5. MATERIALS AND METHODS

5.1. Collection and authentication of plant material

Barleria prionitis (*B.prionitis*) leaf was collected from local market of Komarapalaym located in Tamilnadu state, India, during the month of November- December. It was dried under shade at temperature not exceeding 40 °C. The *Barleria prionitis* splint was linked and verified by the botanist Dr.M. Kannan, Head, Department of Botany, Vivekanandha council of trades and Science for Women, Tiruchengode, Namakkal District., Tamil Nadu.

5.2. Preparation of ethanolic extract

The leaf of *B.prionitis* was dried in the shade. Using a mixer grinder, the dried material was ground into powder. The powder was then passed through sieve no. 40 to ensure uniformity. For extraction, this leaf powder was utilised. For 24 hours, the extract was extracted in a soxhlet extractor with 90% ethanol as the solvent. To obtain the extract, the contents of a round bottom flask were evaporated under decreased pressure at 40°C. The extract was then placed in a sterile container and labelled [17].

5.3. Determination of *in vitro* anticholinesterase activity (AChE)

The Ellman colorimetric method was used to determine the acetyl-cholinesterase inhibition assay. 415 µL of Tris-HCl buffer 0.1 M (pH 8), 10 µL of solution of Ethanolic extract of Barleria prionitis leaf (EEBPL) in methanol with different concentrations, and 25 µL of enzyme (acetyl-cholinesterase) solution containing 0.5 U/mL were incubated for 15 minutes at room temperature in a total volume of 1 mL. 75 µL of AChI (acetyl-thiocholine) 1.83 mM solution and 475 µL of DTNB (5,5-dithiobis-2-nitrobenzoic acid) 3 mM solution were added, and the final combination was incubated for 30 minutes at room temperature. A UV-Visible 752 spectrophotometer was used to test the mixture's absorbance at 412 nm. As a positive control, Donepezil hydrochloride was used. The percentage of enzyme activity inhibition was determined [15].

5.4. Determination of *in vitro* DPPH radical scavenging activity

1 mL of 0.1 mM DPPH in methanol solution was added to 3 mL of the solution to varied concentrations of extracts in methanol (25, 50, 100, 200, and 400 g/mL). The mixture was shaken and set aside in a dark area for 30 minutes. The absorbance was then measured with a spectrophotometer at 517 nm. The EEBPL radical's DPPH radical scavenging potential was then determined [18].

5.5. Dose and drug solution

According to previous findings, *B.prionitis* is non-toxic up to 2.5 g/kg in rats [19]. As a result, the current investigation was conducted at two dose levels, namely 200 and 400 mg/kg body weight. To make the test medication, the needed amount of EEBPL was dissolved in distilled water to achieve the desired dose in 1 mL solution.

5.6. Animals

Sprague Dawley rats (100–150 g) of either sex were housed under standard laboratory conditions at temperature 25 ± 2°C and 55 ± 5% relative humidity with a regular 12h light:12 h dark cycle. Animals were given standard ready made rat pellet diet purchased from Vengadeswara agency, Bangalore and water *ad libitum*. The study was approved by (JKKMMRFCP/IAEC 2021/008), JKKMMRF Annai Sampooraniammal College of Pharmacy, Komarapalayam.

Composition of standard pellets	
Nutrients	**Percent of 100 g pellet**
Carbohydrate	48
From sucrose	0
Fat	4
Protein	23
Fiber	4.5
Calorie per 100 g	320

5.7. Experimental groups

The animals were divided into five groups, each with six animals. Group III received donepezil hydrochloride (DH) (3 mg/kg, p.o.) every 24 hours for 14 days [20]. Groups IV and V were given 200 and 400 mg/kg p.o doses of EEBPL orally every 24 hours for 14 days in a row. On day 15, neurodegeneration was induced by administering Lipopolysaccharide (LPS) (1 mg/kg/i.p) in normal saline to groups II to V [21]. Animals were subjected to behavioural tests two hours after receiving lipopolysaccharide before being slaughtered and the brain removed for biochemical analysis.

Table 10. Experimental groups

GROUP (n=6)	TREATMENT
I	Distilled water
II	Lipopolysaccharide (LPS) (1 mg/kg/i.p)
III	LPS (1 mg/kg/i.p) + Donepezil hydrochloride (DH) (3 mg/kg, p.o.)
IV	LPS (1 mg/kg/i.p) + EEBPL (200 mg/kg, p.o)
V	LPS (1 mg/kg/i.p) + EEBPL(400 mg/kg, p.o)

5.8. Learning and Memory Tests

5.8.1. Elevated plus maze test

The animals were placed individually 30 minutes after neurodegeneration induction at the end of either open arm, and the time it took the animal to travel from open to closed arm (transfer latency) was recorded. The time passed between the animal being placed on the open arm and all four legs being inside the enclosed arms was recorded as transfer latency. The transfer latency was measured again 24 hours after the first exposure [22].

5.8.2. Open field test

The open field habituation task approach was used to assess the rat's exploratory behaviour. The open field arena was divided into 16 squares: four inner squares and twelve squares along the walls. Following acclimatisation to the lab and the open field apparatus, rats were placed individually in the centre of the open field, and the parameters recorded for 5 minutes included the number of squares crossed, i.e. Crossings of lines and rearing [23].

5.9. Biochemical Study

5.9.1. Preparation of brain homogenate

The rats will be anesthetized with moderate ether anaesthesia, and their brains were swiftly dissected and stored in ice-cold saline. After that, a 10% w/v brain homogenate was made by homogenising

it in ice-cold phosphate buffer saline (pH 8, 0.1M). The homogenate was then spun for 10 minutes at 3000 rpm in a chilled centrifuge, and the supernatant was separated and used for biochemical estimates [24].

5.9.2. Estimation of Acetylcholine (ACh) content

The rat brain homogenate was placed in a boiling water bath for 5 minutes to stop the action of the Acetylcholinesterase enzyme and to release the bound ACh. The tissues were then homogenised in 1mL distilled water. 1 mL of alkaline hydroxylamine hydrochloride was added to the homogenate, followed by 1 mL of 50% hydrochloric acid solution. The contents were properly combined and centrifuged. In a spectrophotometer, 0.5 mL of 0.37 M ferric chloride solution was added to the supernatant, and the brown colour developed was measured at 540 nm against a reagent blank (1 mL of alkaline hydroxylamine hydrochloride + 1 mL of 50% hydrochloride + 1 mL of distilled water + 0.5 mL of 0.37 M ferric chloride solution). Acetylcholine content was quantified as moles of ACh/gm wet tissue weight [25].

5.9.3. Estimation of Acetylcholinesterase (AChE) activity

The activity of AChE in the aliquot of the rat brain homogenate was estimated. The aliquot will be mixed with phosphate buffer saline (pH 8). To this, the substrate acetylthiocholine iodide and dithiobisnitrobenzoic acid (DTNB) reagent will be added. Acetyl thiocholineiodide will be hydrolyzed to thiocholine and acetate by AChE. Thiocholine replied with DTNB reagent to produce unheroic colour. The rate of color development will be the measure of the AChE activity. A kinetic profile of the enzyme activity will be studied spectrophotometrically at 412 nm at the interval of 15 sec. The enzyme activity will be expressed as the mol of substrate hydrolyzed/min/gm tissue [26].

5.9.4. Estimation of Superoxide dismutase (SOD) activity

SOD activity was measured in an aliquot of rat brain homogenate. The incubation solution included sodium pyrophosphate buffer (pH 8.3; 0.052 M; 1.2 mL), Phenazine methosulfate (PMS) (186 mol), Nitro bluetetrazolium (NBT) (300 mol), and NADH (780 mol; 0.2 mL). NADH was added to start the reaction, which was then incubated for 90 seconds at 37°C. The reaction was stopped by adding glacial acetic acid (1 mL), n-butanol (4 mL), shaking strongly, centrifuging at 4000 rpm for 1 minute, and reading the upper butanol layer at 560 nm against a butanol blank [27].

5.10. Statistical analysis

Graph Pad Instat software was used for the statistical analysis. Results were expressed in mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test.

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REFERENCES

[1] Yogendra K Gupta, Jatinder Katyal and Sangeetha Gupta. Status of Neuropharmacology Research in India. *Proc Indian NatnSci Acad***.** 2018:15-31. https://doi.org/10.16943/ptinsa/2018/49309

[2] Anand R, Gill KD, Mahdi AA. Therapeutics of Alzheimer's disease: past, present and future. Neuropharmacology.2014 76: 27-50. https://doi.org/10.1016/j.neuropharm.2013.07.004

[3] Divya Singh and Sukanya Hembrom. Neuroprotective Effect of Flavonoids: A Systematic Review. International Journal of Aging Research. 2018, 1:26.

[4] Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet Neurology.2008; 7(9): 812-826. https://doi.org/10.1016/S1474-4422(08)70169-8

[5] Ramirez Bermudez J. Alzheimer's disease: critical notes on the history of a medical concept. Arch Med Res.2012; 43(8): 595-599. https://doi.org/10.1016/j.arcmed.2012.11.008

[6] Seven recent papers amplify advances in Alzheimer's research [Internet]. National Institute on Aging. U.S. Department of Health and Human Services; [cited 2022Nov17]. Available from: https://www.nia.nih.gov/news/seven-recent-papers-amplify-advances-alzheimers-research (accessed on 18 March 2023)

[7] Maji AK, Bhadra S, Mahapatra S, Banerji P, Banerjee D. Mast cell stabilization and membrane protection activity of Barleriaprionitis L. Pharmacogn J. 2011;3:67–71. https://doi.org/10.5530/pj.2011.24.13

[8] Ata A, Kalhari KS, Samarasekera R. Chemical constituents of Barleriaprionitis and their enzyme inhibitory and free radical scavenging activities. Phytochemlett, 2009;2:37–40. https://doi.org/10.1016/j.phytol.2008.11.005

[9] Pathak Shardul, Vivek Gokhale. Evaluation of the effect of kurantaka churna (*barleria prionitis* linn.) in experimental model of high fat diet induced obesity. International Ayurvedic Medical Journal.2017; 5 (4) : 1153-1158.

[10] Gök N, Akıncıoğlu A, Erümit Binici E, Akıncıoğlu H, Kılınç N, Göksu S. Synthesis of novel sulfonamides with anti-Alzheimer and antioxidant capacities. Archiv der Pharmazie. 2021 Jul;354(7):2000496. https://doi.org/10.1002/ardp.202000496

[11] Banerjee S, Banerjee S, Jha GK, Bose S. Barleria prionitis L.: An Illustrative Traditional, Phytochemical and Pharmacological Review. The Natural Products Journal. 2021 Jun 1;11(3):258-74. https://doi.org/10.2174/2210315510666200131114525

[12] Jiayi Zhao, Wei Bi, ShuXiao, XinLan, Xiaofeng Cheng, Jiawei Zhang, Daxiang Lu, Wei Wei, Yanping Wang, Hongmei Li, Yongmei Fu &Lihong Zhu. Neuroinfammation induced by lipopolysaccharide causes cognitive impairment in mice. Scientific Reports. 2019 9:5790. https://doi.org/10.1038/s41598-019-42286-8

[13] Kapoor A, Shukla S, Kaur R, Kumar R, Lehra KS, Kapoor S. Preliminary phytochemical screening and antioxidant activity of whole plant of Barleria prionitis Linn. Int. J. Adv. Pharm. Biol. Chem. 2014;3(2):410-9.

[14] Curcio CA, Kemper T, Nucleus rape dorsalis in dementia of the Alzheimer type: Neurofibrillary changes and neuronal packing density. J Neuropathol Exp Neurol, 1984; 43 (4): 359–368. https://doi.org/10.1097/00005072-198407000-00001

[15] I.A. Owokotomo, O. Ekundayo, T.G. Abayomi A.V. Chukwuka. In-vitro anti-cholinesterase activity of essential oil from four tropical medicinal plants. Toxicology Reports-2. 2015: 850–857. https://doi.org/10.1016/j.toxrep.2015.05.003

[16] Penumala M, Zinka RB, Shaik JB, Amooru Gangaiah D. In vitro screening of three Indian medicinal plants for their phytochemicals, anticholinesterase, antiglucosidase, antioxidant, and neuroprotective effects. BioMed research international. 2017 Oct 24;2017. https://doi.org/10.1155/2017/5140506

[17] Harborne JB. Phytochemical Methods. A Guide to modern techniques of plant Analysis. 3rded. New Delhi, *Springer* (*India*) *Pvt. Ltd*; 1998; 5-16.

[18] Pant DR, Pant ND, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of Pterocarpus marsupium Roxburgh. Journal of Intercultural Ethnopharmacology. 2017 Apr;6(2):170. https://doi.org/10.5455%2Fjice.20170403094055

[19] Reema Dheer, Pradeep Bhatnagar. A study of the antidiabetic activity of *Barleria prionitis* Linn. Indian J Pharmacol. 2010; 42 (2):70-73. https://doi.org/10.4103/0253-7613.64493 10.4103/0253-7613.201016

[20] Pattanashetti LA, Taranalli AD, Parvatrao V, Malabade RH, Kumar D. Evaluation of neuroprotective effect of quercetin with donepezil in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology. 2017 Jan 1;49(1):60-4. https://doi.org/10.4103/0253-7613.201016

[21] Gomathi S, Sundaram RS, Vijayabaskaran M, Kannan C, Sambathkumar R. Pedalium murex Linn leaves against LPS-induced oxidative stress, anxiety and depression behavioural alterations in rats. Research Journal of Pharmacy and Technology. 2017;10(5):1333-8. https://doi.org/10.5958/0974-360X.2017.00236.0

[22] Achliya G, Barabde U, Wadodkar S, Dorle A. Effect of Bramhi Ghrita, an polyherbal formulation on learning and memory paradigms in experimental animals. Indian journal of pharmacology. 2004 May 1;36(3):159-62.

[23] Adebiyi OE, Olopade FE, Olopade JO, Olayemi FO. Behavioural studies on the ethanol leaf extract of Grewia carpinifolia in Wistar rats. African health sciences. 2016 May 9;16(1):339-46. https://doi.org/10.4314/ahs.v16i1.45

[24] Prakash Ramakrishnan, Panneerselvam Perumal, Dhivya Ramadoss, Parthiban Ramalingam, Subash Chandra Boss Vijayarangan. Amelioration of Cognitive Deficits, Oxidative Damage, Neurochemical Alteration by *Bauhinia purpurea* (stem bark) on Scopolamine Induced Amnesia. J Young Pharm, 2020; 12(3) : 231-236. https://doi.org/10.5530/jyp.2020.12.66

[25] SanthiKrupa D, ChLochana, Padmalatha K. Antialzheimer's Potential of *Abrus pectoris* hydro alcoholic root extract. J. Pharm. Sci. & Res. 2020; 12(7), 914-919.

[26] Erukainure OL, Ijomone OM, Sanni O, Aschner M, Islam MS. Type 2 diabetes induced oxidative brain injury involves altered cerebellar neuronal integrity and elemental distribution, and exacerbated Nrf2 expression: therapeutic potential of raffia palm (Raphia hookeri) wine. Metabolic brain disease. 2019 Oct;34:1385-99. https://doi.org/10.1007/s11011-019-00444-x

[27] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J BiochemBiophys. 1984; 21:130-132.