



Evaluation of Fluoride-Induced Neuro Degenerative Defences in *Cyclea peltata* Lam Roots

Rajkumar S¹, Sambathkumar R^{*1}, Gomathi Swaminathan², Vijayabaskaran M¹, Kannan C¹

¹Department of Pharmaceutical Chemistry, J.K.K Nattraja College of Pharmacy, Komarapalayam - 638183, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India

²Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy, JSS Academy of Higher Education & Research, Ooty-643001, Tamil Nadu, India



Article History:

Received on: 08 Nov 2020

Revised on: 10 Dec 2020

Accepted on: 17 Dec 2020

Keywords:

Cyclea peltata,
Fluoride,
Antioxidants,
Neurodegeneration

ABSTRACT

Many diseases have become easier to diagnose and treat as a result of advancements in medical science and technology, but strokes, which have multiple etiologies and mechanisms, continue to be difficult to treat. Synthetic drugs are notorious for causing a slew of unavoidable side effects. Herbal drugs have a wide range of mechanisms of action and are typically free of side effects, making them excellent alternatives to synthetic drugs for stroke treatment. *Cyclea peltata* (Lam) Hook f. Thoms roots were studied for their neuroprotective properties against fluoride-induced neurodegeneration in rats. The rat brain homogenate was investigated for the levels of non-enzymatic antioxidants like norepinephrine and serotonin to analyse the health of the brain. Both the hormones norepinephrine and serotonin levels were restored due to the treatment with ethanol extract of the plant. The antioxidant enzyme levels like SOD, CAT, GSH and GPx in the rat brain were estimated, and the results were similar to the non-enzymatic levels. The elevation of antioxidant enzymes indicates that the extract had an antioxidant mechanism that is responsible to help in the prevention of neurodegeneration of rats. In the brain tissues treated with *Cyclea peltata* extract, there was a significant increase in antioxidant enzymes and a reduction in lipid peroxidation, confirming the antioxidant mechanisms responsible for stroke prevention in extract-treated groups. The root extract of *Cyclea peltata* was found to show a reasonable inhibitory effect on neurodegeneration when delivered at a dose of 200mg/kg.

*Corresponding Author

Name: Sambathkumar R

Phone: 98427 79911

Email: sambathkumar.r@jkkn.ac.in

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.4756>

Production and Hosted by

IJRPS | <https://ijrps.com>

© 2020 | All rights reserved.

INTRODUCTION

Most patients die or suffer from morbidity and mortality as a result of brain disorders and strokes. In many cases, permanent or temporary disabilities, if not death, are the result. The majority of stroke victims experience physical and mental disabilities (Mozaffarian *et al.*, 2015). Brain disorders are the leading cause of death worldwide, accounting for approximately 5 million deaths each year. Stroke affects two out of every 1000 patients, and it is more common in the elderly population. According to some estimates, the number of stroke cases will reach 1.4 billion by 2050 (Kinsella and Monk, 2009). Many diseases have become easier to diag-

nose and treat as a result of advances in science and technology in the medical field, but strokes, which have multiple ethology and mechanisms, continue to pose challenges during treatment (Dirnagl, 2006; Green and Shuaib, 2006). Stroke is usually caused by a complex set of mechanisms and pathways that, despite modern medicine, are still difficult to identify and target.

Only one receptor or pathway is targeted by the synthetic drugs used to treat and prevent stroke. They are effective in strokes caused by a single mechanism, but in strokes caused by multiple etiologies, they are ineffective. As a result, there is a need to look into other options for stroke prevention and treatment, as well as develop drugs that treat multiple pathways. Synthetic drugs, on the other hand, are known to have a plethora of unavoidable side effects. Herbal drugs have a variety of mechanisms of action and are usually free of side effects, making them excellent alternatives to synthetic drugs in the treatment of stroke (George and Steinberg, 2015).

Cyclea peltata (Lam) Hook f. Thoms is generally called as the velvet leaf plant and is a shrub which twins on high trees. It is native to south-east Asia and India. Ancient literary works like sandhaniyamakashaya mentioned about the uses of the plant roots (Vaidya Bhagwan Dash and Ram Karan Sharma, 2013). It is commonly used to treat skin disorders, diarrhoea and GI upset. Works are published which prove the antiulcer, wound healing, antioxidant and antibacterial activities of the plant (Shine et al., 2009; Jyothiabrahamt and Den-nisthomas, 2012; Meena and Santhy, 2015). Having in mind the important medicinal properties of the plant, the roots of *Cyclea peltata* (Lam) Hook f. Thoms had been investigated for neuroprotective potency against the fluoride induction of neurodegeneration in experimental rats.

MATERIALS AND METHODS

Plant material

Cyclea peltata Lam roots were obtained from Thiruvananthapuram district of Kerala, India and authenticated by Dr. B. Duraiswamy, Head and Professor of Department of Pharmacognosy, JSS College of Pharmacy, Ooty. A herbarium specimen had been deposited at the J.K.K. Nattraja College of Pharmacy Herbarium (JSSCP-OOTY/COG/306, dated: 13.11.2018).

Preparation of Extract

Cyclea peltata Lam. roots were meticulously washed with clean water and dried in the dark for 1 week at room temperature. They are then made into a

fine powder and stored at ambient temperature. The crushed drugs were filtered via sieves 40 and 80. Ground items of the same size found in the two sieves were obtained and stored in a clean container for future use. A total of 1 kilogramme of shaded and dried plant roots of *Cyclea peltata* Lam. was extracted using soxhlet with petroleum ether, ethyl acetate, chloroform, and 90% v/v ethanol. A rotary vacuum evaporator was used to evaporate each extract. The extracted extracts from each solvent were weighed, and the percentage yield compared with the weight of the crude drug was calculated. The extract's consistency and colour were noted. All of the solvents utilized in this project were of the analytical grade.

Preliminary Phytochemical Analysis

All the 4 extracts of *Cyclea peltata* Lam. roots were submitted to chemical tests for the evaluation of various plant chemical constituents (Harborne, 2005; Krishnaswamy, 2003; Chatwal et al., 2019; Kasture et al., 2008).

In vitro Free Radical Scavenging Activity

DPPH free radical scavenging potency

A 1 mM DPPH free radical solution mixed in methanol solution was prepared, and 1 mL of this mixture was poured into different concentrations of *Cyclea peltata* Lam. root extracts. The solution was vigorously mixed and allowed to rest for 30 minutes at ambient temperature in the dark, and absorbance was estimated at 517 nm using a UV spectrophotometer and calculated (Blois, 1958). For the control, 1 mL of 1 mM DPPH radical solution was mixed with 1.0 mL of methanol.

$$\text{DPPH free radical Scavenging Activity Percentage\%} = \frac{(\text{Control Abs} - \text{Extract Abs})}{(\text{Control Abs})} \times 100$$

Reducing ability

The reducing capacity of *Cyclea peltata* Lam extracts was estimated using Oyaizu's technique (Oyaizu, 1986). *Cyclea peltata* Lam root extracts (12.5, 25, 50, 100, and 200 mg) were combined with phosphate buffer (2.5mL, 0.2M, pH6.6) and ferricyanide of potassium [K₃Fe(CN)₆] in 1 mL of distilled water (2.5mL, 1%). For 20 minutes, the mixture was allowed to rest at 50°C. A fraction (2.5mL) of TCA (10%) was prepared to the mixture, that was subsequently subjected to centrifugation at 1000xgm for 10 minutes. The topmost layer of solution (2.5 mL) was combined with double distilled water (2.5mL) and FeCl₃ (0.5mL, 0.1 per cent), and absorbance at 700 nm was estimated with a spectrophotometer. The higher the absorbance of the reaction mix-

ture, the larger the reducing power. *Cyclea peltata* Lam ethanol extract was chosen for pharmacological effects depending on phytochemicals and in vitro free radical scavenging results.

Invivo Pharmacological Studies

Animals

The rats utilized in the research were Sprague Dawley (150-200 gm). Animals were obtained from the J.K.K. Natraja College of Pharmacy in Kumarpalayam and housed in regular circumstances. During the experiment, regular commercially available food was served with unlimited water.

The animals were housed in a neat and dry polycarbonate cage in a properly ventilated rat house with a 12-hour light/dark cycle. This research was approved by the institutional animal ethics committee (Reg. No: JKKNCP/IAEC/PhD/01/2018)).

Oral Toxicity Invivo Study

The acute toxicity investigation was done out in accordance with OECD guidelines 423. In each group, three animals of the same sex were employed. Each group received EECP at a dose of 5,50,300, and 2000mg/kg b.w.

The animals were fasted overnight before the extract was given to them. For 14 days, animals were monitored for signs and symptoms of poisoning.

Experimental design

The rats were divided into 5 groups at random, and EECP was given to them for 30 days (p.o). The neurodegeneration was then triggered for 30 days by administering fluoride (120 ppm) in normal saline. Animals were exposed to behavioural tests two hours following Fluoride treatment, and the brain was removed for biochemical analysis.

Group I is the control group. For 30 days, the animals were given 0.1 ml of normal saline orally.

Group II is in charge of disease control. For 30 days, fluoride (120 ppm) was administered.

Group III is the standard group. For 30 days, Indomethacin (0.5 mg/kg) was combined with Fluoride (120 ppm).

Group IV is the treatment group. For 30 days, EECP (100 mg/kg) was combined with fluoride (120 ppm).

Group V is the treatment group. For 30 days, EECP (200 mg/kg) was combined with fluoride (120 ppm). Animals were exposed to behavioural examinations and biochemical examination at the end of the therapy. All of the values are statistically analyzed.

Behavioural Studies

Water maze test

The water maze was made up of a round-shaped tank with a radius of 50 cm and a height of 20cm above the water surface level. A round platform was inserted in 2cm beneath the water's level. During the experiment, the water was rendered opaque with a titanium dioxide solution and held at roughly 23°C. The animals were trained for 5 days in a row, with 3 trials each day and an interval of 6-10 minutes. Each test began at 1 of 4 different polar locations, with a different pattern each day. The platform's swimming length was measured till it found the platform (Morris, 1984).

Radial-arm maze test

The device was an elevated 8-arm maze radial with arms radiating from a centre 26 cm diameter platform. Each arm is 56 cm long, 5 cm wide, and has 2cm high rails running the length of it. The maze is well-lit, and there were various indications. Food pellets were placed at the ends of the arms as a reward. To enable the rats to run in the maze, they were only fed once a day, and their weight was kept at 85 % of their free-feeding weight during the test. For 24 days, animals were trained in the maze to collect food pellets on a daily basis. The test was ended after 8 selections, and the rats had to get the most number of treats with the fewest amount of errors (Olton and Samuelson, 1976).

Biochemical Analysis

Preparation of brain tissue homogenate

Following behavioural observations, all of the animals were decapitated and slaughtered. The brains were rapidly removed and weighed after being rinsed in ice-cold sterile isotonic saline. With 0.1M tris-HCL buffer, tissue homogenates of 10% (w/v) were produced (pH 7.4). Centrifugation of the homogenate at 1000 rpm for 20 minutes at 5°C yielded the supernatant, which was used for biochemical analysis.

In vivo non-enzymatic antioxidant studies in brain hippocampus

Estimation of Neurotransmitters [Norepinephrine (NE) and Serotonin]

Schlumpf *et al.* (1974) described a method for estimating norepinephrine (NE) and serotonin (Kakkar *et al.*, 1984). Iodine solution was added to the aqueous phase of the brain homogenate for oxidation, and sodium thiosulfate solution was added after that. Excitation-emission spectra were read at 395-485 nm to estimate Norepinephrine after the reaction medium was heated at 100°C for 6minutes. In a

Table 1: DDPH free radical scavenging capacity of the extract

S.No	Concentration ($\mu\text{g/ml}$)	%inhibition				
		Ascorbic acid	Pet Ether Extract	Ethyl acetate Extract	Chloroform Extract	Ethanol Extract
1	12.5	42.17	28.46	21.76	32.61	39.92
2	25	51.04	32.1	34.47	39.57	42.2
3	50	67.83	48.24	41.92	47.12	51.78
4	100	80.13	52.17	50.06	54.46	73.12
5	200	94.19	59.44	52.98	62.7	89.21
6	IC ₅₀ value	11.95	115.75	147.59	95.94	43.58

Table 2: Effect of extracts on Reducing ability

S.No	Concentration ($\mu\text{g/ml}$)	% inhibition				
		Standard (Gallic acid)	Pet Ether Extract	Ethyl acetate Extract	Chloroform Extract	Ethanol Extract
1	12.5	33.45	29.65	28.78	25.28	34.67
2	25	47.78	34.52	37.21	34.87	45.89
3	50	58.02	46.13	47.23	42.98	56.16
4	100	63.56	52.45	58.34	50.02	67.92
5	200	74.09	60.73	61.91	59.45	61.99
6	IC ₅₀ value	47.92	111.66	97.87	123.83	50.95

Table 3: Effect of ethanol extract on behavioural studies

Groups	Swimming length (cm)	Number of errors
Control	21.60 \pm 0.6	3.47 \pm 0.11
Fluoride	38.29 \pm 1.9 ^{a***}	6.44 \pm 0.18 ^{a***}
Indomethacin	32.95 \pm 2.1 ^{b**}	3.18 \pm 0.15 ^{b**}
EECP 100mg/kg	36.29 \pm 1.9 ^{b**}	5.20 \pm 0.14 ^{b***}
EECP 200mg/kg	30.84 \pm 1.4 ^{b**}	3.55 \pm 0.12 ^{b***}

Values are represented as mean \pm SEM, n=6. a-Group II (Fluoride control) was compared with Group I (control). b-Treated groups III, IV, V was compared with Group II. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001)

Table 4: Effect of EECP on Non-Enzymatic Antioxidant Levels in Fluoride induced neuro-degeneration in rat brain

Groups	Norepinephrine (NE)	Serotonin
Control	1.33 \pm 0.02	0.96 \pm 0.01
Fluoride	0.56 \pm 0.96 ^{a***}	0.42 \pm 0.008 ^{a***}
Indomethacin	0.96 \pm 0.18 ^{b*}	0.77 \pm 0.74 ^{b***}
EECP 100mg/kg	0.94 \pm 0.10 ^{b*}	0.60 \pm 0.04 ^{b*}
EECP 200mg/kg	1.08 \pm 0.04 ^{b**}	0.81 \pm 0.01 ^{b***}

Values are represented as mean \pm SEM, n=6. a-Group II (Fluoride control) was compared with Group I (control). b-Treated groups III, IV, V was compared with Group II. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001)

Table 5: Effect of EECP on Enzymatic Antioxidant Levels in Fluoride induced neuro-degeneration in rat brain

Groups	SOD U/mg	CAT (μ moles of catalase/mg protein)	GSH (nm/min/mg protein)	GPx (μ moles of NADPH/mg protein/min)
Control	46.66 \pm 1.80	1.67 \pm 0.11	134.5 \pm 1.73	4.36 \pm 0.09
Fluoride	25.5 \pm 0.85 ^{a***}	1.31 \pm 0.09 ^{a***}	92.5 \pm 1.07 ^{a***}	1.36 \pm 0.13 ^{a***}
Indomethacin	40.48 \pm 1.04 ^{b***}	1.69 \pm 0.06 ^{b***}	130.3 \pm 3.88 ^{b***}	3.50 \pm 0.15 ^{bns}
EECP 100mg/kg	31.45 \pm 1.08 ^{b**}	1.50 \pm 0.10 ^{b**}	122.7 \pm 2.44 ^{b**}	2.57 \pm 0.14 ^{b**}
EECP 200mg/kg	42.10 \pm 1.67 ^{b*}	1.49 \pm 0.10 ^{b**}	133.8 \pm 2.37 ^{b***}	3.45 \pm 0.15 ^{b**}

Values are represented as mean \pm SEM, n=6. a-Group II (Fluoride control) was compared with Group I (control). b-Treated groups III, IV, V was compared with Group II. (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001)

spectrofluorimeter, the fluorophore formed by heating the reaction mixture to 100°C for 10 minutes after adding the O-phthaldialdehyde reagent was detected at 360-470 nm for serotonin.

In vivo enzymatic antioxidant studies in brain hippocampus

Estimation of superoxide dismutase (SOD)

Phenazinemetosulphate (PMS) and decreased nicotinamide adenine dinucleotide, in their presence, SOD was measured by inhibiting the synthesis of blue formazan dye from nitro blue tetrazolium (NBT) (NADH). Sodium pyrophosphate buffer (pH8.3; 0.052M; 1.2ml), PMS (186mol), NBT 300mol), and NADH (780mol; 0.2ml) were used in the incubation mixture. The reaction was started by adding NADH, which was then incubated for 90seconds at 37°C. The reaction was stopped by adding 1mL glacial acetic acid and 4mL n-butanol, shaking vigorously for 1minute, centrifuged at 4000rpm for 1minute, and the upper butanol layer was read at 560nm against a butanol blank (Kakkar *et al.*, 1984).

Estimation of catalase (CAT)

The capacity of CAT to block H₂O₂ oxidation was used to determine its CAT value (H₂O₂). 2.25ml potassium phosphate buffer (65mmol, pH7.8) was added to 100l brain homogenate or sucrose (0.32M) and incubated for 30minutes at 25°C. The reaction was started by the addition of H₂O₂ (7.5mmol; 650l). It took 2-3minutes to measure the change in absorbance at 240 nm. CAT units of protein were used to express the results (Beers and Sizer, 1952).

Estimation of glutathione peroxidase (GPx)

The activity of GPx was determined using the Lawrence and Burk technique. In a phosphate buffer saline, a 100l aliquot of the supernatant was combined with a 700l reaction mixture containing 1mmol EDTA, 1mmol NaN₃, 0.2mmol NADPH,

and 1mmol glutathione in a phosphate buffer saline and 100l (10 U/100 l) glutathione reductase (Lawrence and Burk, 1976). After vortexing the tubes, they were incubated at room temperature for 5 minutes. After incubation, each tube received 100l of 0.2mmol H₂O₂ to start the reaction, and the absorbance was measured at 340nm every 30seconds for 3minutes using a Spectronic-20 spectrophotometer (Spectronic Instruments). Using an extinction coefficient of 6.22103mol⁻¹cm⁻¹, changes in absorbance rate were translated into 1nmol of NADPH oxidized/min/mg protein.

Estimation of glutathione reductase (GSH)

In a phosphate buffer saline, a 100l aliquot of the supernatant was combined with a 700l reaction mixture containing 1mmol EDTA, 1mmol NaN₃, 0.2mmol NADPH, and 1mmol glutathione, and 100 l(10 U/100 l) glutathione was oxidized (Beutler *et al.*, 1963). After vortexing the tubes, they were incubated at room temperature for 5minutes. After incubation, each tube received 100 l of 0.2mmol H₂O₂ to start the reaction, and absorbance was measured at 340 nm every 30 seconds for 3 minutes using a Spectronic-20 spectrophotometer. Using extinction coefficient of 6.22103l^{mol}-1cm⁻¹, changes in absorbance rate were translated into 1nmol of NADPH oxidized/min/mg protein.

Histopathological studies

The rats were severely sedated with a required dose of ketamine (150mg/kg) and perfused with glutaraldehyde in 0.1M phosphate buffer (PB, pH7.4) followed by 100ml of 0.1%PB containing 10% sucrose through the ascending aorta at the conclusion of the behavioural trials. The brain was removed after perfusion, the fore brain and brain stem were produced, immersed in paraffin, and then thin sections were cut in the thickness of 30m in a microtome and collected in PB (0.1M). For histological evaluation of the hippocampus under the

light microscope, sections were Nissl stained with 0.1 percent cresyl violet (Khuwaja *et al.*, 2011). To examine the neuronal size, morphologies, and number per high power field, sections were taken and processed using image analysis software.

Statistical analysis

Graph Pad Prism (version 5.0) was used to perform the statistical analysis. One-way (ANOVA), which is followed by Dunnet's test, was performed.

The results were represented as mean \pm SEM. The significance of the test results were represented by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

RESULTS

Preliminary studies on the phytochemistry showed the presence of chemicals like alkaloids, terpenoids, flavonoids, saponins and phenolic compounds in four different extracts.

In vitro, free radical scavenging activity showed the ethanol extract of *Cyclea peltata* (EECP) were significantly active against the free radicals.

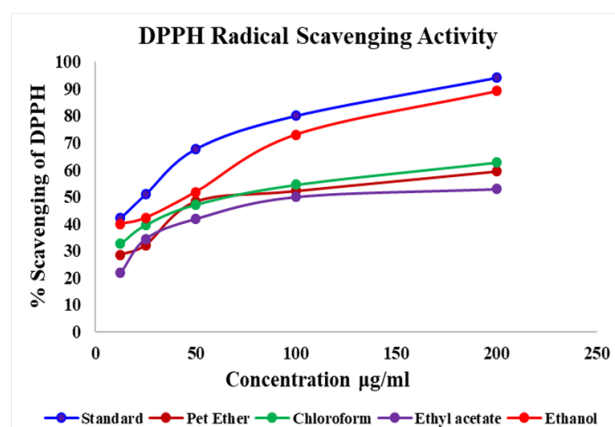


Figure 1: DPPH free radical scavenging capacity of the extract

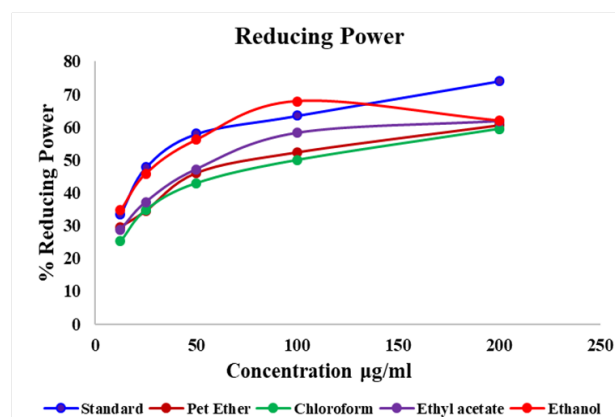


Figure 2: Effect of extracts on the reducing ability

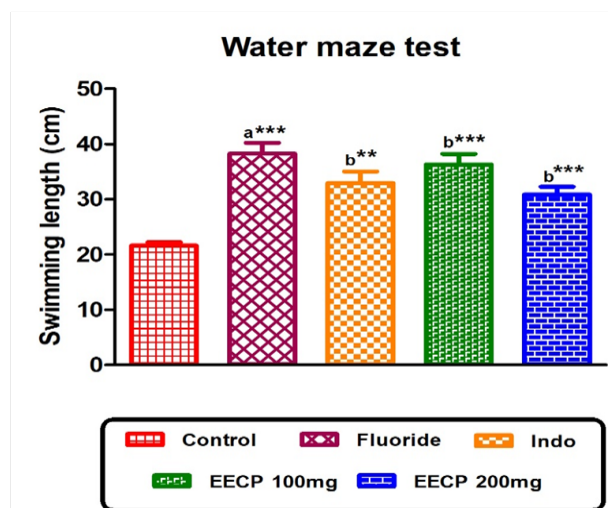


Figure 3: Effect of ethanol extract in Water maze test

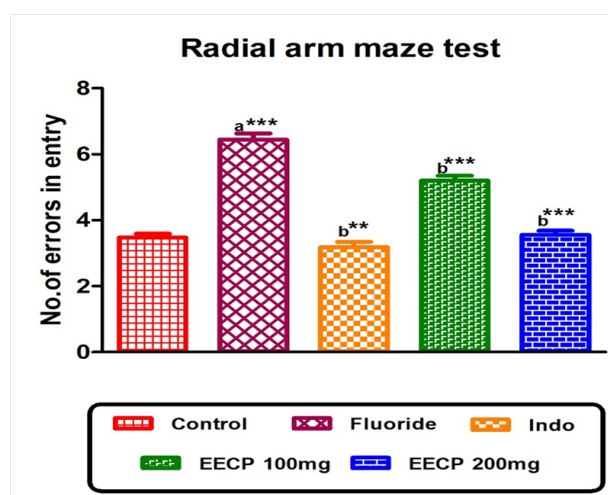


Figure 4: Effect of ethanol extract in Radial arm test

In Vitro antioxidant Activity

DPPH free radical scavenging capacity

All the extracts had been tested for antioxidant activity invitro in DPPH free radical scavenging mechanism. The test was performed at various concentration of extracts like 12.5,25,50,100 and 200µg/ml, which was compared to the standard drug, ascorbic acid. The highest % inhibition was shown at a concentration of 200µg/ml in all the extracts as given in Table 1 and Figure 1. Ethanol extract showed the highest activity of 89.21% inhibition at 200µg/ml, which was comparable to the standard drug at the same concentration of 200µg/ml.

Reducing Power

All the extracts had been tested for antioxidant activity by testing its reducing ability of free radicals. The test was performed at various concentration of

extracts like 12.5,25,50,100 and 200 μ g/ml, which was compared to the standard drug, ascorbic acid. The highest % inhibition was shown at a concentration of 200 μ g/ml in all the extracts, which was similar as given in Table 2 and Figure 2. Ethanol extract showed the highest activity of 61.99% inhibition at 200 μ g/ml, which was comparable to the standard drug at the same concentration of 200 μ g/ml, which showed 74.09%. Interestingly, all the extract showed similar activity in the reducing ability.

Behavioural Studies

The performance of the animals treated with extracts in the water maze test showed dose dependant activity which was highest at 200mg/kg and least at 100mg/kg, which was comparatively similar to the standard drug indomethacin. The induction of neurodegeneration was significant with the treat of fluoride. There was significant activity seen in the extract compared to the induction group, and similar activity was seen in the extract compared to the standard group, of which the data is represented in Table 3 and Figure 3. In Figure 3, a-Group II (Fluoride control) was compared with Group I (control). b- Treated groups III, IV, V was compared with Group II. (*P<0.05;**P<0.01;***P<0.001;****P<0.0001).

A similar activity was also seen in the radial arm maze test too. The number of errors made by the animals in the induction group were significantly higher compared to the normal groups. Animals treated with ethanol extract showed a significantly better activity compared to the induction group, and the activity was significantly low compared to the standard group rat at a dose of at 100mg/kg. Contrarily extract-treated groups at 200mg/kg showed significantly similar activity as a standard drug-treated group, as showed in Figure 4. In Figure 4, a-Group II (Fluoride control) was compared with Group I (control). b- Treated groups III, IV, V was compared with Group II. (*P<0.05;**P<0.01;***P<0.001;****P<0.0001).

Biochemical Analysis

The rat brain homogenate was investigated for the levels of non-enzymatic antioxidants like norepinephrine and serotonin to analyse the health of the brain. Interestingly, the rats treated with ethanol extract at 200mg/kg showed a remarkable increase in the hormone level, which is greater than the standard drug, indomethacin. The values were significantly similar compared to the induction group. Both the hormones norepinephrine and serotonin levels were restored due to the treatment with ethanol extract of the plant. The values were tabulated in Table 4.

The antioxidant enzyme levels like SOD, CAT, GSH and GPx in the rat brain were estimated, and the results were similar to the non-enzymatic levels. The elevation of antioxidant enzymes indicates that the extract had an antioxidant mechanism that is responsible to help in the prevention of neurodegeneration of rats. The activity was significantly similar and even higher than the standard drug, which are shown in Table 5.

CONCLUSION

Cyclea peltata Lam was studied to determine the mechanism of neuroprotective activity against fluoride-induced neurodegeneration. In the brain tissues treated with *Cyclea peltata* extract, there was a significant increase in antioxidant enzymes and a reduction in lipid peroxidation, confirming the antioxidant mechanisms responsible for stroke prevention in extract-treated groups. Overall, at a dose of 200mg/kg, the root extract of *Cyclea peltata* was found to have a significant inhibitory effect on neurodegeneration. This study opens the door to further research into the isolation of chemical constituents responsible for the activity and their standardization for medical use in the treatment of acute and chronic stroke cases.

ACKNOWLEDGEMENT

This work is a part of Ph.D. Thesis of The Tamilnadu Dr. M.G.R. Medical University, Chennai. The authors are grateful to The Tamilnadu Dr. M.G.R. Medical University, Chennai and J.K.K. Nattraja College of Pharmacy, Komarapalayam, for providing facilities and great support for the fruitful achievement of this research.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

- Beers, R. F., Sizer, I. W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195(1):133-140.
- Beutler, E., Duron, O., Kelly, B. M. 1963. An improved method for the determination of blood glutathione. *The Journal of Laboratory and Clinical Medicine*, 61:882-888.

- Blois, M. S. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, 181(4617):1199–1200.
- Chatwal, G. R., Sham, K., Anand 2019. Instrumental Methods of Chemical Analysis. 5th ed. Mumbai. page 794. Himalaya publishing house.
- Dirnagl, U. 2006. Bench to Bedside: The Quest for Quality in Experimental Stroke Research. *Journal of Cerebral Blood Flow and Metabolism*, 26(12):1465–1478.
- George, P. M., Steinberg, G. K. 2015. Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. *Neuron*, 87(2):297–309.
- Green, A. R., Shuaib, A. 2006. Therapeutic strategies for the treatment of stroke. *Drug Discovery Today*, 11:681–693.
- Harborne, J. B. 2005. A Guide to modern techniques of plant analysis. *Phytochemical Methods*, 22:5–16.
- Jyothiabrahamt, Dennisthomas 2012. Antibacterial activity of medicinal plant *Cycleapeltata* (Lam) Hooks &Thoms. *Asian Pacific Journal of Tropical Disease*, 2(1):280–284.
- Kakkar, P., Das, B., Viswanathan, P. N. 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*, 21:130–132.
- Kasture, A. V., Wadodkar, S. G., Mahadik, K. R., More, H. N. 2008. Pharmaceutical Analysis. 13th ed. Pune. page 252. Nirali Prakashan.
- Khuwaja, G., Khan, M. M., Ishrat, T., Ahmad, A., Raza, S. S., Ashafaq, M., Javed, H., Khan, M. B., Khan, A., Vaibhav, K., Safhi, M. M., Islam, F. 2011. Neuroprotective effects of curcumin on 6-hydroxydopamine-induced Parkinsonism in rats: Behavioral, neurochemical and immunohistochemical studies. *Brain Research*, 1368:254–263.
- Kinsella, M. T., Monk, C. 2009. Impact of Maternal Stress, Depression and Anxiety on Fetal Neurobehavioral Development. *Clinical Obstetrics and Gynecology*, 52(3):425–440.
- Krishnaswamy, N. R. 2003. Chemistry of Natural Products. A laboratory handbook. 1st ed. Hyderabad, Universities press (India) Pvt. Ltd. page 134.
- Lawrence, R. A., Burk, R. F. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications*, 71(4):952–958.
- Meena, J., Santhy, K. S. 2015. Evaluation of the antioxidant potential of methanol extract of *Cycleapeltata* in DAL Model. *The Pharma Innovation Journal*, 4(1):71–75.
- Morris, R. 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 11:47–60.
- Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. 2015. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation*, 131(4):29–322.
- Olton, D. S., Samuelson, R. J. 1976. Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes*, 2(2):97–116.
- Oyaizu, M. 1986. Anti-oxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44:307–315.
- Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P. G., Hefti, F. 1974. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. *Biochemical Pharmacology*, 23(17):2437–2446.
- Shine, V. J., Latha, P. G., et al. 2009. Gastric anti-secretory and antiulcer activities of *Cycleapeltata* (Lam.) Hook. f. & Thoms. In rats. *Journal of Ethnopharmacology*, 125(2):350–355.
- Vaidya Bhagwan Dash and Ram Karan Sharma 2013. *Agnivesa's Caraka Samhitha*. 1:89. Reprint ed. Varanasi: Chaukhambha Sanskrit Series Office.