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Neuroprotective effect of Ethanolic leaf extract of *Carissa macrocarpa* (Eckl.) A.DC.) against Scopolamine Induced Memory Impairment in Rats

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ABSTRACT: The important phytoconstituents found in Carissa macrocarpa make it a promising medicinal plant with a long history of usage in traditional medicine. Therefore, the purpose of this study was to determine whether or not an ethanolic extract of Carissa macrocarpa leaves could produce neuroprotective effect on rats in Scopolamine induced memory loss. The antioxidant capacity was measured in-vitro using the ferric reducing antioxidant power (FRAP) and 1-diphenyl-2-picrylhydrazyl (DPPH) assays. Before receiving a single intraperitoneal injection of Scopolamine 1mg/kg, b.w. on day 15, rats were pre-treated with 200 and 400 mg/kg of ethanolic leaf extract of Carissa macrocarpa (EECM) of body weight for 14 days. The extent of memory loss was determined by administering a set of behavioural tests, including the Morris water maze and radial arm maze tests. At the end of the experiment, the rats were slaughtered, and the hippocampal region of their brains were removed and analysed the concentrations of acetylcholinesterase, nitric oxide, and protein. Using the DPPH assay, the IC50 values for EECM was 79.63±4.24μg, and that its total antioxidant activity was 804.24±23.42 μmol Fe (II)/g extract. In the behavioural tests of the Morri's water maze and the 8-arm radial maze, mice treated with EECM at 200 and 400 mg/kg showed a neuroprotective effect, with lowered acetylcholinesterase, nitric oxide, and protein levels (P<0.001). Positive neuroprotective benefits of an ethanolic extract of Carissa macrocarpa leaves against Scopolamine-induced memory impairment in rats were found in the current investigation.

Keywords: *Carissa macrocarpa*, Scopolamine, Behavioural studies, Memory impairment, Neuroprotective.

INTRODUCTION

Dementia is a syndrome that is characterised by the slow development and persistent decrease of higher cognitive ability. Dementia is currently ranked as the sixth largest cause of death among all diseases and is one of the primary causes of impairment and dependency among older people all over the world (WHO, 2021). There are a wide variety of distinct types of dementia. Alzheimer's disease is the most frequent kind, and it may be responsible for between 60 and 70 percent of all cases. There are currently more than 55 million people living with dementia across the globe, and each year there are over 10 million new cases reported and more than 60 percent of them residing in low- and middle-income nations. This number is projected to increase to 78 million in 2030 and 139 million in 2050 as a result of the rising share of elderly persons in the population of practically every country (Vasudevan *et al*., 2007). Till date, there is no medication that can cure dementia that is available Although many new treatments are currently being researched and tested in clinical trials of varying stages, the anti-dementia drugs and disease-modifying therapies that have been created to date have limited efficacy and are mostly labelled for Alzheimer's disease.

Extensive study reveals that several plant-derived compounds and traditional Oriental herbal treatments exhibit cognitive-enhancing qualities. These chemicals and remedies come from plants. Extracts of Ginkgo biloba and numerous alkaloidal, and consequently poisonous, plant-derived cholinergic compounds are currently among the most used therapies for dementia. There are several non-toxic plant species that have a long history of use as medicines for treating cognitive impairments, including those that come with advancing age. An animal model for neurodegenerative illnesses

was developed by analysing the effects of a wide variety of substances on animals' behaviour patterns to determine their mode of action. Some of them include ethanol, colchicine, heavy metals, scopolamine, lipopolysaccharide, streptozotocin, and okadaic acid (Srinivasan *et al*., 2021).

Similar to atropine and hyoscyamine, scopolamine is a tropane alkaloid that was identified from plants belonging to the Solanaceae family. These chemicals all structurally resemble the naturally occurring neurotransmitter acetylcholine. Scopolamine influences the function of the parasympathetic nervous system and acts on smooth muscles that are responsive to acetylcholine but do not have cholinergic innervation. It does this through a mechanism known as competitive inhibition of muscarinic receptors. As a result, it causes memory impairment in rodents. Recent research has shown that Scopolamine causes an increase in the formation of reactive oxygen species, which in turn generates oxidative stress and leads to memory impairment. An injection of scopolamine, which causes cognitive abnormalities that are like those reported in Alzheimer's disease, can be used to test the cholinergic theory. Scopolamine-induced dementia is widely used to evaluate the potential of therapeutic agents to treat cognitive impairment (Kohnen-Johannsen *et al.*, 2019).

Carissa macrocarpa also known as Natal plum, is native to South Africa (Chopra *et al.,* 1956). It is a member of the Dogbane or Apocynaceae family. *Carissa macrocarpa* is a prickly evergreen, drought-resistant, dense, spiny, dwarf spreading shrublet with leathery, dark-green, glossy leaves. Traditionally, the plant is used in Chinese and ayurvedic medicines. it is used in the treatment of liver diseases, diarrhea and venereal diseases. Phytoconstituents such as amino acids, phenolic and terpenoid were isolated from it, the following pharmacological activities such as antibacterial and adhesion (Roshila *et al*., 2011), neuroprotective (Orabi *et al*., 2021) and antioxidant (Souilem *et al*., 2019) effects were reported. In light of this, the purpose of the current study was to investigate the neuroprotective impact of leaf extracts from *Carissa macrocarpa* against the memory impairment generated by scopolamine in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals: Scopolamine (SC), Piracetam, 1,1-diphenyl-2-picrylhydrazyl (also known as DPPH), and Folin-Ciocalteu reagent were all acquired from Sigma-Aldrich Chemicals Private Limited in Bengaluru, India.

Plants materials: In the month of May in the year 2020, the fresh leaves of the *Carissa macrocarpa* plant were gathered in the Singaraya Konda region of Andhra Pradesh. After being air-dried in the shade and tray-dried in controlled conditions, the leaves were used. With the help of a mechanical grinder, the plant's dried leaves were ground into a rough powder. After going through sieve no. 40, the powder was placed in a container with a tight-fitting lid for later extraction. The plant components were analysed by the Botanical Survey of India in Tamil Nadu and the Agri University in Coimbatore, Tamil Nadu, to determine their identity and verify their authenticity. For the sake of future research, a voucher specimen with the designation KSCM-1 has been filed away in the Department of Pharmacology at the Vinayaka Mission's College of Pharmacy in Salem, Tamil Nadu.

Preparation of extracts: Following that, the leaves were dried in the shade at room temperature for ten days, after which they were ground into a coarse powder and placed in an airtight container. Around 500 g of coarsely crushed leaves were used in this experiment. The leaves were put through a continuous hot percolation process using a variety of solvents that increased in polarity from least to most, including pet ether, chloroform, acetone, ethanol, and water. After drying the extracts in the rotary evaporator, the samples were analysed to determine the levels of numerous phytochemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates.

Animals: For the purpose of the study, adult Wistar healthy male rats that had reached the age of 8 weeks and weighed 200 g each were employed. Animals used in experiments were obtained from Srivenkateshwara Enterprises in Bangalore, India, which are on the list of approved suppliers of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were housed in polypropylene cages that had enough ventilation and were kept at a temperature of 25º C with humidity levels ranging from 55 to 65%. The rats had been provided with a regular diet of pellets and had unrestricted access to water during the experiment.

Preparation of animals: The animals were chosen at random, marked to enable for individual identification, and then kept in their cages for at least 5 days prior to the administration of the dose in order to allow for acclimatisation to the environment of the laboratory. Before each test, the animals were required to abstain from food and water for at least 12 h. The experimental protocols were reviewed by the Institutional Animals Ethical Committee (P.Col/37/2021/IAEC/VMCP), and approved. According to the CPCSEA rules for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals, every experiment was carried out in the morning. For the purpose of oral drug administration in experimental animals, the conventional orogastric cannula was utilised.

Determination of in-vitro antioxidant activity

DPPH radical scavenging activity: EECM were evaluated using the DPPH technique in order to determine their level of antioxidant activity (Salari *et al.,* 2019). The extract at concentrations of 20, 40, 60, 80, and 100 µg/mL was combined with three millilitres of methanolic solution containing 0.1 mM concentrations of DPPH radicals. After waiting for thirty minutes, the absorbance was measured at 517 nm.

Using the calculation, we were able to determine the percentage of activity that was inhibited.

$$\% \, inhibition = \frac{A_o - A_e}{A_o} \, x \, 100$$

Where, Ao = absorbance without extract; Ae = absorbance with extract.

The findings were expressed using the IC50 values, which refers to the sample concentration that was necessary to suppress 50% of the DPPH concentration.

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay is an innovative technique for determining the level of antioxidant power possessed by the sample. The procedure that was used in this investigation was exactly the same as the one reported by Prasad *et al.* (2010). It is predicated on the capacity of antioxidants to convert Fe+3 to Fe+2 in the presence of TPTZ (2, 4, 6-tripyridyl-s-triazine), thereby producing an intensely blue complex composed of Fe+2 and TPTZ. After mixing the FRAP solution (3 mL) with 100 ml of the EECC, the mixture was heated to 37° C for 10 min. At 593 nm, absorbance was determined for various doses (0.2, 0.4, 0.8, or 1 mg/mL) of extract in FRAP reagent. Following a comparison of the absorbance of the samples to a standard curve constructed from FeSO4, the FRAP values were calculated and represented as mmol Fe (II) per mg of extract.

Pharmacological studies Experimental design: There were a total of 30 healthy male Wistar rats weighing 200 g that were randomly split up into five groups, with six rats assigned to each group. Group I serves as the normal control and is given 0.1 mL of normal saline to consume orally every day for a period of thirty days. Group II, which serves as the disease control, received a single dose of SC (1 mg/kg) administered intraperitoneally on day 15 (Jagdish *et al.*, 2015). A positive control was used in Group III, which was given SC (1 mg/kg) in addition to Piracetam (200 mg/kg) for a period of 14 days. Groups IV and V received SC (1 mg/kg) of EECM plus either 200 or 400 mg/kg over the course of 14 days. On the final day of the experiment, once the results of the behavioural investigations were analysed, the animals were sacrificed with ether anaesthesia, and their brains were removed, and put through a series of biochemical tests.

Behavioural tests for learning and memory Morris water maze test: According to Vorhees and Williams (Vorhees and Williams 2006), the Morris Water Maze (MWM) test is a method that can be used to evaluate spatial or location learning. It is made up of an open circular pool that is 100 cm in diameter and 50 cm in diameter in height, and the interior surface of the pool is completely featureless. A circular platform was concealed two cm below the surface of the water. The 500 mL of milk served as a cover for the circular pool of water that had been heated to a constant temperature of 23±1° C and placed inside the pool. Every one of the animals underwent training that consisted of three sessions a day, with an interval of five to ten min between each session, five days a week. Each test began from one of four polar positions that were predetermined, and the order of the trials varied from day to day. The calculation of the escape latency is the initial stage in the learning process. It refers to the amount of time it took for the animal to get to the platform. The current research looked at a number of other criteria, such as the length of the path walked and the amount of time spent in each quadrant.

Radial arm maze test: According to a researcher (Gomathi *et al*. 2017), one way to evaluate an animal's working memory is to put them through a radial arm maze. It is a equipment made out of wood that features a radial maze with eight elevated arms that spreads outward from a central platform that is 26 cm in diameter. Each arm has a length of 60 cm, a height of 2 cm, and a breadth that holds along the length of the arm and measures 5 cm. The examination was carried out in a room that was adequately lit and had a variety of indications. During the course of the experiment, each of the arms was loaded with pellets, and the procedure was carried on until either all of the food pellets were gathered, or 10 min had elapsed, whichever came first. Over the course of two weeks, each animal was taught to collect the pellet on a daily basis. The test was stopped after eight choices, and the animals now have to make more accurate selections while making fewer mistakes. During the course of the research project, the performance of the animals was evaluated using the following parameters: the number of correct choices, the number of errors, the test time in seconds, and the total number of errors. This was done prior to collecting all of the food pellets from the eight different arms.

Biochemical studies: At the end of the behavioural studies, each rat was sacrificed using ether as anaesthetic, and the hippocampus region of each rat's brain was removed and examined. It was homogenised using a Potter–Elvehjem homogenizer at 0 °C with 0.1M phosphate buffer at a pH of 8. After that, the homogenate was centrifuged at 10,000 rpm for five min at a temperature of 4 °C in order to obtain a liquid that was completely clear. This liquid was then utilised for the biochemical measurements of acetylcholinesterase, nitric oxide (NO) and protein.

Estimation of acetylcholinesterase: The method of Ellman et al., with a slight modification (Ellman *et al*., 1961), was used to measure the concentration of acetylcholinesterase in the brain. After adding 0.1 mL of brain homogenate, 6 mL of sodium phosphate buffer with a pH of 8, 0.2 mL of acetylthiocholine iodide, and 5,5'-dithio-bis-(2-nitrobenzoic acid (also known as the Ellman reagent), the mixture was stirred. At a wavelength of 412 nm, the variations in the absorbance of the combination were measured.

Estimation of nitric oxide: The approach developed by Green et al. (Green *et al*., 1982) was utilised to determine the nitric oxide concentration found in the brain. In this procedure, equal volumes of brain homogenate and Griess reagent (1% sulphanilamide, 2% H_3PO_4, 0.1% N-(1-naphthyl) ethylenediamine-HCl)

were allowed to react at room temperature for 5 minutes. The reaction was carried out in order to determine whether or not the brain homogenate contained N-(1-naphthyl) ethylenediamine-HCl. A brilliant reddish-purple coloured azo dye was produced as a result, and its spectrophotometric measurement was taken at 540 nm.

Estimation of Protein: Lowry *et al.'s*, conventional method of estimation was utilised in order to calculate the levels of protein found in the brain homogenates (Lowry *et al.*, 1951).

Statistical analysis: The findings were reported as the mean±SEM, and they were subjected to statistical analysis using Graphpad Instat software. The one-way ANOVA was followed by Tukey's multiple comparison tests. When P was less than 0.001, statistical significance was attributed to the differences.

RESULTS AND DISCUSSION

Percentage yield and Phytochemical screening: The percentage yield of the extracts was calculated, and the results showed that pet ether, chloroform, acetone, and ethanol extracts, as well as aqueous extracts, had respective values of 1.2, 1.45, 1.18, 11.22 and 7.28%w/w, respectively. The extracts made using acetone, ethanol, and aqueous all contained carbohydrates, phenols, saponins, terpenoids, tannins, and flavonoids. There was evidence of the presence of alkaloids and terpenoids in the chloroform extract. The petroleum ether extract contained a variety of gums in addition to fixed oils. According to the results of the phytochemical study, there is evidence of the existence of bioactive chemicals, which suggests that this could be a possible source for the treatment of neurological illnesses. since of this, the ethanolic extract of the leaves of *Carissa macrocarpa* was chosen for the neuroprotective investigations as it had the highest percentage yield and the most promising phytochemical data.

***In-vitro* antioxidant studies DPPH radical scavenging activity:** In this experiment, the DPPH free radical scavenging activity of the EECM was tested. Various concentrations of the extracts (20, 40, 60, 80, and 100 µg/ml) were examined and tested. The EECM was able to inhibit 91.45% of the DPPH radical at 100µg/ml, but the standard (ascorbic acid) was only able to inhibit 91.38% of the radical at the same concentration. Even at the lowest concentration tested, which was only 20 µg/ml, the extract was able to scavenge DPPH radicals. Ascorbic acid served as the benchmark compound, and its ability to inhibit DPPH radicals was found to be rising and concentration dependent. The EECM had an IC50 value of 79.63 µg/mL, while ascorbic acid had a value of 18.60 µg/mL.

Total antioxidant activity by FRAP method: In- vitro total antioxidant activity for EECM was estimated by ferric reducing antioxidant power method using ascorbic acid as standard. The EECM showed the Frap activity of 804.24±23.42 µmol Fe (II)/g extract, whereas ascorbic acid showed 1045.67±17.15 /µmol Fe (II)/g.

Morris water maze test: In this test, spatial learning and memory were evaluated by assessing the escape latency, path length, and time spent in the quadrant. These metrics reflect an improvement in cognitive function, therefore the longer the path and the less time it takes to escape the quadrant, the better. Table 1 demonstrates how EECM influences both the learning and memory of spatial relationships. When compared to the normal group animals, the group II SC treated animals showed significantly increased escape latency, which demonstrated neurotoxicity and had a P value that was significantly higher than 0.001. Piracetam was administered to the animals in group III, and as a result, their overall escape latency was drastically cut down when compared to the animals in group II ($P<0.001$). While administration of EECM at doses of 200 and 400 mg/kg, b.w reduced the escape latency in a dose-dependent manner, the difference between the EECM-treated animals and the SC-treated animals was statistically significant ($P<0.001$). When compared to the animals in the normal group, the SC-treated animals demonstrated a statistically significant increase in the total distance travelled over the course of the test. When compared to SC-treated mice, piracetam-treated animals had a statistically significant reduction in the total distance travelled, with a P value that was significantly less than 0.001. The treatment groups that received a low dose (200 mg/kg, b.w.) and a high dose (400 mg/kg, b.w.) displayed a large reduction in the path length travelled in comparison to the animals in group II, with a P-value that was less than 0.001. In terms of the amount of time spent in the quadrant, animals with memory impairment induced demonstrated significantly less time spent in the quadrant in comparison to normal animals used as controls. When compared to the animals in group II, the positive control animals exhibited a significant increase in the amount of time they spent. While animals given EECM demonstrated a discernible increase in their time spent in the quadrant, this change was not statistically significant. The EECM considerably decreased the metrics such as escape latency ($P< 0.001$), path length travelled ($P<0.001$), and improved the time spent in quadrant ($P<0.001$) when given at high doses of 400 mg/kg, body weight. As a result, the deficit in long-term memory that was caused by SC was improved by the EECM treatment in a manner that was dose-dependent.

Radial arm maze test: The effect of EECM on working memory was determined using the radial arm maze method. In this, the total number of errors in entry and exploration time was checked to assess its effects and the results were shown in table.1.

The total number of errors in the entry was significantly increased in SC treated animals as compared to the control group $P < 0.001$. But Piracetam intended to reduce the total number of errors in entry. However, there was a significant ($P < 0.001$) dose-dependent

reduction of a total number of errors in the entry on animals treated with EECM 200 mg/kg,b.w when compared with those treated with a high dose of 400 mg/kg, b.w. In exploration time, there was a significant decrease was observed in EECM 400 mg/kg, b.w treated animals, as compared to EECM 200 mg/kg, b.w treated animals. similarly, Piracetam also reduced the exploration time While, SC treated animals showed increased exploration time as compared to control group animals ($P < 0.001$). The EECM at high doses (400mg/kg, b.w) significantly reduced the total number of errors in entry and exploration time ($P < 0.001$) and ameliorate the SC induced working memory deficit.

Biochemical studies

Acetylcholinesterase enzyme: Table 2 displays the findings of an investigation into the inhibitory effect of EECM on acetylcholinesterase enzyme. The significant levels of inhibition of acetylcholinesterase seen in SC-treated neurotoxic rats indicate that these animals suffered from neurodegenerative consequences. When compared to SC-treated mice, the inhibition levels were considerably lower in animals that had been treated with piracetam. However, EECM treated groups at 200 and 400mg/kg, b.w. revealed a significant reduction in inhibitory activity when compared to disease control animals with a P value that was more than 0.001. When compared to animals that were not affected by the disease, this demonstrates the neuroprotective effects of EECM.

Nitric oxide: The impact that EECM had on the amount of nitric oxide was outlined in table.2. P 0.001 indicates that there was a statistically significant rise in the levels of NO that were found in SC-treated animals in comparison to the control animal's levels. Piracetam treatment resulted in a significant reduction of these levels, which are now close to normal. The increased levels of nitric oxide were dramatically reduced after treatment with EECM at doses of 200 and 400 mg/kg, body weight respectively. This reduction was dose-dependent.

Table 1. Effect of ethanolic leaf extracts of *Carissa macrocarpa* (EECM) on spatial learning and working memory.

Groups	Dose	Morri's water maze			Radial arm maze	
		Escape latency (S)	Path length travelled (cm)	Time spent in quadrant (S)	Number of errors	Exploration time (S)
Control	1 ml	20.83±0.71	29.10±0.46	75.21±0.25	2.48±0.64	13.26±0.35
Scopolamine	1mg/kg, i.p	95.10±0.49a	65.38±0.30a	38.86±0.64a	10.24±0.32a	44.15±0.26a
SC+Piracetam	200mg/kg, p.o	29.45±0.16b	33.50±0.20b	68.30±0.56b	4.16±0.35 b	21.31±0.42 b
SC+EECM	200mg/kg,p.o	51.40±0.79b	49.23±0.58b	39.15±0.23b	6.42±0.13 b	27.11±0.29 b
SC+EECM	400mg/kg, p.o	34.25±0.36b	38.27±0.52b	63.28±0.18b	4.92±0.10 b	22.11±0.45 b

Values are expressed as mean± SEM of 6 animals. Data were analysed by One-way ANOVA followed by Tukey's multiple comparison tests. a P<0.001, indicates that Group II (negative control) was compared with group I(control). b P<0.001 indicates that Group III, IV, and V was compared with group II.

Table 2. Effect of ethanolic leaf extracts of *Carissa macrocarpa* (EECM) on and biochemical studies.

Groups	Dose	Ache (µg/min/mg protein)	Nitric oxide (µmol/g tissue)	Total Protein (g/dl)
Control	1 ml	62.33±0.84	0.76±0.02	4.03±0.13
SC	1mg/kg	105.33±0.88a	5.07±0.14a	9.01±0.08a
SC+ PIR	0.5mg/kg	66.17±0.83b	1.51±0.01b	5.13±0.15b
SC+EECM	200mg/kg	89.16±1.01b	3.65±0.07b	7.05±0.09b
SC+EECM	400mg/kg	71.16±0.60b	1.86±0.02b	6.20±0.10b

Values are expressed as mean ±SEM. Datas were analysed by One-way ANOVA followed by Tukey's multiple comparison tests. a P<0.001, indicates that Group II (negative control) was compared with group I(control). b P<0.001 indicates that Group III, IV and V was compared with group II.

Total protein: Table 2 displays the results of an investigation on the impact of EECM on protein levels. When compared to animals that were not affected by the condition, the protein level in the group that was given SC therapy saw a considerable increase. However, pre-treatment with Piracetam and EECM at doses of 200 and 400 mg/kg, b.w. considerably decreased these high levels in comparison to disease control animals.

The purpose of this study was to investigate whether or not ethanolic leaf extracts of *Carissa macrocarpa* have a neuroprotective effect against memory impairment caused by SC. *Carissa macrocarpa* is a traditional medicinal plant that has been shown to have a wide range of therapeutic effects. These benefits can be linked to the presence of bioactive chemicals in various portions of the plant, including flavonoids, triterpenoids, proteins, carbohydrates, phenols, saponins, and glycosides. Therefore, the purpose of the current investigation was to explore the neuroprotective effects of an ethanolic leaf extract of *Carissa macrocarpa* and its action against the cognitive impairment that was generated by SC in rats.

According to the findings of the phytochemical study, the sample contained sterols, carbohydrates, saponins, tannins, proteins, terpenoids, phenolic compounds, and flavonoids. Free radicals are one of the primary contributors to the death of neurons in a variety of

neurological conditions, including but not limited to seizure disorders, schizophrenia, cerebral ischemia, Parkinson's disease, and Alzheimer's disease (Uttara *et al.,* 2009). The *in-vitro* antioxidant potential of EECM was investigated in this work using the DPPH and FRAP assay methods, and the results showed that it exhibited promising antioxidant activity in a dose-dependent manner. The ability to decrease Fe^{3+} to Fe^{2+} was likewise much higher at concentrations ranging from 0.2–1 mg/mL when tested using the FRAP method. The results are coincided with the earlier findings, as the antioxidant property is directly related to the phenolic compounds (Awika *et al.,* 2003).

This study, which primarily focused on the effects of EECM on SC, generated behavioural alterations in the brain of the rat. These changes in behaviour were measured by the rat's spatial learning and memory as well as its working memory by putting it through the Morris Water Maze test and the Radial arm maze test, respectively. According to Jarrard *et al.,* 1984 research, the Morris water maze is a test that is designed to particularly examine a memory test that is dependent on the hippocampus (Jarrard *et al*., 1984). According to the findings of our research, giving an intraperitoneal injection of 1 mg/kg SC leads to a considerable impairment in both spatial learning and memory. This effect was observed in all of the test subjects. This impairment was evidenced by greater escape latencies to reach the platform and path length travelled, as well as poor time spent in the quadrant by the SC treated animals. This finding was consistent with the findings of several research. (Lee *et al.,* 2013) This suggests that as a result of exposure to SC, there is a severe deterioration in the brain, which correlates to deficiencies in episodic memory and recognition capacity. The rats that were given EECM at doses of 200 and 400 mg/kg b.w. showed significantly improved learning and shorter escape latencies in comparison to the rats that were given SC. As a result, these behavioural anomalies were improved with EECM treatment, and both spatial learning and memory were recovered. Therefore, the findings of the current investigation provide evidence in support of our hypothesis that EECM can improve the deficit in spatial memory caused by SC injection. The ability to keep information active in one's working memory for a brief period of time after it has been received is an essential cognitive function. Working memory in the spatial domain. According to Cassel *et al.,* the radial-arm maze test is one of the more prominent methods that are used to examine working memory in rats (Cassel *et al.,* 1998). This investigation was evaluated by counting the number of mistakes made when entering the arms and timing how long it took to explore before finding the food pellets. When compared to the animals in the control group, the animals that were given SC treatment exhibited a greater rise in the amount of time spent exploring as well as an increase in the number of counting errors. This demonstrates the alterations that occur in memory impairment in disease control mice. When contrasted with the SC-treated animals, however, the EECM-treated animals demonstrated a statistically significant reduction in the number of errors that occurred during the entry and exploration time periods. These findings provided further evidence that treatment with EECM is able to mitigate the negative effects of SC on working memory.

The function of the central cholinergic system is critically important to neurocognition. According to a researcher, a significant reduction in acetylcholine causes substantial behavioural alterations, which were shown in a wide variety of neurological illnesses (Kumar *et al.,* 1996). In the current research, the induction of memory loss by SC results in changes in behaviour due to an increase in the activity level of the acetylcholinesterase enzyme. The ameliorating effects of EECM against cholinergic alterations are suggested by the reduction in the amount of acetylcholinesterase inhibition that was caused by the treatment with EECM for 15 days.

Nitric oxide is implicated in a wide range of neurological conditions, including Alzheimer's disease and Parkinson's disease, and plays a significant role in a number of key physiological processes. Numerous pieces of evidence pointed to the fact that nitric oxide produces an excitotoxic effect. High levels of nitric oxide play an important role as a critical mediator in neurological illnesses (Schulz *et al.,* 1995). This is because excited neurons kill a significant number of other neurons. It is well knowledge that acute and chronic inflammatory disorders are linked to increased levels of certain proteins. In this research it was found that the region of the rat brain known as the hippocampus contained large levels of protein in SC-treated animals. It is possible that the EECM's antioxidant capability is responsible for the pre-treatment with EECM causing a considerable drop in the amounts of protein. Piracetam, on the other hand, brought these levels back to near normal.

CONCLUSION

The findings of the current investigation demonstrated that an ethanolic extract of the leaves of *Carissa macrocarpa* exhibited potentially neuroprotective properties against the SC-induced memory impairment in rats. It's possible that this is because of the flavonoid's antioxidant capabilities. The findings of the current investigation demonstrated that an ethanolic extract of the leaves of *Carissa macrocarpa* exhibited potentially neuroprotective properties against the SC-induced memory impairment in rats. One possible explanation for this is that the flavonoids, tannins, and polyphenols that are contained in it all have antioxidant capabilities.

FUTURE SCOPE

The promising neuroprotective effects of ethanolic extract of leaves of *Carissa macrocarpa* investigated in this study can be exploited for isolation and characterization studies to identify the novel compounds responsible for its neuroprotective activity.

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Conflict of interest. None

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