Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

Phytochemical And Neuro Pharmacological Evaluation Of *Cyclea Peltata* Lam Roots

Rajkumar S[1], Sambathkumar R[1]*, Gomathi Swaminathan[2], Kannan C[1]
[1]Department of Pharmaceutical Chemistry, J.K.K Nattraja College of Pharmacy, Komarapalayam - 638183.Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India.
*Corresponding Author
Name: Dr. Sambathkumar R,
J.K.K Nattraja College of Pharmacy, Komarapalayam – 638183
Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Tamilnadu, India.
E-mail: sambathkumar.r@jkkn.ac.in
Contact: 98427 79911

ABSTRACT
Brain damage is one of the horrible conditions that cause death and permanent harm to the people involved. Brain injury victims gradually undergo physical and functional disabilities. The product of several mechanisms that affect the brain is brain injury. But the medicines meant to remedy it work in one or two mechanisms, essentially limiting them to the treatment of degeneration. Drugs derived from natural products are found in many ways to be effective in the treatment of stroke, also having less side effects. Cyclea peltata plant extracts have been investigated for antioxidant efficacy in this current research and the best one has been assessed for the prevention of brain injury to fluoride. The extracts also shown a dose-dependent neruoprotective function in the forced swim test and loco engine activity. The measurement of biochemical parameters has resulted in the determination of potential mechanisms for neuroprotection. It can be argued that the neruoprotective role of the extract may be attributed to the estimated antioxidant activity of the extract in the overall antioxidant activity and the prevention of lipid peroxidation in brain tissue.

Keywords: brain damage, nervous injury, neuroprotection, cyclea, forced swim

INTRODUCTION
One of the terrible conditions that cause death and permanent injury for the afflicted persons is brain injury. Patients with brain injury eventually have physical and functional disorders (Mozaffarian D et al. 2015). It is the world's leading cause of casualties, amounting to around 5.6 million a year. Per year, 2 patients out of every 1000 are affected by stroke and elderly people are more vulnerable to the disease. Data forecasts a drastic rise in the estimated graph of impacted individuals ranging from 0.5 billion in 2009 to 1.5 billion in 2050 (Kinsella et al., 2009). While there are different technologies and options that are successful in diagnosing and treating stroke, it is difficult to avoid the disease in a timely manner and ischemic acute stroke treatment is still a difficult challenge. A single pathway, rather than multi-dimensional

processes and channels, does not induce nervous degeneration (Dirnagl et al., 2006; Green et al., 2006).

Brain injury is the outcome of multiple processes that damage the brain. But the medications intended to cure it act in one or two systems, restricting them effectively to the treatment of degeneration. In consideration of the multifactorial pathophysiology of nervous disorders, drug formulation and production can therefore be focused in such a manner that they function in a variety of pathways. There is also an urgent need to synthesize such medications that successfully avoid and cure without inducing side effects. Drugs obtained from herbal sources are considered to be effective in the treatment of stroke in many respects and often cause less side effects.

Cyclea peltata, Menispermaceae, is locally referred to as 'Padathaali.' It is a highly branched climbing shrub with tuberous roots, peltate leaves, greenish yellow flowers and drupaceous fruits occurring in south and eastern India. In the treatment of jaundice, stomachache, fever, nephrolithiasis (Christina et al, 2002), asthma (Valiathan, 2003), and type 2 diabetics, the tuberous roots of the plant are included (Kirana& Srinivasan, 2010). It is documented that C. peltata roots contain tetrandrine, a bisbenzylisoquinolinedioxine alkaloid well known for its antioxidant function (Ng et al., 2006). In this current study, Cyclea peltata plant extracts have been examined for antioxidant activity and the strongest one has been evaluated for the prevention of fluoride brain harm.

Materials and Methods

Phase-I:Phytochemical Studies

Collection & Authentication of Plant

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

Procedure For Extraction

The roots of Cyclea peltata Lam. were specifically washed with tap water and dried at room temperature beneath dimness for one week. They were then ground into powder and held at room temperature. The pulverized materials were moved by sieve numbers 40 and 80. For additional usage, the crushed materials of equal size present between those two sieves were gathered and packaged in an airtight jar. Approximately 1 kg of shaded and dry Cyclea peltata Lam. plant roots were successively harvested with petroleum ether, chloroform, ethyl acetate and 90 percent v/v ethanol in soxhlet. Using a rotational vacuum evaporator, each extract was evaporated. The extract obtained from each solvent was measured and the percentage yield was determined according to the dry weight of the roots of the plant. The purity and colour of the extract have been established. All of the solvents used were of analytical grade for this work (Harborne, 2005).

Preliminary Study of Phytochemistries

For the detection of different plant constituents, all four extracts of Cyclea peltata Lam. roots have been subjected to qualitative studies (Krishnaswamy, 2003).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

In vitro estimation of Free Radical Scavenging

The total activity of antioxidants

Complete antioxidant activity was calculated according to the thiocyanate system of Cyclea peltata Lam. Roots (Mitsuda et al., 1966). For the stock solution, 20 mg extracts of Cyclea peltata Lam. were dissolved in 20 mL of water. Cyclea peltata Lam extracts (12.5, 25, 50, 100 and 200 mg) or normal samples with a potassium phosphate buffer of 2.5 mL (0.04 M, pH 7.0) were applied to the potassium phosphate buffer of 2.5 mL of linoleic acid emulsion (0.04 M, pH 7.0). At 37 ° C in the dark, each solution was then incubated. Each solution was stirred for 3 min at intervals during the incubation. 0.1 mL of the incubation solution was added to the test tube holding 4.7 mL of ethanol; 0.1 mL FeCl3 and 0.1 mL thiocyanate were transferred. The solution was then incubated for a duration of 5 min. Finally, the peroxide value was calculated by a spectrophotometer reading of the absorbance at 500 nm (8500 II, Bio-CromGmbh, Zurich, Switzerland). Peroxides were formed through the oxidation of linoleic acid, and Fe2+ to Fe3+ were oxidized by these compounds. With SCN, which has a maximum absorption at 500 nm, Fe3+ ions have formed a complex. Therefore, higher absorption values demonstrated greater oxidation of linoleic acid. The solutions without the inclusion of extracts or standards from Cyclea peltata Lam were used as blank samples. The emulsion of five milliliters of linoleic acid composed of 17.5 gm of Tween-20, linoleic acid 15.5 mL, and potassium phosphate 0.04M buffer (pH 7.0). In the other hand, 2.5 mL of linoleic acid emulsion and 2.5 mL of potassium phosphate buffering were used in the 5 mL control (0.04 M, pH 7.0). The average of the duplicate studies was all data on the overall antioxidant function. The lipid peroxidation inhibition percentage was determined according to the following equation:

$$\text{Percent inhibition} = [A0 - A1/A0] \times 100$$

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample of *Cyclea peltata* Lam.

Nitric Oxide Radical Inhibition Assay

At physiological pH, nitric oxide formed from sodium nitroprusside in aqueous solution interacted with oxygen and produced nitrite ions that were determined by the Griess reaction. The reaction mixture (3 mL) contained sodium nitroprusside (10 mmol) in saline buffered phosphate (PBS) and the separate extract and control concentrations were incubated for 30 minutes in a water bath at 25 °C. 1.5 mL of the mixture was extracted after incubation and 1.5 mL of the Griess reagent was then applied. Using a spectrophotometer at 546 nm, the absorption of the shaped chromophore was evaluated (Samak et al., 2009; Takeda et al., 1993).

NO Scavenging activity % = [(Control Absorbance – Extract Absorbance) / Control Absorbance] x 100.

For control 1.0 mL of buffer was added to 3 mL of 10 mmol sodium nitroprusside and the rest of the procedure was the same.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

Phase-II: Pharmacological Studies

Animals

The tests were performed using Sprague dawley rats (150-200 gm). Animals were collected and preserved under normal housing conditions from the J.K.K. Nattraja College of Pharmacy, Kumarapalayam. During the trial, a normal commercially usable diet was given with water ad libitum. The animals were housed in polycarbonate cages that were clean and dry and kept in a well ventilated animal house with a light and dark period of 12 hours. The institutional animal ethics committee (Reg. No: JKKNCP/IAEC/PhD/01/2018)) has approved this report.

Acute Oral Toxicity

Acute toxicity tests have been carried out in compliance with OECD Recommendations 423. In each group, three animals of the same sex were working. The EECP was administered at 5, 50, 300 and 2000 mg/kg b.w, respectively, for each category. Before the extract administration, the animals were fasted over night. For any signs and symptoms of poisoning, animals were observed on a daily basis for 14 days.

Experimental design of experimental design

Animals were randomly allocated to five classes and EECP was administered for a 30-day duration (p.o). Neurodegeneration was then caused by fluoride administration (120 ppm) in regular saline for a duration of 30 days. Animals were exposed to behavioral tests two hours after the injection of fluoride and eventually sacrificed, and the brain was removed for biochemical study.

Group I: Group of controls. For 30 days, the animals were administered 0.1 ml of regular saline orally.

Class II: Management of an infection. For 30 days, the fluoride (120 ppm).

Category III: group of the norm. For 30 days, Indomethacin (0.5 mg/kg) + Fluoride (120 ppm).

Group IV: group of care. For 30 days of EECP (100 mg/kg) + Fluoride (120 ppm).

Group V: group of care. For 30 days of EECP (200 mg/kg) + Fluoride (120 ppm).

Animals exposed to behavioral experiments and biochemical tests is conducted at the conclusion of the therapy process. Both the meanings are subject to the study of statistics.

Studies on behaviour

General research into behaviour

Changes in body weight, consumption of food and drink have been assessed.

Anxiety and Depression behavioural assessments

The rats were observed in a square open field arena (68x68x45 cm) fitted with 2 rows of 8 infrared light-sensitive photocells mounted 40 and 125 mm above the turf, respectively. The photocells were spaced 90 mm apart, and 25 mm away from the wall was the last photocell in a series. In a ventilated, sound-attenuating enclosure, tests were carried out in the dark. Photocell beam interruptions were collected by a microcomputer and the number of motions was measured within five minutes (Saelens et al., 1986).

The Forced Swimming Test

Within a vertical Plexiglas cylinder containing 15 cm of water held at 25 ° C, Rats were individually forced to swim. Initially, rats put in the cylinders became intensely aggressive for the first time, actively swimming in circles, attempting to scale the wall or diving to the ground. They started to subside and to be interspersed with stages of immobility or floating of rising duration after 2-3 minutes of operation. Immobilization reached a peak after 5-6 minutes,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

where the rats remained immobile for around 80 per cent of the time. The rats were removed after 15 minutes in the water and allowed to dry in a heated enclosure (32 ° C) prior to being returned to their home cages. 24 hours later, they were again put in the cylinder and the overall time of immobility was assessed during a test of 5 minutes (Porsolt et al., 1991).

BIOCHEMICAL ANALYSIS

The Nitric Oxide Assay (NO)

In acid medium and in the presence of nitrite, the assay of nitric oxide content was conducted in brain homogenates. N-(1-naphthyl) ethylenediamine was mixed with the processed nitrous acid diazotize sulphanilamide. There was a vivid reddish-purple hue of the resulting azo-dye that was measured at 540nm by spectrophotometry (Lowry et al., 1951).

Estimation of protein

The tissue homogenate protein concentrations is calculated using the conventional Lowry procedure using bovine serum albumin as the standard. According to this process, the colour shift of the sample solution is proportional to the concentration of protein that can be measured using colorimetric methods. In this case, brain homogenates were taken from the EECP and combined with a 10 mL buffer (N/10 acetate and N/10 acetate sodium). Then at 2500 rpm it was centrifuged and supernatant was accumulated. About 0.5 mL of supernatant was taken and 0.5 mL of purified water was added, and about 5 mL of alkaline solution (NaOH+ sodium potassium tartarate) and 0.5 mL of foline reagent is added to this solution. An optical density of 600 nm has been measured.

Brain MDA & Lipid Peroxide Calculation (LPO)

To approximate the total sum of the lipid peroxidation (LPO) product, the Ohkawa method (Ohkawa et al., 1979) was used. In terms of TBARS, the LPO was calculated and malondialdehyde (MDA) was taken to describe the TBARS. The incubation mixture composed of 0.5 mL of supernatant brain homogenate, 0.2 mL of 8 percent sodium dodecyl sulphate, 1.5 mL of 20 percent acetic acid solution (adjusted to pH 3.5 with 1N NaOH / 0.1N HCl) and 1.5 mL of 0.9 percent aqueous thiobarbituric acid solution (adjusted to pH 7.4 with 1N NaOH / 0.1N HCl), and up to 5.0 mL of double purified water was prepared and then heated in boiling water. The red chromogen was separated into 5 mL of the n-butanol and pyridine mixture after cooling and was centrifuged for 10 min at 4000 rpm. At 532 nm, the absorbance of the organic layer was measured. As an external norm, 1, 2, 3, 3-tetraethoxypropane (TEP) was used and the amounts of lipid peroxide were expressed as MDA / gm protein μmol. In the above method, the calibration curve of TEP was prepared using 80-240 nmol of TEP as the norm over which linearity was obtained (Ellman et al., 1961).

Results and discussion

Phytochemical analysis

Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, terpenoids, 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.

Total antioxidant activity

The *invitro* antioxidant activity of the extracts was estimated in thiocyanate method in various concentrations like 12.5, 25, 50, 100 and 200 μg/mland the IC50 values were represented in table 1. Out of all the extracts pet ether extract had the highest IC50 value of 197.5 indicating

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

that nearly 200 µg/ml of extract is required to show 50% inhibition. Whereas ethanol extract showed the least IC50 of 123 µg/ml. This indicates that the plant extracts ranged from 120-190 µg/ml which were not significantly different from each other but from the table it can be advocated that ethanol extract exhibited the potent antioxidant activity compared to other extracts.

Table 1. Total antioxidant activity of various extracts of *Cyclea peltata* (EECP)

S.No	Concentration (µg/ml)	% inhibition				
		Standard (Ascorbic acid)	Pet Ether Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
1	12.5	40.21	13.17	27.18	11.22	22.1
2	25	54.65	25.41	32.42	27.72	30.12
3	50	68.98	29.21	41.70	39.82	44.75
4	100	79.34	32.83	50.37	50.15	52.68
5	200	93.72	49.87	58.42	61.23	59.23
6	**IC_{50} value**	**8.675**	**197.52**	**128.03**	**136.43**	**123.32**

Nitric oxide radical scavenging activity

The plant extracts were estimated for antioxidant activity using nitric oxide free radical scavenging capacity. This method uses antioxidant ability of the extract to counteract the nitric oxide radicals generated from sodium nitroprusside using Griess reaction. The results were represented in figure 1. Out of all the extracts, ethanol extract showed the best activity in combating the nitric oxide free radicals. The least activity was exhibited by chloroform and pet ether extracts. Overall all the extracts showed a dose dependant increase in the activity.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

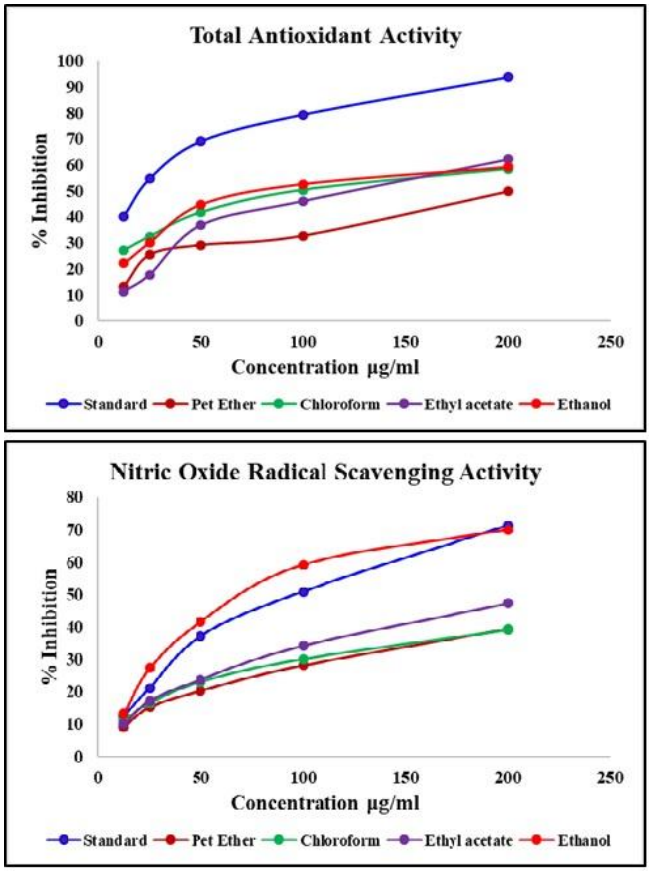

Fig. 1. Total antioxidant activity and nitric oxide scavenging activity of extracts

Acute oral toxicity study

Acute toxicity study was performed according to OECD-guidelines 423 by administering ethanol extract of the plant to each group at various doses of 5, 50, 300 and 2000 mg/kg b.worally respectively. Animals were observed regularly for 14 days for any signs and symptoms of toxicity. After 14 days of the study there was no sign of toxicity and animals exhibited no change in the behaviour and had a normal metabolism. Signs of toxicity like redness of eyes, itching of skin, tail flicking etc were not observed thus indicating that the extract can be safely used till 2000mg/kg and the dose was fixed at 200 mg/kg orally in further experiments.

Effect of ethanol extract of *Cyclea peltata on*General behaviour of rats

In the experiments fluoride was used an induction agent for brain damage and after induction the general activity of the animals was observed. Food intake, water intake and changes in body weight weremonitored. There was a significant reduction in all the three parameters in the induced groups compared to rest of the groups. This indicates that the brain damage was induced effectively. There was significant recovery or prevention of damage in extract treated and standard, indomethacin treated groups wherein the food intake, water intake and overall body weight were hiked compared to the induced group. Overall all the other groups except fluoride treated group are consistent with the control group of animals.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

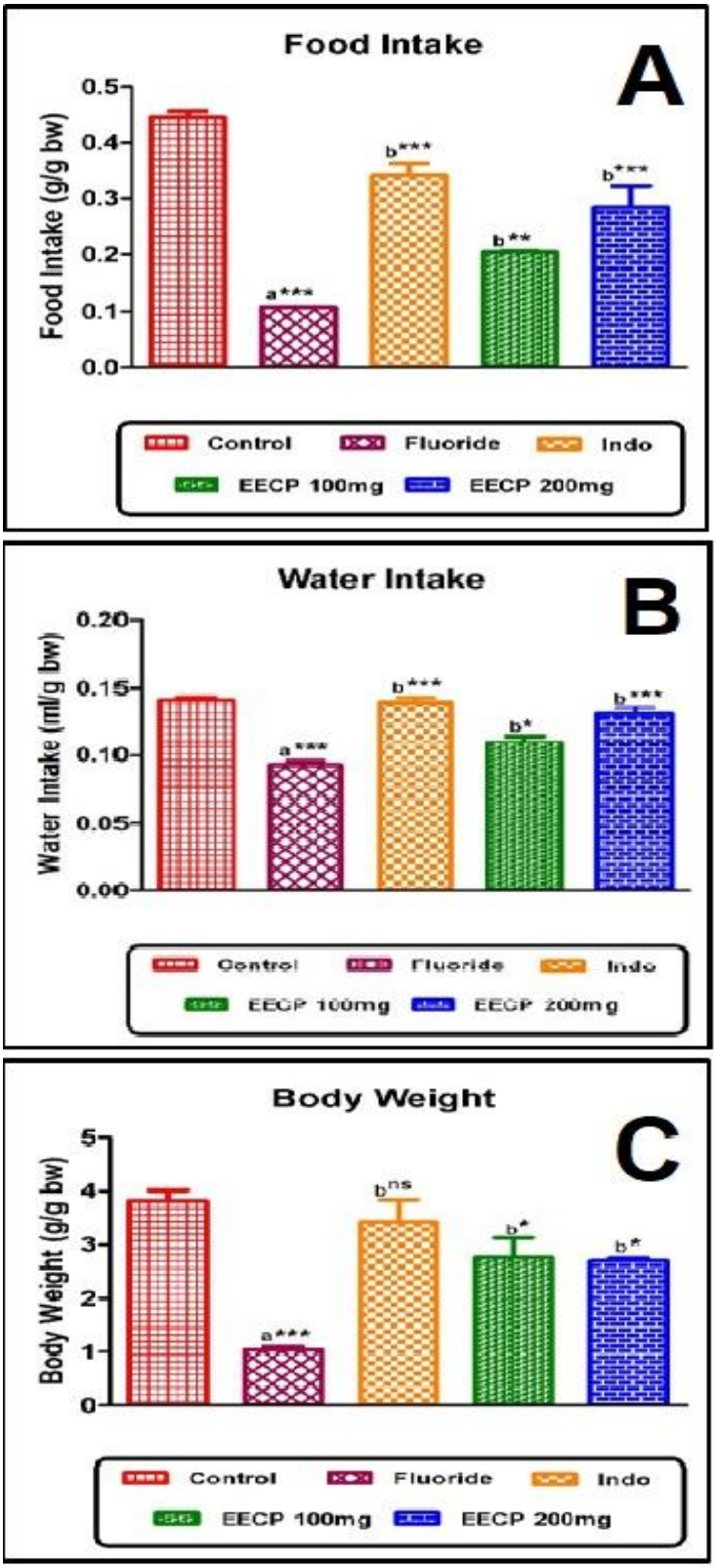

Fig. 2. Effect of Extract on food intake, water intake and body weight

Statistical comparison: Values are expressed as mean±SEM, n=6 in each group. One-way ANOVA followed by Dunnett test was performed. 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)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

Effect of ethanol extract of *Cyclea peltata* onLocomotor Activity and Forced Swimming

The ethanol extract was administered into the animals in two doses 100 and 200mg/kg orally and investigated for prevention of brain damage with fluoride. The results were given in table 2. The locomotor activity was estimated by calculating the number of movements within 5mins of time. Ethanol extract treated groups showed an increase in the movements compared to the fluoride treated groups. It was comparable to the standard drug, indomethacin. There was dose based activity noticed in the extract treated group. Similar results were exhibited in the forced swin test also. Time duration of immobility was estimated for specific time in which the animals did not show any movement. The fluoride treated groups had a significant non movement time interval of 43 sec where as the standard group showed a less duration of 19 sec which was comparably similar to the extract treated group at 200mg/kg. Usually rats move quicker and when induced brain damage using fluoride they tend to not move due to the lack of coordination in the nervous system resulting in the gait disturbance. They may undergo physical and psychological shock at the moment which was successfully prevented using the extract of the plant. The treatment of the animals using the ethanol extracts resulted in the prevention of the brain damage and thus restoring the normal movements comparable to the control group. The comparative results were represented in figure 3.

Table 2. **Locomotor Activity and Forced Swim test using ethanol extract of *Cyclea peltata***

Groups	**Number of movements (in 5mins)**	**Immobility time on day 30 (in sec**
Control	22.41±0.15	19.78±0.49
Fluoride	$7.85\pm0.48^{a***}$	$43.12\pm0.98^{a***}$
Indomethacin	$20.20\pm0.41^{b***}$	$19.23\pm0.69^{b**}$
EECP 100mg/kg	$13.44\pm0.72^{b**}$	$28.47\pm0.39^{b***}$
EECP 200mg/kg	$17.93\pm0.31^{b***}$	$19.49\pm0.68^{b***}$

Statistical comparison: Values are expressed as mean±SEM, n=6 in each group. One-way ANOVA followed by Dunnett test was performed. 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)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

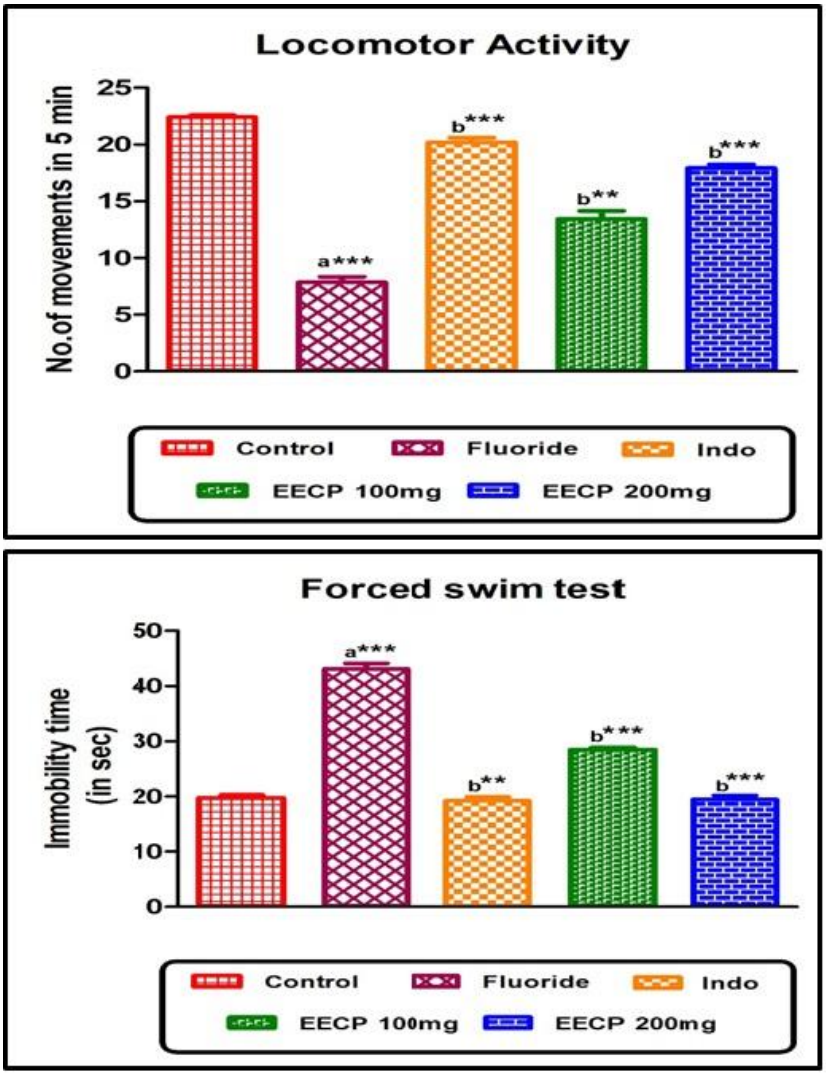

Fig. 3. Effect of Extract on Locomotor activity and forced swim test

Statistical comparison: Values are expressed as mean±SEM, n=6 in each group. One-way ANOVA followed by Dunnett test was performed. 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)

Effect of extract on the biochemical parameters of brain tissue

After the experiments on rats, the brain tissue was carefully isolated and estimated for various paramters like Nitric oxide content, total proteins content and lipid peroxidation. The nitric oxide content was lower in the control groups and the fluoride induced significant oxidation in animals. Extract at 200mg/kg prevented the formation of nitric oxide and the activity was significantly similar to the standard drug. The same results were shown in the other parameters too like total proteins and lipid peroxidation too. The extracts could successfully prevent the formation of oxidative free radicals and induce brain damage which is due to lipid peroxidation. This was also prevented significantly using the extract at a dose 200mg/kg and it was comparable to the standard drug. Overall, it can be suggested that the antioxidant mechanisms of the extract was the underlying mechanism in preventing the brain damage in rats.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 212 - 223
Received 05 March 2021; Accepted 01 April 2021.

Table 3. Effect of extract on the Biochemical parameters of brain tissue

Groups	**Nitric Oxide µmol/g tissue**	**Total proteins mg/dL**	**Lipid peroxidation nmol/mg protein**
Control	0.90± 0.09	1.79±0.20	54.75±2.14
Fluoride	$4.00\pm0.10^{a***}$	$1.01\pm0.12^{a***}$	$95.56\pm3.15^{a***}$
Indomethacin	$1.34\pm0.08^{b**}$	1.56 ± 0.15^{b}	46.30 ± 3.3^{b}
EECP 100mg/kg	$2.26\pm0.16^{b***}$	$1.59\pm0.15^{b*}$	$76.02\pm1.7^{b***}$
EECP 200mg/kg	$1.96\pm0.16^{b***}$	$1.69\pm0.20^{b*}$	$61.36\pm0.94^{b***}$

Statistical comparison: Values are expressed as mean±SEM, n=6 in each group. One-way ANOVA followed by Dunnett test was performed. 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)

CONCLUSION

Brain injury is the outcome of multiple processes that damage the brain. But the medications intended to cure it act in one or two systems, restricting them effectively to the treatment of degeneration. There are still major side effects of synthetic medications, so the emphasis was moved to the use of natural drugs for the same reason. In this analysis, Cyclea peltata plant extracts were investigated for the defense of the brain against fluoride damage. In the forced swim test and loco engine activity, the extracts demonstrated a dose-dependent neruoprotective activity. The assessment of biochemical parameters has culminated in the evaluation of alternative neuroprotection pathways. It may be asserted that the extract's neruoprotective function could be due to the extract's approximate antioxidant activity in overall antioxidant activity and the avoidance of lipid peroxidation in brain tissue. This opens up a new field for exploring the elucidation of the molecular patterns and pathways that may be responsible for the activity.

AKNOWLEDGEMENT:

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, Chennaiand J.K.K. Nattraja College of Pharmacy, Komarapalayam for providing facilities and great support for the fruitful achievement of this research.

REFERENCES

1. Harborne J.B, Phytochemical Methods. A Guide to modern techniques of plant Analysis. 3rded. New Delhi, Springer (India) Pvt. Ltd, 22, pp.5-16, 2005.
2. Krishnaswamy, N.R, Chemistry of Natural Products. A laboratory hand book. 1sted. Hyderabad, Universities press (India) Pvt. Ltd, pp.15, 2003.
3. Chatwal, G.R,Anand, S.K,Instrumental methods of Chemical Analysis. 5thed. Mumbai, Himalaya publishing house; pp.567, 2003.
4. Kasture, A.V,Wadodkar, S.G,Mahadik, K.R, More, H.N,Pharmaceutical Analysis. 9thed. Pune, NiraliPrakashan, pp.16, 2003.

5. Mitsuda, H;Yasumoto, K;Iwami, K:Antioxidative action of indole compounds during the autoxidation of linoleic acid.Nihon EiyoShokuryo Gakkai-Shi. 19, 3, 210-214, 1996.
6. Samak, G;Shenoy, R;Manjunatha, S;Vinayak, K: Superoxide and hydroxyl radical scavenging actions of botanical extracts of Wagateaspicata,Food Chem. 115, 2, 631-634, 2009.
7. Takeda, Y; Matsumoto, T;Terao, H;Shingu, T;Futatsuishi, Y;Nohara, T:Orthosiphol D and E, minor diterpenes from Orthosiphonstamineus. Phytochemistry. 33, 2, 411-415, 1993.
8. Saelens, J.K;Kovacsics, G.B; Allen, M.P: The influence of the adrenergic system on the 24-hour loco motor activity pattern in mice. Arch. Int.Pharmacodyn.Ther. 173, 411-416, 1986.
9. Porsolt, R.D,Lenègre, A, McArthur, R.A, Pharmacological models of depression. In: Olivier B Mos, Slangen JL, editors. Animal models in psychopharmacology. New York, pp.137-139, 1991. Springer publishers.
10. Lowry, O.H;Rosebrough, N.J; Farr, A.L; Randall, R.J: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275, 1951.
11. Ellman, G; Courtney, K; Andres, V; Featherstone, R: A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem.Pharmacol. 7, 2, 88-95, 1961.
12. Ohkawa, H;Ohishi, N; Yagi, K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal.Biochem. 95, 2, 351-358, 1979.
13. Mozaffarian, D: Heart disease and stroke statistics-2015 update: a report from the American Heart Association. Circulation. 2015.
14. Green, A.R;Shuaib, A: Therapeutic strategies for the treatment of stroke. Drug. Discov. Today. 11, 681–693, 2006.
15. Dirnagl, U: Bench to bedside: the quest for quality in experimental stroke research. J. Cereb. Blood. Flow.Metab. 26, 1465–1478, 2006.
16. Kinsella, M.T; Monk, C: Impact of maternal stress, depression & anxiety on fetal neurobehavioral development. Clin. Obst.Gyn, 52, 3, 425, 2009.
17. Christina, A.J.M;Packia Lakshmi, M; Nagarajan, M; Kurian, S: Modulatory effect of Cyclea peltata Lam. on stone formation induced by ethylene glycol treatment in rats. Methods Find. Exp.Clin.Pharmacol. 24, 77-79, 2002.
18. Valiathan, M.S, The Legacy of Charaka, Orient Longman Private Ltd., Chennai, pp.365-367, 2003.
19. Kirana, H; Srinivasan, B.P: Effect of Cyclea peltata Lam. roots aqueous extract on glucose levels, lipid profile, insulin, TNF-α and skeletal muscle glycogen in type 2 diabetic rats. Indian J .Exp. Biol. 48, 499-502, 2010.
20. Ng, L.T; Chiang, L.C; Lin, Y.T; Lin, C.C:Antiproliferative and apoptotic effect of tetrandrine on different human hepatoma cell lines. Am, J, Chinese, Med, 34, 125-135, 2006.