Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Neuroprotective Effect of Ethanolic Extract of *Pedalium murex* Linn Leaf in 3-Nitropropionic acid Induced Neurodegeneration

Suresh VELAYUTHAM [1] *, Arunprasanth Sundararaju [1], Thamotharan GOVINDHASWAMY [1], Muthukumaran Gurupackiyam [1], Anbukarasi Balakrishnan [1], Mohamed Yasar ABDUL [2], Chetan ASHOK [2], Srikanth JEYABALAN [2] *

[1] Department of Pharmacology, Faculty of Pharmacy, JKK Munirajah Medical Research Foundation, Annai Sampoorani Ammal College of Pharmacy, Komarapalayam – 638138, Nammakkal, India.
[2] Department of Pharmacology, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai - 600052, India.
* Corresponding Author. E-mail: srikanth.j@sriramachandra.edu.in (S.J.); Tel. +91-9094020093

Received: 12 November 2022 / Revised: 02 March 2023 / Accepted: 03 March 2023

ABSTRACT: This study was designed to investigate the effect of ethanolic extract of *Pedalium murex* linnleaf (EEPML) in 3-Nitropropionic acid (3-NPA) induced neurodegeneration in a Sprague Dawley rat model. EEPML at a dose of 200 and 400mg/kg was given orally to desired group of animals for a period of 14 days. Neurodegeneration was induced by administering 3-Nitropropionic acid (10 mg/kg/i.p) on 14th day. Two hours after drug administration on 14th day of drug treatment, parameters such as *in vitro* anticholinesterase activity, DPPH Radical Scavenging Activity, behavioural test for memory and learning (Rotarod test, Open field test), Acetylcholine (ACh) content, Acetylcholinesterase (AChE) activity, Superoxide dismutase (SOD) level in brain homogenate were analysed. EEPML at both doses, i.e., 200 and 400 mg/kg, decreased the acetylcholinesterase in neurodegenerated rats. The EEPML 400 mg/kg/i.p had more pronounced effect on memory, learning test and motor coordination. The EEPML also decreased the body weight of neurodegenerated animals. Acetylcholine was found to be decreased in untreated neurodegenerated rats due to neuronal inflammation. The EEPML at both doses increased the acetylcholine level in neurodegenerated rats. Modulation of Acetylcholine level by the EEPML is related with its potential anti-oxidant and cholinesterase inhibitory activity.

KEYWORDS: Acetylcholinesterase; Pedalium murex Linn; superoxide dismutase; Huntington's disease.

1. INTRODUCTION

Huntington's disease (HD) is an inherited (autosomal dominant) disorder characterised by choreichyperkinesias (dance-like movements of limbs and rhythmic movements of tongue and face) and dementia with progressive brain degeneration. It is caused by a genetic error in huntingtin gene and subsequent abnormal synthesis of a huntingtin protein that contains several repeats of polyglutamine, in which GABA ergicstriatonigral pathway is impaired, which leads to large decreases in striatal GABA concentrations, whereas somatostatin and dopamine concentrations are relatively preserved [1].

The HTT gene on chromosome 41 harbours a CAG trinucleotide repeat that expands in HD, an autosomal-dominant progressive neurological disease. People who have one HTT allele with 40 or more CAG repeats are almost always affected by the disease, whereas people who have both alleles with less than 36 CAG repeats are not affected by the disease. Symptoms develop insidiously, either as a movement disorder manifest by brief, jerk like movements of the extremities, trunk, face, and neck (chorea) or as personality changes or both [1, 2]. According to estimates, there are 5 HD cases for every 100,000 persons [3].

The Pedaliaceae family includes Pedalium murex Linn. It is the most valuable medicinal herb, possessing a wide range of medical characteristics. *P. murex* is employed in medical systems such as Ayurveda,

How to cite this article: Velayutham S, Sundararaju A, Govindhaswamy T, Gurupackiyam M, Balakrishnan A, Abdul MY, Ashok C, Jeyabalan S. Neuroprotective Effect of Ethanolic Extract of Pedalium murex Linn Leaf in 3-Nitropropionic acid Induced Neurodegeneration. J Res Pharm. 2023; 27(4): 1388-1401

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.426

Folk medicine, Unani, and Siddha. *P. murex* is known by several common and vernacular names, including Bada Gokhuru in Hindi, Brihat Gokshuraka in Sanskrit, Land Caltrops in English, Anainerunji in Tamil, Kadva Gokhru in Gujarati, Gokhura in Marathi, Ananerinnil in Malayalam, and Baraghokru in Bengali. It is found in India, Tropical Africa, Pakistan and Sri Lanka. It is mostly found in Tamil Nadu, Rajasthan, Uttar Pradesh, Gujarat, and Punjab in India [4]. In Indian system of medicine, the plant *P. Murex* is being used as antiulcer, antimicrobial, antibacterial, antihepatotoxic, antioxidant and immune modulatory, antidermatophytic, antipyretic, aphrodisiac, aldose reductase inhibitory, antitussive, antihyperlipidemic, insecticidal and antifeedant successfully for centuries, Activation of immune system in response to stress and infection or produces profound neurophysiological, neuroendocrine and behavioural changes [5].

A number of studies has accredited the curative effect of *P. murex* due to its high content of flavonoids, phenols, glycosides, terpenoids, tannins, steroids, and saponins [6]. According to reports, flavonoids make up the majority of the material taken from the *P. murex* plant's stem, flowers, roots, and leaves [7]. Flavonoids reduce free radical production, some are known to promote angiogenesis. These pathways can be explored as potential treatments options for HD. Flavonoids can offer an alternative therapy choice in prevention and treatment of HD [8]. But there is non-existence of studies related with protective effect of *P. murex* in neurodegenerative diseases in human and experimental animal models too. Till now, some of the paradigms has been not used at all in the evaluation of *P. murex* leaf extract against behavioural consequences of rats in 3-NPA induced neurodegeneration. Hence, a special attention is focused to understand the treatment of ethanol extract from this plant against 3-NPA induced neurodegenerative diseases.

2. RESULTS

2.1. Percentage yield of *P. murex*

Table 1. Percentage yield of ethanolic extract of *P. murex*

Plant part	Consistency	Colour	Percentage yield
Leaf	Semisolid (pasty)	Dark green	7.34%

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

Table 2. *In vitro* AChE inhibitory effect of EEPML and donepezil hydrochloride

Concentration (µg/ml)	% Inhibition of AChE by EEPML	% Inhibition of AChE by Donepezil hydrochloride
12.5 µg/ml	18.54±0.37	42.38±0.75
25 µg/ml	31.81±0.45	70.55±0.41
50 µg/ml	35.71±0.23	80.33±0.17
100 µg/ml	38.56±0.21	86.26±0.14
200 µg/ml	43.78±0.42	90.19±0.10
400 µg/ml	48.29±0.25	93.31±0.16
IC_{50} value	7.09µg/ml	0.52µg/ml

Values are expressed in mean ± SEM (n=3)

Both EEPML and Donepezil hydrocholoride produced a dose depended increase in *in vitro* AChE inhibitory activity. EEPML has shown 38.56 ± 0.21% inhibition at 100 µg/ml while Donepezil hydrocholoride has shown 86.26±0.14% inhibitionat 100 µg/ml. IC_{50} value of EEPML and standard donepezil against AChE was found to be 7.09 µg/ml and 0.52 µg/ml respectively.

2.3. Percentage inhibition of EEPML on invitro DPPH radical scavenging activity

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

Concentration (µg/ml)	DPPH Free radical scavenging activity by EEPML	DPPH Free radical scavenging activity by Ascorbic acid
12.5 µg/ml	40.20±0.20	20.17±0.37
25 µg/ml	52.67±0.13	29.55±0.29
50 µg/ml	62.25±0.08	54.02±0.12
100 µg/ml	67.28±0.22	63.61±0.25
200 µg/ml	73.43±0.19	69.27±0.10
400 µg/ml	74.89±0.11	72.06±0.03
IC_{50} value	2.79µg/ml	4.37µg/ml

Values are expressed in mean ± SEM (n=3)
DPPH - 2,2-diphenyl-1-picrylhydrazyl

Both EEPML and Ascorbic acid produced a dose depended increase in DPPH free radical scavenging effect. EEPML has shown 67.28±0.22% inhibition at 100 µg/ml while Ascorbic acid has shown 63.61±0.25% inhibition at 100 µg/ml. IC_{50} value of EEPML and Ascorbic acid against DPPH Free radical scavenging activity was found to be 2.79 µg/ml and 4.37 µg/ml respectively.

2.4. Effect of EEPML on body weight

Table 4. Effect of EEPML on body weight

Effect of EEPML and Donepezil Hydrochloride on Body Weight			
Treatment Group (n=6)	Initial Weight	End of Week -1	End of Week -2
Distilled water	179.33±2.11	182.33±1.41	188.67±2.17
3-NPA(10 mg/kg/i.p)	182.00±1.16	185.67±1.59	190.67±1.52
3-NPA(10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	184.67±1.33	191.67±1.20	196.67±1.12
3-NPA(10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	184.00±1.71	178.67±1.43*	173.00±0.86***
3-NPA(10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	184.67±1.52	175.33±2.17***	165.00±1.98***

*p<0.05; **p<0.01;***p<0.001, ^{ns}p>0.05
Disease group is compared against the Normal group.
All the treated groups are compared against the disease group

Journal of Research in Pharmacy
Research Article

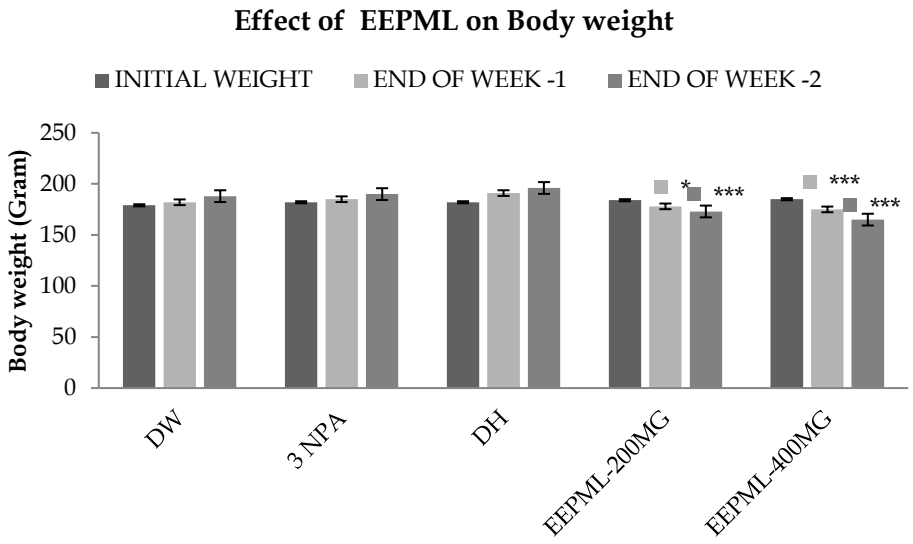

Figure 1. Effect of EEPML on body weight

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

EEPML 200 mg and 400 mg treated group animals have shown significant decrease in body weight when compared with disease group animals at the end of week 1 ($p<0.001$) and week 2 ($p<0.001$). Donepezil hydrochloride treated group animals have not shown significant decrease in body weight when compared with disease group animals at the end of week 1 and week 2 (Table 4).

2.5. Effect of EEPML on locomotor activity

Table 5. Effect of EEPML on locomotor activity

Treatment group (n=6)	Time spent on revolving rod in Rotarod apparatus (sec)
Distilled water	311.33±3.71
3-NPA (10 mg/kg/i.p)	114.17±3.73***
3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	266.00±2.63***
3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	129.17±1.17*
3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	234.67±3.04***

*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.

http://dx.doi.org/10.29228/jrp.426
J Res Pharm 2023; 27(4): 1388-1401

Journal of Research in Pharmacy
Research Article

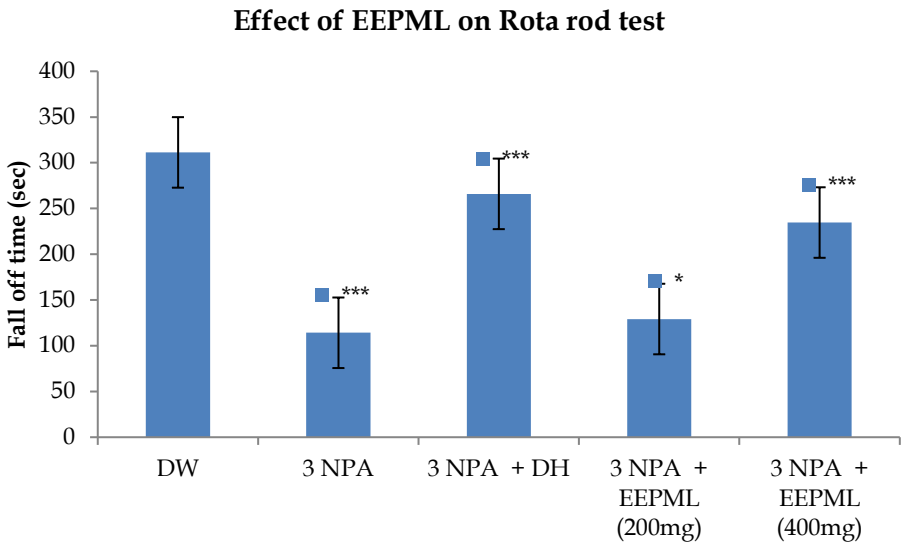

Figure 2. Effect of EEPML on rotarod test

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Normal group is compared against disease group
All the treated groups are compared against the disease group.
DW-Distilled water, 3 NPA – 3-Nitropropionic acid (10 mg/kg/i.p), 3 NPA + DH – 3 NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), 3 NPA + EEPML (200 mg) - 3NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), 3 NPA + EEPML (400 mg) - 3NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)

3-Nitropropionic acid (10 mg/kg/i.p) only treated animal showed highly significant decrease in fall off time when comparing to normal control group (p<0.001). Donepezil hydrochloride (3 mg/kg/p.o) and EEPML (400 mg/kg/p.o) treated animal showed highly significant increase in fall off time when comparing to disease control group (p<0.001).

2.6. Effect of EEPML on open field test

Table 6. Effect of EEPML on open field test

Treatment group (n=6)	Number of line crossed in 5 minutes	Rearing time(sec)
Distilled water	33.17±1.82	66.67±0.67
3-NPA (10 mg/kg/i.p)	10.00±0.97 ***	12.50±0.43***
3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	30.17±0.61***	54.17±0.79***
3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	14.83±0.54*	16.17±0.60**
3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	28.00±0.37***	48.00±0.36***

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Normal group is compared against disease group
All the treated groups are compared against the disease group.

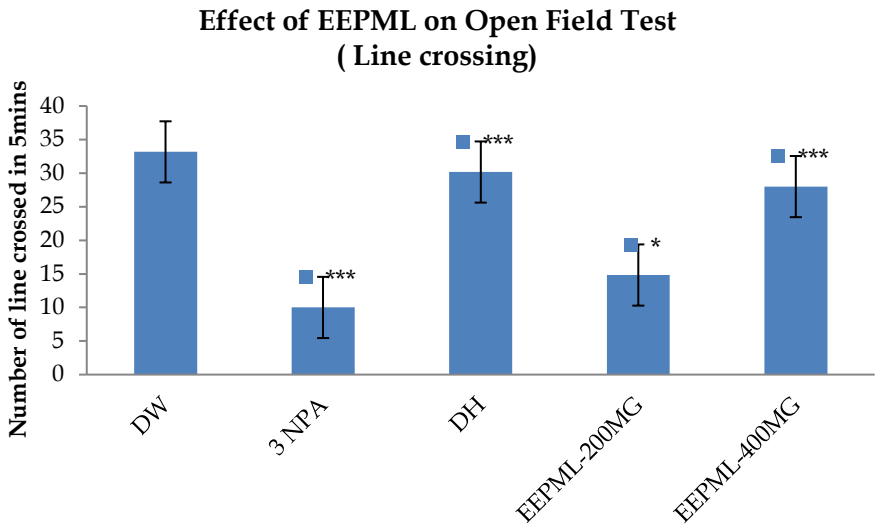

Figure 3. Effect of EEPML on open fiels test (Line crossing)

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Normal group is compared against disease group. All the treated groups are compared against the disease group.
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA(10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

3-Nitropropionic acid (10 mg/kg/i.p) only treated group showed a decrease in number of lines crossed when compared to control animals (p<0.001). A significant improvement in movement activity (increase in number of lines crossed) was observed in 3-nitropropionic acid treated animals administered with EEPML (400 mg/kg) and Donepezil hydrochloride (3 mg/kg/p.o) (p<0.001).

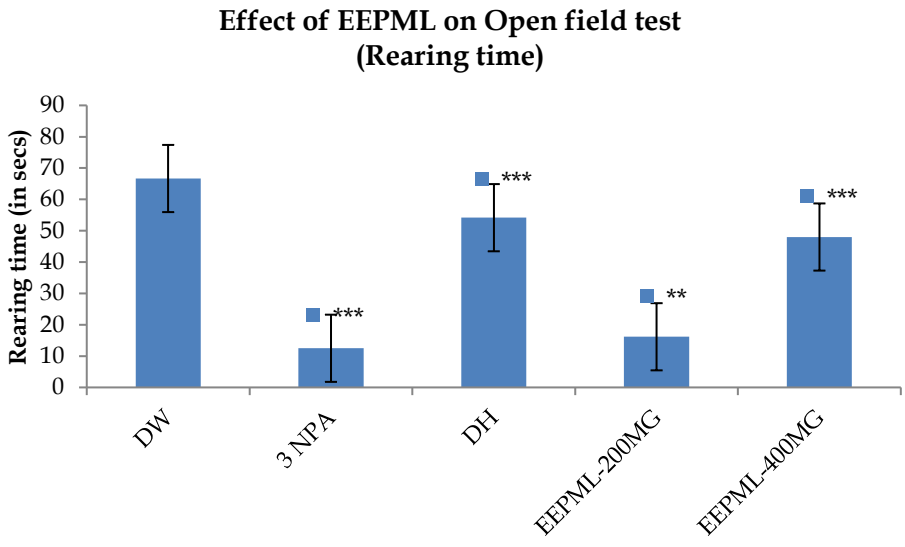

Figure 4. Effect of EEPML on open field test (Rearing time)

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Normal group is compared against disease group. All the treated groups are compared against the disease group.
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

3-Nitropropionic acid (10 mg/kg/i.p) only treated group showed a decrease in rearing when compared to control animals ($p<0.001$). A significant improvement in movement activity (rearing) was observed in 3-Nitropropionic acid treated animals administered with EEPML (400 mg/kg) and Donepezil hydrochloride (3 mg/kg/p.o) ($p<0.001$).

2.7. Effect of EEPML on Acetylcholine Level (ACh)

Table 7. Effect of EPML on Acetylcholine Level (ACh)

Treatment group (n=6)	µ moles of Acetylcholine/ g wet weight of tissue
Distilled water	5.05±0.08
3-NPA (10 mg/kg/i.p)	2.35±0.08***
3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	4.23±0.05***
3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	2.63±0.08*
3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	4.00±0.05***

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Disease group is compared against normal group
All the treated groups are compared against disease group

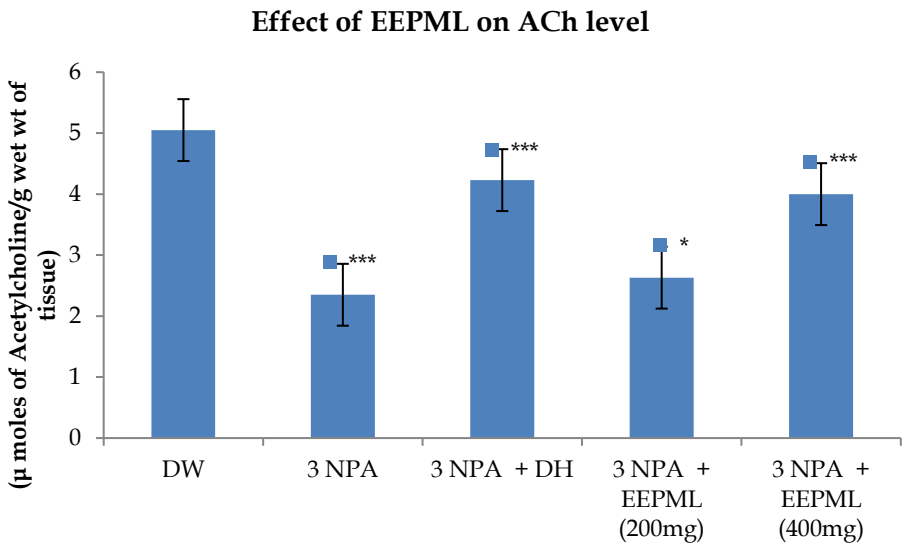

Figure 5. Effect of EEPML on Ach level

*p<0.05; **p<0.01;***p<0.001, nsp>0.05
Disease group is compared against normal group
All the treated groups are compared against disease group
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

3-Nitropropionic acid only treated group showed significant decrease in acetylcholine level ($p<0.001$) when comparing to normal group. Donepezil (3 mg/kg/p.o) and EEPML (400 mg/kg/p.o) treated group showed significant increase in acetylcholine when comparing to 3-Nitropropionic acid only treated group

2.8. Effect of EEPML on Acetylcholinesterase (AChE) activity

Table 8. Effect of EEPML on Acetylcholinesterase activity

Treatment group (n=6)	Acetylcholinesterase (AChE) activity (M/min/g protein)
Distilled water	0.27±0.003
3-NPA (10 mg/kg/i.p)	0.32±0.003***
3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	0.27±0.003***
3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	0.30±0.003**
3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	0.26±0.003***

*p<0.05; **p<0.01; ***p<0.001, nsp>0.05
Disease group is compared against normal group
All the treated groups are compared against disease group

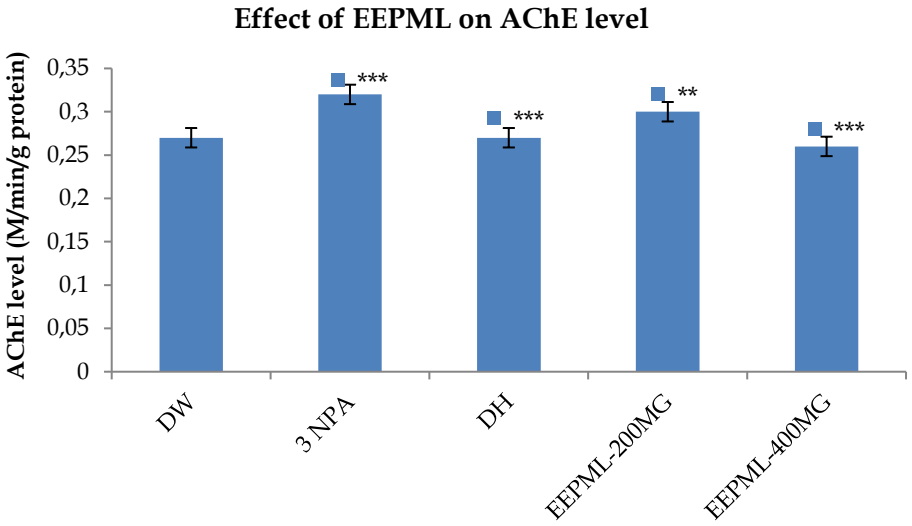

Figure 6. Effect of EEPML on AChE level

*p<0.05; **p<0.01; ***p<0.001, nsp>0.05
Disease group is compared against normal group. All the treated groups are compared against disease group
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

3-Nitropropionic acid only treated group showed significant increase in acetylcholinesterase level (p<0.001) when comparing to normal group. Donepezil (3 mg/kg/p.o) and EEPML(400 mg/kg/p.o) treated group showed significant decrease in acetylcholinesterase level (p<0.001) when comparing to 3-Nitropropionic acidonly treated group.

2.9. Effect of EEPML on superoxide dismutase (SOD) level

Table 9. Effect of EEPML on superoxide dismutase (SOD) level

Treatment group (n=6)	Superoxide dismutase (SOD) level (U/mg protein)
Distilled water	3.7±0.06
3-NPA (10 mg/kg/i.p)	2.8±0.04***
3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o)	3.4±0.04***
3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o)	3.0±0.04*
3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o)	3.02±0.03**

*p<0.05; **p<0.01; ***p<0.001, nsp>0.05
Disease group is compared against normal group
All the treated groups are compared against disease group

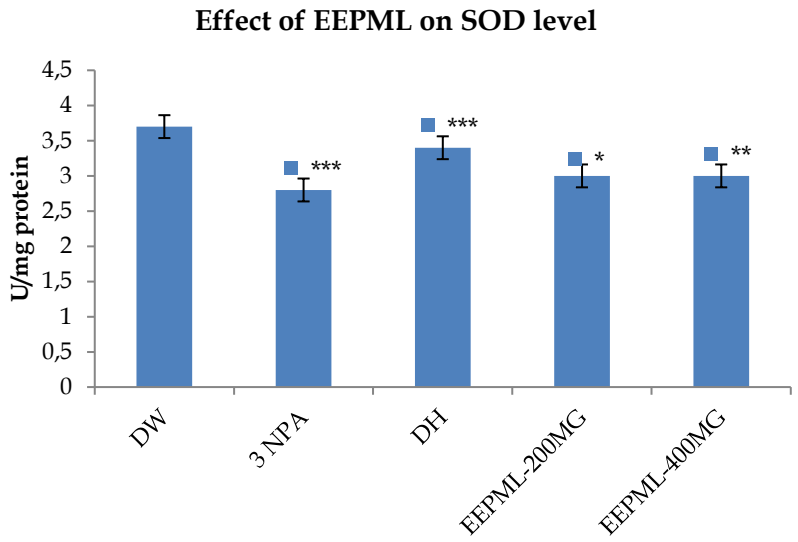

Figure 7. Effect of EEPML on SOD level

*p<0.05; **p<0.01; ***p<0.001, nsp>0.05
Disease group is compared against normal group
All the treated groups are compared against disease group
DW: Distilled water, 3 NPA : 3-Nitropropionic acid (10 mg/kg/i.p), DH : 3-NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg/p.o), EEPML-200 mg: 3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg/p.o), EEPML-400 mg: 3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg/p.o).

3-Nitropropionic acid only treated group showed significant decrease in SOD level ($p<0.001$) when comparing to normal group. Donepezil (3 mg/kg/p.o) and EEPML (400 mg/kg/p.o) treated group showed significant increase in SOD level ($p<0.001$) when comparing to 3-Nitropropionic acid only treated group.

3. DISCUSSION

The mitochondrial enzyme succinate dehydrogenase is inhibited by 3-NPA, which mimics a downstream process of cell death seen in selective excitotoxic striatal pathology in Huntington's disease (HD). It was discovered that 3-NPA intoxication triggers a mitochondrial permeability transition brought on by Ca^{2+}. Therefore, 3-NPA may be utilised as a HD animal model in experiments [9].

3.1. Effect of EEPML on *in vitro* AchE enzyme activity

Different cognitive domains are impacted by HD over the course of the illness. Executive function, mental flexibility, psychomotor performance, attention, working memory, and emotion detection are among the cognitive domains that are affected [10]. Acetylcholinesterase inhibition has been related to improvement of some cognitive (memory, attention)and behavioural (apathy, agitation, hallucinations) symptoms [11]. The critical role of cholinesterases in neural transmission makes them a key target of a large number of cholinesterase-inhibiting drugs relevant to the treatment of neurodegenerative disorders [12]. To evaluate the potential of the EEPML as an anti-Huntington drug, its AchE inhibitory activity was quantified. As shown in Table 2, the EEPML showed significant AchE inhibitory effect ($p<0.05$) (Figure 6) when compared to the standard (Donepezil hydrochloride) and the cholinesterase inhibitory activity occurred in a dose-dependent manner.

3.2. Anti-oxidant effect of EEPML

Reactive oxygen species (ROS) assault DNA and result in mutation, which causes pathological disorders like dementia, the ageing process, and neurodegeneration. Antioxidant is employed in the treatment of disorders brought on by ROS. Flavonoids and other phenolic substances have been found to have substantial antioxidant action [13]. Due to their redox characteristics, the phenolic compounds in herbs operate as antioxidants, acting as reducing agents, hydrogen donors, free radicals, quenchers, and metal chelators [14]. Ascorbic acid and EEPML both increased the DPPH free radical scavenging effect in a dose-dependent manner. Ascorbic acid showed a 63.61% inhibition at 100 g/ml, whereas EEPML demonstrated a 67.28% inhibition. (Table 3). High antiradical capabilities of EEPML may be due to phenolic compounds (containing phenolic hydroxyls) being present. Other compound flavonoids, such as flavones, flavanols, and condensed tannins, are secondary metabolites with antioxidant activity due to the presence of free OH groups, especially 3-OH for the avoidance of oxidative stress, which may be the cause of DPPH activities.

3.3. Effect of EEPML on body weight

Our findings that the body weight of the EEPML 200 mg and 400 mg treated group animals had decreased significantly by the end of weeks 1 and 2 ($p<0.001$) when compared to the disease group animals are consistent with Mukundh N et al.'s 2008 finding that the administration of an ethanolic extract from Pedalium murex fruits caused a significant decrease in the body weight of animals with hypercholesterolemia [15]. When compared to the disease group animals at the end of weeks 1 and 2, the Donepezil hydrochloride treated group animals did not exhibit a significant decrease in body weight.

3.4. Effect of EEPML on Motor coordination

There were flavonoids, glycosides, tannins, carbohydrates, phenolic compounds, triterpenoids, and saponins found in EEPML after preliminary phytochemical screening. The open-field test (OFT) is frequently employed to evaluate emotionality and locomotor function. The OFT was utilised to rule out these erroneous effects that might be related to hyperkinesia [5]. Because EEPML was administered in our study, there was an increase in locomotor activity, demonstrating that EEPML's unique effects on the behavioural model are indicative of anti-immobility activity. Rotarod testing is a popular screening technique for assessing rodent locomotor activity (caused by changes in muscle relaxation). Gamma-aminobutyric acid (GABA) concentration increases have been reported to have CNS depressive effects [16].

The improvement in locomotion suggests that EEPML may work through GABA receptors. The antagonistic impact of EEPML on the GABA/benzodiazepine receptor complex may be the cause of the reported effect of the drug on muscle (increase in fall-off time). The studied antioxidant pretreatments were successful in reducing 3-Nitropropionic acid-induced brain oxidative stress as evidenced by the quenching of free radicals and elevation of the antioxidant SOD. EEPML reduces anxiety in the Rotarod and open field test (Figure 7).

In a dose-dependent manner, EEPML significantly attenuated the behavioural effects. While significant protection was elicited by all treatments, EEPML 400 mg/kg delivered the best outcomes.

4. CONCLUSION

We chose the ethanol extract for pharmacological screening after discovering that EEPML shown considerable *in vitro* results of acetylcholinesterase inhibition and *in vitro* free radical scavenging activities. Results of behavioural tests for motor coordination, agitated levels of acetylcholine, and SOD in this study

showed that 3-NPA caused memory and learning problems and abnormal movement in rats. These symptoms were found to be reversed by EEPML ($p<0.05$) when compared to groups that had been treated with Donepezil hydrochloride. A gradual increase in the pharmacological activity was observed and EEPML at 400 mg/kg delivered the best outcomes. These findings suggested that the existence of strong antioxidants in EEPML may make it a possible candidate for 3-NPA-induced brain injury. However, more in-depth investigation is required to pinpoint the precise components and clarify any potential mechanisms of action underlying the anti-huntington effect of EEPML. These recent discoveries might be used to develop neuroprotection techniques that are more successful than present treatments.

5. MATERIALS AND METHODS

5.1. Collection and authentication of plant material

In the months of November and December, *Pedalium murex* linn (*P. murex*) leaf was procured from the neighbourhood market in Komarapalaym in the Tamilnadu state of India. It was dried in the shade at a temperature that didn't get above 40°C. The botanist Dr. M. Kannan, Head of the Department of Botany at the Vivekanandha College of Arts and Science for Women in Tiruchengode, Namakkal Dt., Tamil Nadu, identified and verified the Pedalium murex linn (*P. murex*) leaf.

5.2. Preparation of ethanolic extract

Under cover, the *P. Murex* leaf was dried. Using a mixer grinder, the dried material was ground into powder. To obtain consistent powder, this powder was next put through sieve number 40. This leaf powder was extracted using a power mill. 90% ethanol was used as the solvent for 24 hours of extraction in a Soxhlet extractor. To obtain the extract, the round bottom flask's contents were evaporated at 40°C under reduced pressure. After that, the extract was put into a clean container and labelled [17].

5.3. Determination of *in vitro* Anticholinesterase activity (AchE).

The Ellman colorimetric method was used to determine the acetylcholinesterase inhibition assay. A total of 1 ml of a mixture including 415 l of 0.1 M Tris-HCl buffer (pH 8), 10 l of various concentrations of EEPML solution in methanol, and 25 l of acetyl-cholinesterase enzyme solution were incubated for 15 min at room temperature. AchI (acetyl-thiocholine) solution containing 1.83 mM was dissolved in 75 l, and 475 l of DTNB (5,5-dithiobis-2-nitrobenzoic acid) solution containing 3 mM was added. The finished mixture was then incubated at room temperature for 30 min. The mixture's absorbance was determined at 412 nm using a UV-Visible 752 spectrophotometer. As a positive control, Donepezil hydrochloride was utilised. It was calculated what proportion of enzyme activity was inhibited [18].

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

Three millilitres of the solution to various concentrations of extracts in methanol were combined with one millilitre of a 0.1 mM solution of DPPH in methanol. After shaking, the mixture was left in a pitch-black chamber for 30 minutes. A spectrophotometer was then used to measure the absorbance at 517 nm. The EEPML radical's potential to scavenge DPPH radicals was then established [19].

5.5. Dose and drug solution

According to earlier reports, *P. murex* was found to be non-toxic upto 2.5 g/kg in rats [4]. Hence, the present study was carried at two dose levels, i.e., at 200 and 400 mg/kg body weight. To prepare the test drug, required quantity of the EEPML was dissolved in distilled water to have a desired dose in 1mL solution [20]. 3-NP was dissolved in 5% dimethyl sulfoxide saline (pH 7.4) [21].

5.6. Animals

Sprague Dawley rats (100-150 g), of either sex, were kept in typical lab settings at a temperature of 25°C and a relative humidity of 55%, with a regular cycle of 12 hours of light and 12 hours of darkness. Animals were provided free access to tap water and a conventional rat pellet diet. The Institutional Animal Ethical Committee (IAEC) (JKKMMRFCP/IAEC/2021/001), JKKMMRF College of Pharmacy, Komarapalayam, approved the study protocol.

5.7. Experimental groups

Six animals each made comprised each of the five groups that were randomly assigned to the animals. For 14 straight days, Group III received doses of donepezil hydrochloride (3 mg/kg, p.o.) every 24 hours [22]. Every 24 hours for 14 days straight, EEPML doses of 200 and 400 mg/kg, p.o. were given orally to groups IV and V, respectively. On day 14, group II to V received an injection of 3-Nitropropionic acid (10 mg/kg/i.p.) in normal saline to cause neurodegeneration [20]. Animals were given behavioural tests two hours after receiving 3-Nitropropionic acid, and then euthanised by mild halogenated ether anesthesia and the brain was removed for biochemical study.

Table 10. Experimental groups

Group I	Normal control group (Distilled water)
Group II	Disease 3-Nitropropionic acid (3-NPA) (10 mg/kg/i.p)
Group III	Standard group treated with 3NPA (10 mg/kg/i.p) + Donepezil hydrochloride (3 mg/kg, p.o.)
Group IV	3-NPA (10 mg/kg/i.p) + EEPML (200 mg/kg, p.o)
Group V	3-NPA (10 mg/kg/i.p) + EEPML (400 mg/kg, p.o)

5.8. Motor coordination and grip strength tests

5.8.1. Rotarod test

Rats' grip strength and motor coordination were tested using a rotarod apparatus; the animals had previously received training to become acclimated to the device. The fall time was recorded, and the cutoff time was 300s [21].

5.8.2. Open field test

The open field habituation task approach was used to assess the rat's exploratory behaviour. Four inner squares and twelve outer squares along the walls made up the 16 squares that made up the open field arena. Rats are placed individually in the middle of the open field after becoming acclimated to the lab and the open field apparatus. For five minutes, parameters such as the number of squares crossed, or line crossings, and rearing were monitored [24].

5.9. Biochemical study

5.9.1. Preparation of brain homogenate

The rats weregiven a mild isoflurane anesthesia, which is a halogenated ether before having their brains swiftly removed and preserved in ice-cold saline. Then, it was homogenised in ice-chilled phosphate buffer to create 10% w/v brain homogenate (pH 8, 0.1M). The homogenate was then spun in a chilled centrifuge for 10 minutes at 3000 rpm to separate the supernatant, which was then used for the biochemical calculations [25].

5.9.2. Estimation of Acetylcholine (Ach) content

In order to stop the Acetylcholinesterase enzyme from working and to release the bound Ach, the rat brain homogenate was put in a boiling water bath for five minutes. In 1ml of distilled water, the tissues were then homogenised. 1 ml of alkaline hydroxylamine hydrochloride and 1 ml of a 50% hydrochloric acid solution were each added to the homogenate. The components were carefully combined before centrifuging. The brown colour developed was read 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) in a spectrophotometer after the addition of 0.5 ml of 0.37 M ferric chloride solution to the supernatant. The amount of acetylcholine was given as moles of Ach/gm of tissue's moist weight [26].

5.9.3. Estimation of Acetylcholinesterase (AchE) activity

An estimate was made of the AchE activity in the rat brain homogenate aliquot. Phosphate buffer will be combined with the aliquot (pH 8). Acetylthiocholine Iodide Substrate and Dithiobisnitrobenzoic Acid (DTNB) Reagent will be added to this. AchE will hydrolyze acetyl thiocholineiodide into thiocholine and acetate. Yellow colour was produced by the DTNB reagent's reaction with thiocholine. The AchE activity will

be gauged by how quickly colours develop. Spectrophotometric analysis of the enzyme activity's kinetic profile will be done at 412 nm every 15 seconds. The mol of substrate hydrolyzed/min/gm tissue will be used to express the enzyme activity [27].

5.9.4. Estimation of superoxide dismutase activity (SOD)

An estimate was made of the SOD activity in the rat brain homogenate aliquot. The incubation mixture contained Phenazine methosulfate (PMS) (186 mol), Nitro bluetetrazolium NBT (300 mol), and nicotinamide adenine dinucleotide (NADH) (780 mol) in addition to sodium pyrophosphate buffer (pH 8.3; 0.052 M; 1.2 mL). NADH was added to start the reaction, which was then incubated for 90 seconds at 37 °C. The addition of glacial acetic acid (1 mL) and n-butanol (4 mL), along with a vigorous shake and centrifugation at 4000 rpm for one minute, were used to stop the reaction. The upper butanol layer was then read at 560 nm against a butanol blank. [28].

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.

Acknowledgements: The authors would like to express gratitude to the management of JKKMMRF, AnnaiSampoorani Ammal College of Pharmacy and Sri Ramachandra University for providing us with all the facilities for the successful completion of the research.

Author contributions: Concept – S.V., T.G., M.G.; Design – S.V., A.S., T.G., M.G., A.B.; Supervision – S.V., T.G.; Resources – S.J., T.G., M.G.; Materials – T.G., M.G., A.S., A.B..; Data Collection and/or Processing – A.S., A.B., M.G.; Analysis and/or Interpretation – T.G., M.G., A.S., A.B.; Literature Search – S.V., A.B., A.S., T.G., M.G., M.A., C.A.; Writing – A.S., A.B., M.A.; Critical Reviews – S.V., A.S., T.G., M.G., A.B., M.A., C.A., S.J.

Conflict of interest statement: The authors declare no conflict of interest in the manuscript.

REFERENCES

[1] Komal, Parul Sharma, Firoz Khan, Lubhan and Vipin Kumar Garg. To investigate the neuroprotective effect of Crocin against Colchicine and Aluminium Chloride ınduced cognitive disfunction and oxidative damage in rodents. Adv Pharmacol Clin Trials. 2019; 4(3): 000163. https://doi.org/10.23880/apct-16000163.

[2] Keller CG, Shin Y, Monteys AM, Renaud N, Beibel M, Teider N, Peters T, Faller T, St-Cyr S, Knehr J, Roma G. An orally available, brain penetrant, small molecule lowers huntingtin levels by enhancing pseudoexon inclusion. Nature Commun. 2022;13(1):1150. https://doi.org/10.1038/s41467-022-28653-6.

[3] Wiprich MT, Bonan CD. Purinergic signaling in the pathophysiology and treatment of Huntington's disease. Front Neurosci. 2021:787. https://doi.org/10.3389/fnins.2021.657338.

[4] Tyagi P, Ranjan R. Comparative study of the pharmacological, phytochemical and biotechnological aspects of Tribulus terrestris Linn. and Pedalium murex Linn: An overview. Acta Ecol Sin. 2023; 43(2): 223-233. https://doi.org/10.1016/j.chnaes.2021.07.008.

[5] Gomathi S, Shanmuga SR, Vijayabaskaran M, Kannan C, Sambathkumar R.*Pedalium murex Linn* leaves against LPS-induced oxidative stress, anxiety and depression behavioural alterations in rats. Res J Pharm Tech. 2017; 10(5): 1333-1338. https://doi.org/10.5958/0974-360X.2017.00236.0.

[6] Mandavia DR, Patel MK, Patel JC, Anovadiya AP, Baxi SN, Tripathi CR. Anti-urolithiatic effect of ethanolic extract of Pedalium murex linn. fruits on ethylene glycol-induced renal calculi. Urol J. 2013;10(3):946-952.

[7] Choudhary S, Kaurav H, Chaudhary G. Gokhru (Tribulus Terrestris and Pedalium Murex): Medicinal Importance of Chota Gokhru And Bada Gokhru in Ayurveda and Modern Science. Asian J Pharm Clin Res. 2021; 14(6): 6-13. https://doi.org/10.22159/ajpcr.2021.v14i6.41366.

[8] Khan H, Ullah H, Tundis R, Belwal T, Devkota HP, Daglia M, Cetin Z, Saygili EI, Campos MD, Capanoglu E, Du M. Dietary flavonoids in the management of huntington's disease: Mechanism and clinical perspective. EFood. 2020 Feb;1(1):38-52. https://doi.org/10.2991/efood.k.200203.001.

[9] Milutinović A. Lithium chloride could aggravate brain injury caused by 3-nitropropionic acid. Bosn J Basic Med Sci. 2016;16(4):261-267. https://doi.org/10.17305/bjbms.2016.1206.

[10] Ali Reza ASM, Hossain MS, Akhter S, Rahman MR, Nasrin MS, Uddin MJ, Sadik G, Khurshid Alam AHM. In vitro antioxidant and cholinesterase inhibitory activities of Elatostema papillosum leaves and correlation with their phytochemical profiles: a study relevant to the treatment of Alzheimer's disease. BMC Complement Altern Med. 2018;18(1):123. https://doi.org/10.1186/s12906-018-2182-0.

[11] Rosca EC, Simu M. Montreal Cognitive Assessment for evaluating cognitive impairment in Huntington's disease: A systematic review. CNS Spectr. 2022 Feb;27(1):27-45. https://doi.org/10.1017/S1092852920001868.
[12] Vecchio I, Sorrentino L, Paoletti A, Marra R, Arbitrio M. The state of the art on acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. J Cent Nerv Syst Dis. 2021; 13:11795735211029113. https://doi.org/10.1177/11795735211029113.
[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. IJAPBC. 2014; 3(2): 410-419.
[14] Sakthidevi G, Mohan VR, Jeeva S. *In vitro* antioxidant activity of leaf extracts of *Alangium salvifolium* (C.F) warf (Alangiaceae). Biosci Disc. 2014; 5: 74-81.
[15] Balasubramanian MN, Muralidharan P, Balamurugan G. Anti hyperlipidemic activity of *Pedafium murex* (Linn.) fruits on high fat diet fed rats. Int J Pharmacol. 2008; 4 (4): 310-313. https://doi.org/10.3923/ijp.2008.310.313
[16] Tirumalasetti J, Patel M, Shaikh U, Harini K and Shankar J. Evaluation of skeletal muscle relaxant activity of aqueous extract of *Nerium oleander* flowers in Albino rats. Indian J Pharmacol. 2015;47:409-13. https://doi.org/10.4103/0253-7613.161265.
[17] Harborne JB. Phytochemical Methods. A Guide to modern techniques of plant Analysis. 3rd ed. New Delhi, *Springer* (*India*) *Pvt. Ltd*; 1998;5-16.
[18] Owokotomo IA ¸ Ekundayo O, Abayomi TG, Chukwuka AV. In-vitro anti-cholinesterase activity of essential oil from four tropical medicinal plants. Toxicol Rep. 2015;2: 850–857. https://doi.org/10.1016/j.toxrep.2015.05.003.
[19] Pant DR, Pant ND, Saru DB, 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. J Intercult Ethnopharmacol. 2017; 6(2): 170-176. https://doi.org/10.5455/jice.20170403094055.
[20] Gomathi S, Sundaram RS, Annapandian VM, Vijayabaskaran M. Neuroprotective effect of *Pedalium murex* Linn. Leaf against lipopolysaccharide ınduced behavioural disorders in rat. Pharmacogn J. 2017; 9(6): 957-962. https://doi.org/10.5530/pj.2017.6.150.
[21] Mehan S, Monga V, Rani M, Dudi R and Ghimire K. Neuroprotective effect of solanesol against 3-nitropropionic acid-induced Huntington's disease-like behavioral, biochemical, and cellular alterations: Restoration of coenzyme-Q10-mediated mitochondrial dysfunction. Indian J Pharmacol. 2018 ; 50(6): 309-319. https://doi.org/10.4103/ijp.IJP_11_18.
[22] 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 J Pharmacol. 2017;49:60-64. https://doi.org/10.4103/0253-7613.201016.
[23] Kaur N, Jamwal S, Deshmukha R, Gauttamb V and Kumar P. Beneficial effect of rice bran extract against 3-nitropropionic acid induced experimental Huntington's disease in rats.Toxicol Rep. 2015;2: 1222–1232. https://doi.org/10.1016/j.toxrep.2015.08.004
[24] Adebiyi OE, Olopade FE, Olopade JO, Olayemi FO. Behavioural studies on the ethanol leaf extract of *Grewiacarpinifolia* in Wistar rats. Afri Health Sci. 2016;16(1) : 339-346. https://doi.org/10.4314/ahs.v16i1.45
[25] Ramakrishnan P, Perumal P, Ramadoss D, Ramalingam P, Vijayarangan SCB. Amelioration of cognitive deficits, oxidative damage, neurochemical alteration by *Bauhinia purpurea* (stem bark) on scopolamine ınduced amnesia. J Young Pharm. 2020; 12(3) : 231-236. https://doi.org/10.5530/jyp.2020.12.66.
[26] Santhi Krupa D, ChLochana, Padmalatha K. Antialzheimer's Potential of *Abrus pectoris* hydro alcoholic root extract. J Pharm Sci Res 2020; 12(7): 914-919.
[27] Erukainure OL, Ijomone OM, Sanni O, Aschner M and 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 (Raphiahookeri) wine. Metab Brain Dis. 2019; 34:1385–1399. https://doi.org/10.1007/s11011-019-00444-x.
[28] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J BiochemBiophys. 1984; 21:130-132.