IMPROVEMENT OF LEARNING AND MEMORY BY *CROTON HIRTUS* AGAINST SCOPOLAMINE INDUCED AMNESIA IN RATS

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ABSTRACT

Amnesia (most notably, Alzheimer's disease) is often associated with impairments of both working memory and inhibitory control. However, it is unclear whether these are functionally distinct impairments. This investigation examines the possible therapeutic advantages of ethanolic extract of *croton hirtus* (EECH) against scopolamine induced amnesia in rats. In this procedure, animals (male Sprague Dawley rat) weighed 180-220g are grouped in to five containing six each having age of 5-6 months. The group 1 was considered as normal control (vehicle) and were treated with 0.9% normal saline (1ml/kg p.o) from day 1 to 14.Group 2 is considered as negative control treated with scopolamine (1mg/kg, i.p) on days 1stand 3rd . Whereas groups 3 is considered as standard treated with scopolamine (1mg/kg,i.p) on day 1stand 3rdthen treated with Donepezil (5mg/kg,p.o) on day 4 to 14. Group 4 treated with scopolamine (1mg/kg,i.p) on day 1stand 3rdand then treated with low dose of test drug , ethanolic extract of *croton hirtus* (200mg/kg,p.o) on day 4 to 14. Group 5 treated with scopolamine (1mg/kg,i.p) on day 1stand 3rdand then treated with high dose of test drug , ethanolic extract of *croton hirtus*(400mg/kg,p.o) on day 4 to 14. Rats learning and memory abilities were evaluated using a morris water maze test ,elevated plus maze test and passive avoidance test ,Locomtor activity, Forced swim test and acetyl cholinesterase Enzyme in Brain. Our findings reveal the potential of Ethanolic extract of *croton hirtus* as a novel therapeutic agent for the treatment of amnesia.EECH significantly prevented the memory deficit induced byscopolamine.Therefore, EECH may be able to treat cognitive disorders like amnesia associated with Alzheimer's disease (AD).

Key word: Alzheimer's diseas, Donepezil, Scopolamine, *Croton hirtus*

Introduction

Alzheimer's disease (AD) is characterized by increasing memory loss and a steady deterioration in cognitive functioning, which can lead to early death many years after diagnosis. Although the exact causation of Alzheimer's disease is unknown, there is a wealth of evidence indicating that abnormal free radical generation may be the root cause of disease progression, which is accompanied by neuronal destruction. Variations in reactive oxygen species (ROS) and free radical generation can induce changes in cellular structure and function due to oxidative metabolism imbalances. Extrinsic amyloid plaques, intracellular neuro fibrillary tangles (NFT), neuronal degeneration, and cell damage have all been linked to Alzheimer's disease, according to recent study[1]. Ageing, stress from oxidative damage, abnormal mitochondrial structure, and inflammation all contribute to its development. A recent investigation reveals that diabetes, hyperglycemia, and decreased insulin action in the brain may be connected to the sporadic development of Alzheimer's disease (sAD)[2]. It is the most prevalent cause of dementia in elderly people and is recognized as the pandemic of the twenty-first century, exacting great social and economic costs on patients and their families. Furthermore, the aging world population has contributed to a significant increase in the number of persons with AD, which is expected to quadruple by the year 2050[3].

Numerous biochemical and clinical pathways contribute to Alzheimer's disease. The amyloid hypothesis posits that the buildup of amyloid β (Aβ) plaques in the brain is a key factor in the onset of Alzheimer's disease (AD). It suggests that an imbalance between Aβ production and its clearance results in plaque formation, which disrupts neural function and drives neurodegeneration. Aβ is a fragment derived from the larger amyloid precursor protein (APP). Normally, Aβ is removed from the brain efficiently; however, when production exceeds clearance or clearance mechanisms fail, Aβ accumulates. This accumulation leads to plaque formation, which is believed to hinder communication between cells and trigger neuroinflammatory responses, ultimately resulting in the cognitive decline and memory impairment associated with Alzheimer's disease.[4]

Alzheimer's disease (AD) is primarily linked to two significant factors: neuroinflammation and deficits in cholinergic function. The accumulation of Aβ plaques initiates neuroinflammation, activating microglia that release inflammatory cytokines and reactive oxygen species (ROS): which harm cellular structures and exacerbate oxidative stress. This cellular damage contributes to the worsening of cognitive deficits and the progression of AD. According to the cholinergic hypothesis, AD is associated with a reduction in the activity of cholinergic neurons that synthesize acetylcholine. The activity of enzymes like acetyl-cholinesterase (AChE) and Butyryl-cholinesterase (BChE) becomes excessive, leading to an accelerated breakdown of acetylcholine and a further decline in cholinergic function. The resulting loss of acetylcholine in critical brain regions, including the hippocampus and cortex, significantly impacts memory and cognitive abilities.[6]

The exploration of herbal medicine and its believed therapeutic benefits for Alzheimer's disease (AD) have gained progress. The use of Ayurvedic medicinal plants and

their bioactive ingredients is especially significant since many of these plants have a long history of traditional use in the treatment of cognitive decline, memory issues, and overall brain health.[7] Numerous Ayurvedic medicinal plants and their phytocompounds have demonstrated encouraging pharmacological effects related to Alzheimer's disease (AD). The appeal of these plants lies in their ability to address various mechanisms associated with AD, such as neuroinflammation, oxidative stress, the formation of amyloid plaques, and cholinergic dysfunction.[8-9]

Croton hirtus is a medicinal plant known for its various pharmacological characteristics. It has long been utilized in indigenous medicine to treat skin infections, wounds, and gastrointestinal issues. The plant includes bioactive substances such alkaloids, flavonoids, terpenoids, and tannins, which contribute to its medicinal value[10]. Croton hirtus claims to have antibacterial[11], anti-inflammatory[11], and antioxidant properties[12], making it a suitable option for the creation of natural treatments. Our results driven the behavioral tests and acetylcholine levels show that rats had memory and learning impairments, which were relieved by EECH when compared to groups treated with Donepezil hydrochloride and Scopolamine. These findings demonstrate that EECH may improve memory in rats suffering from Scopolamine-induced brain damage, most likely because to its high content of powerful antioxidants[12], notably sesquiterpenoids.

Material and Methods:

Chemicals:

Scopolamine hydrobromide, Donepezil was obtained from Sigma-Aldrich.

Plant Extraction:

After the *Croton hirtus* plant was collected from Malappuram, Kerala, and its identity was verified by Dr. V. Aravindhan, an assistant professor in the Department of Botany at Kongunadu Arts and Science College in Coimbatore, leaves were taken from mature specimens at the same spot and thoroughly cleaned under running tap water removing any remaining materials before being dried in the shade.[13-14]

Prepare of Extraction:

For three days at 45 °C, a total of 100 g of the dried and refined leaves of CH were repeatedly extracted using 80% (v/v) ethanol using a series of 15-minute sonication and two-hour rest cycles. The final product was concentrated using non-fluorescent cotton filtering and a revolving evaporator set at 45 °C and lower pressure. 3.25 g of CH extract remained after freeze drying and vaporization; this residue was added to 10% diluted HCl, thoroughly shaken, and filtered. Standard procedures were used to identify the phytochemical elements in the plant material extract using chemical testing.[14]

Phytochemical screening:

The ethanolic extract of the plant *Croton hirtus*leaves was investigated to Preliminary Phytochemical studies.[15,16]

***In-vivo* Studies:**

Acute toxicity studies:

In accordance with OECD 423 guidelines and findings from acute toxicity studies, a dose of 200 mg/kg (one-tenth of 2000 mg/kg) of leaf extracts was chosen for pharmacological screening related to Alzheimer's disease.

Anti- Alzheimer activity studies:

In this study, male Sprague Dawley rats weighing between 180-220g and aged 5-6 months were divided into five groups, each consisting of six animals. Group 1 served as the normal control, receiving 0.9% normal saline (1ml/kg p.o) from days 1 to 14. Group 2 acted as the negative control, treated with scopolamine (1mg/kg, i.p) on days 1 and 3. Group 3, the standard group, received scopolamine on the same days followed by Donepezil (5mg/kg, p.o) from days 4 to 14. Group 4 was administered scopolamine on days 1 and 3, then given a low dose of the test drug, ethanolic extract of *Croton hirtus* (200mg/kg, p.o) from days 4 to 14. Group 5 also received scopolamine on days 1 and 3, followed by a high dose of the test drug (400mg/kg, p.o) from days 4 to 14. The learning and memory capabilities of the rats were assessed from days 15 to 20 using the Morris water maze test, elevated plus maze test, and passive avoidance test.[17]

Behavioral assessments of Alzheimer rats:

Elevated plus-maze test:

Memory enhancement index screening was performed on rats utilizing the elevated plus maze. Each rat was positioned at the end of an open arm within the maze apparatus. The duration taken for a rat to move into the closed arm was recorded as transfer latency (TL). A cutoff time of 180 seconds was established for each rat, and a retention trial was conducted 24 hours following the initial trial, with transfer latency documented in a manner consistent with prior methodologies. A reduced transfer latency served as a measure of memory enhancement.[18]

Morris Water maze(MWM):

Spatial learning and memory were assessed using the Morris water maze, which involved a two-step procedure. The initial phase, conducted over days 1 to 4, featured a place navigation test where escape latency (EL)—the time taken to reach the hidden platform—

served as a measure of learning and memory. Rats that successfully located the platform were allowed to stay for 20 seconds before being returned to their home cage. If a rat failed to find the platform within 120 seconds, the experimenter would gently guide it to the platform, where it would also remain for 20 seconds. The final trial was designated as the probe test. The second phase occurred on day 5, following the removal of the platform, and involved a spatial probe test to evaluate the rats' ability to recall the location of the platform based on memory.[19]

Passive avoidance test (PAT):

The step-through passive avoidance apparatus featured an illuminated chamber measuring 11.5 cm x 9.5 cm x 11 cm, connected to a darkened chamber of 23.5 cm x 9.5 cm x 11 cm, which had a metal floor capable of delivering foot shocks. A guillotine door separated the two compartments, with the illuminated chamber lit by a 25 W lamp. Initially, each rat was acclimatized in the dimly lit room containing the apparatus for 30 minutes before training. Subsequently, the rat was placed individually in the illuminated chamber, facing away from the door to the dark chamber, and allowed to adjust for one minute. Upon entering the dark chamber, the door closed, and a mild foot shock (0.3 mA, 50 Hz, 5 s) was administered. The rat was then promptly removed and returned to its home cage, with the latency to change compartments recorded. A retention test was performed 24 hours later, repeating the procedure in the illuminated chamber without the foot shock, with a maximum time limit of 300 seconds.[20]

Locomotor activity:

The locomotor behavior of the animals was assessed using an Actophotometer. This device, manufactured by Dolphin, features a digital counter, photocell, and light source to quantify the horizontal movement of the subjects. Each animal was observed in the Actophotometer for a duration of five minutes to establish a baseline activity score. Following treatment with the designated drug, activity scores were recorded at 30 minutes and one-hour intervals. A reduction in activity score was interpreted as an indicator of central nervous system depression or memory impairment.[21]

Forced swim test (FST)

Each rat is placed in a glass beaker with an inner diameter of 15 cm and a depth of 20 cm, filled with tap water at a temperature of 20–25 °C to a depth of 16 cm. This water level is designed to ensure that the rat cannot touch the bottom of the beaker with its hind limbs or tail, necessitating adjustments based on the rat's size. The test duration is 6 minutes, after which the rat is dried and returned to its cage. The procedure is recorded, and independent observers score the immobility time, defined as the minimal movement required to keep the rat's head above water. Previous studies have indicated that cranial irradiation leads to increased immobility times, which are interpreted as signs of heightened depression-like behavior or memory deficits.[22]

Preparation of brain homogenate:

Prior to euthanasia, rats were anesthetized with an intraperitoneal injection of thiopental sodium at a dosage of 20 mg/kg. Following this, the rats underwent transcranial perfusion with 0.9% ice-cold saline, after which the entire brain was extracted. The hippocampus, striatum, and cortex were carefully dissected using sterilized surgical blades and ophthalmic forceps. The dissected brain regions were promptly placed on dry ice and subsequently stored in a deep freezer at −80°C. For brain homogenate preparation, the samples were homogenized in ice-cold phosphate-buffered saline (PBS) at a concentration of 10% w/v. The homogenized samples were then centrifuged at 12,000 × g for 15 minutes at 4°C, and the supernatant was collected for subsequent biochemical analysis.[23]

Estimation of Acetyl cholinesterase Enzyme in Brain:

A total of twenty milligrams of brain tissue per milliliter of phosphate buffer was homogenized using a Potter Elvehjem homogenizer. Approximately 0.4 mL of this homogenate was combined with 206 mL of 0.1 M phosphate buffer. Following this, one hundred microliters of the DTNB reagent were introduced into the photocell, and the absorbance was recorded at 412 nm. Subsequently, acetylthiocholine iodide was added, and the enzyme activity was quantified as micromoles per minute per milligram of tissue.[24]

Statistical Studies:

The outcomes of the learning and memory assessment parameters, including the Morris Water Maze (MWM) test, Passive Avoidance Test (PAT), elevated plus maze test, and brain tissue homogenates (AChE), were reported as mean ± standard error of the mean (SEM). To determine the overall statistical significance ($P<0.05$) among the groups, a one-way analysis of variance (ANOVA) was conducted, followed by the Tukey multiple comparison test.

Result and discussion:

Phytochemical Studies:

Preliminary phytochemical analyses of the ethanolic extract of *Croton hirtus* indicate the presence of various compounds, including alkaloids, carbohydrates, glucosides, flavonoids, proteins, phenolic compounds, terpenoids, tannins, and saponins. The presences of Flavonoids, phenolic compounds and terpenoids are exhibit the anti-oxidants and anti-inflammatory activity.[25] The anti-oxidant activity exhibits the free radical scavenging activity[26] and anti-inflammatory activity reveals inhibits the inflammatory pathway(Eg: TNF-α, IL_2, MAPK/ERK/p38 signaling pathway).[27-28]

***In-vivo* studies**

This study was investigated by the Elevated Plus Maze test illustrated in **Table: 1 &Figure 1** demonstrates the learning capabilities of rats, indicating that the transfer latency for the negative control group is significantly higher than that of the other groups. This suggests that all groups, except for the negative control, exhibit improved memory enhancement.The Morris water maze test illustrated the learning capabilities of rats in the SCOP-treated group, as shown in **Table: 2& Figure 2**. This group exhibited a longer escape latency to reach the hidden platform and spent less time in the target quadrant. In contrast, the EECH-treated groups demonstrated increased escape latency and a greater duration in the target quadrant compared to the negative control group**Table: 3& Figure 3**.The passive avoidance test revealed a notable reduction in escape latency for rats treated with scopolamine. In contrast, rats administered with EECH exhibited significant increases in escape latency compared to the negative control group**Table: 4& Figure 4**.The actophotometer records the frequency of animal crossings over a beam. In this study, the group administered SCOP exhibited reduced locomotor activity compared to the other group. Conversely, the groups treated with EECH at doses of 200 mg and 400 mg demonstrated increased locomotor activity relative to the negative control group**Table: 5& Figure 5**.The immobility time observed in the SCOP-treated group during the forced swim test is greater than that of the other groups. In contrast, the EECH groups exhibited reduced immobility time compared to the negative control group. Increased immobility time is indicative of heightened depressive-like behavior or memory deficits.The EECH can reduce AChE levels while enhancing the availability of ACh. This effect may be attributed to its antioxidant properties.

CONCLUSION

The research investigates the phytochemical and pharmacological characteristics of *Croton hirtus* leaves, identifying the presence of various compounds including alkaloids, carbohydrates, glucose, flavonoids, proteins, phenolic compounds, terpenoids, tannins, and saponins. Notably, elevated levels of terpenoids demonstrate antioxidant properties, which may contribute to improved memory function.The ethanolic extract of *Croton hirtus*demonstrated notable efficacy in inhibiting acetylcholinesterase and exhibiting free radical scavenging activity, prompting its selection for pharmacological evaluation. Behavioral assessments and acetylcholine level measurements indicated memory and learning deficits in rats, which were ameliorated by EECH compared to groups treated with Donepezil hydrochloride and Scopolamine. These findings suggest that EECH may enhance memory in rats with Scopolamine-induced brain damage, likely due to its high concentration of potent antioxidants, particularly sesquiterpenoids.

Acknowledgements:

The author expresses gratitude to Dr. N. Senthilkumar, Principal and Professor, along with Dr. V. Suresh, Head and Professor of the Department of Pharmacology at JKKMMRF's

Annai JKK Sampoorani Ammal College of Pharmacy in Komarapalayam, Namakkal, Tamil Nadu, India, for their technical assistance and insightful guidance.

TableNo.1. Effect of ethanolic extract of *croton hirtus* on elevated plus maze test

GROUP	**TRANSGER LATENCY IN EPM(s)**
CONTROL	10.25±2.23
SCOPOLAMINE(1mg/kg,i.p)	26.15±4.34
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	13.27±1.18 ***
SCOPOLAMINE+EECH(200mg/kg,i.p)	16.24±2.78 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	14.24±4.20 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001 The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group

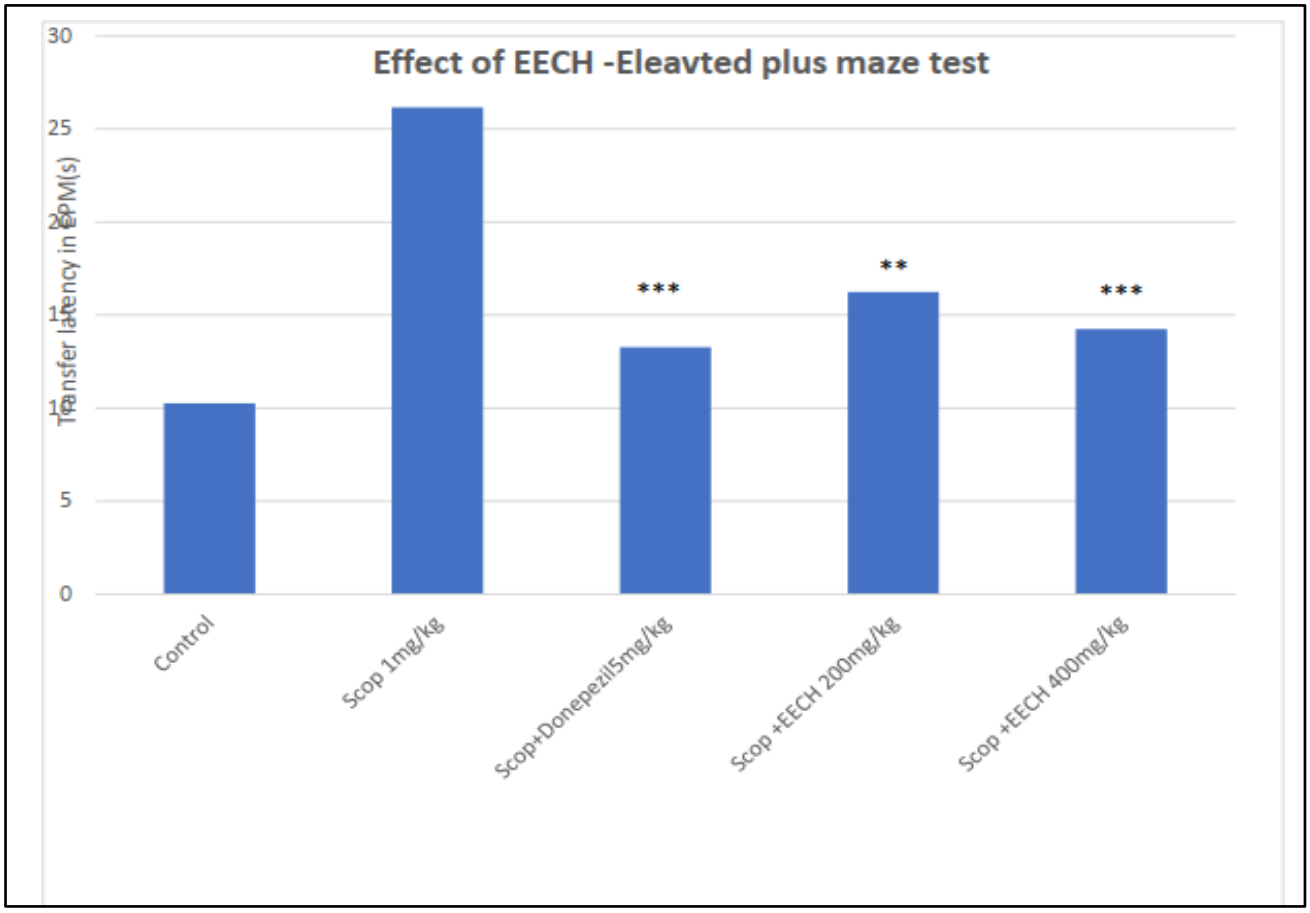


Fig no: 1– Effect EECH – Elevated plus maze test

TableNo.2. Effect of ethanolic extract of *croton hirtus*- Morris water maze test.

GROUP	**TIMESPENTIN TARGET QUADRANT(s)**	**ESCAPE LATENCY(s)**
CONTROL	5.15±1.24	6.6±2.56
SCOPOLAMINE(1mg/kg,i.p)	1.15 ±0.98	12.16±1.78
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	4.28±1.89 ***	6.8 ±3.68 ***
SCOPOLAMINE+EECH(200mg/kg,i.p)	2.62±3.26 **	7.8 ± 4.26 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	3.96±2.46 ***	7.1 ±2.24 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001 The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group

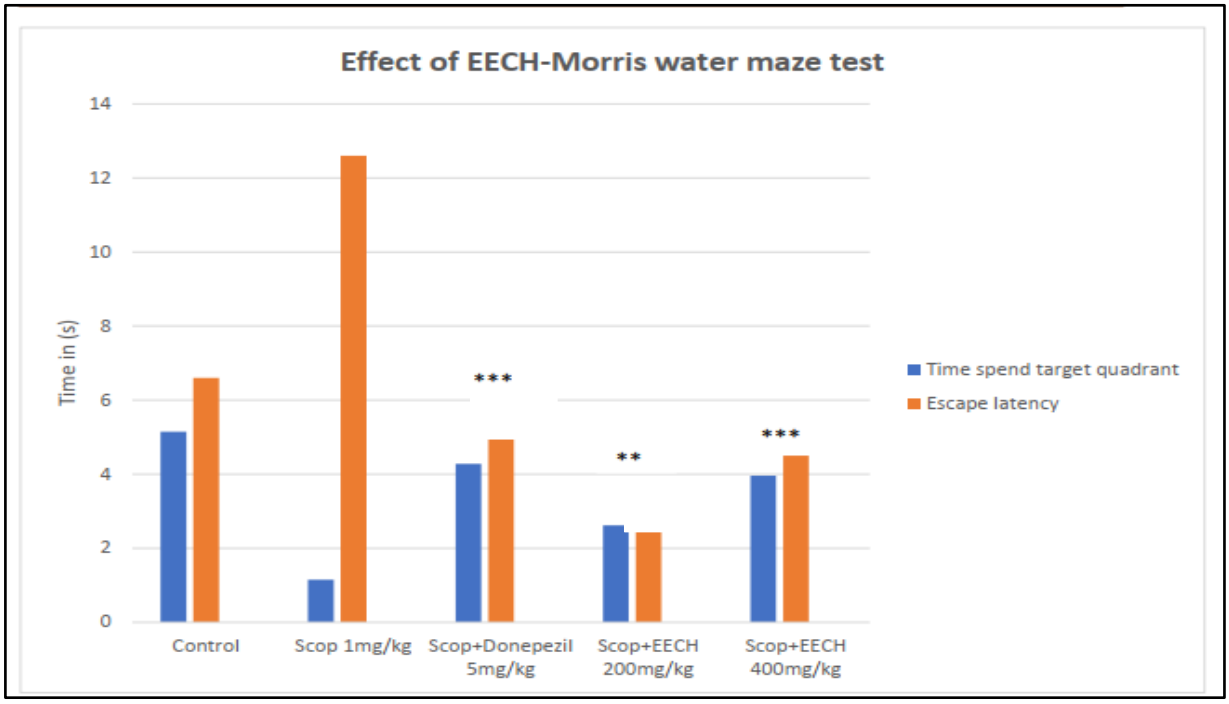


Fig no: 2–Effect EECH - Morri's water maze test

TableNo.3. Effect of ethanolic extract of *croton hirtus*- Passive avoidance test.

GROUPS	**ESCAPELATENCY(s)**
CONTROL	12.16±2.56
SCOPOLAMINE(1mg/kg,i.p)	4.67±1.78
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	11.20±3.68***
SCOPOLAMINE+EECH(200mg/kg,i.p)	7.98 ± 4.26 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	10.98±2.24 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001

The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group

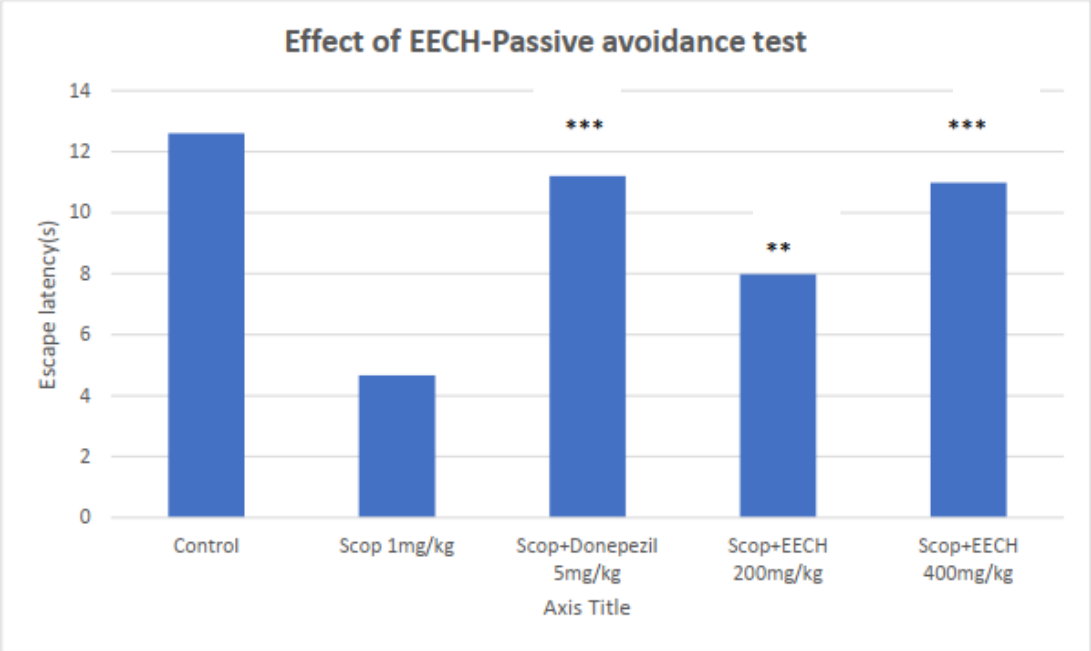


Fig no:3 –Effect EECH- Morri's water maze test

TableNo.4. Effect of ethanolic extract of *croton hiatus* on Act photometer test (Locomotors activity)

GROUPS	**LOCOMOTORACTIVITY(MEAN SCORE IN 5 min)**
CONTROL	80±2.7
SCOPOLAMINE(1mg/kg,i.p)	28±1.2
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	70±2.4 ***
SCOPOLAMINE+EECH(200mg/kg,i.p)	43±1.8 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	60±2.1 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001 The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group

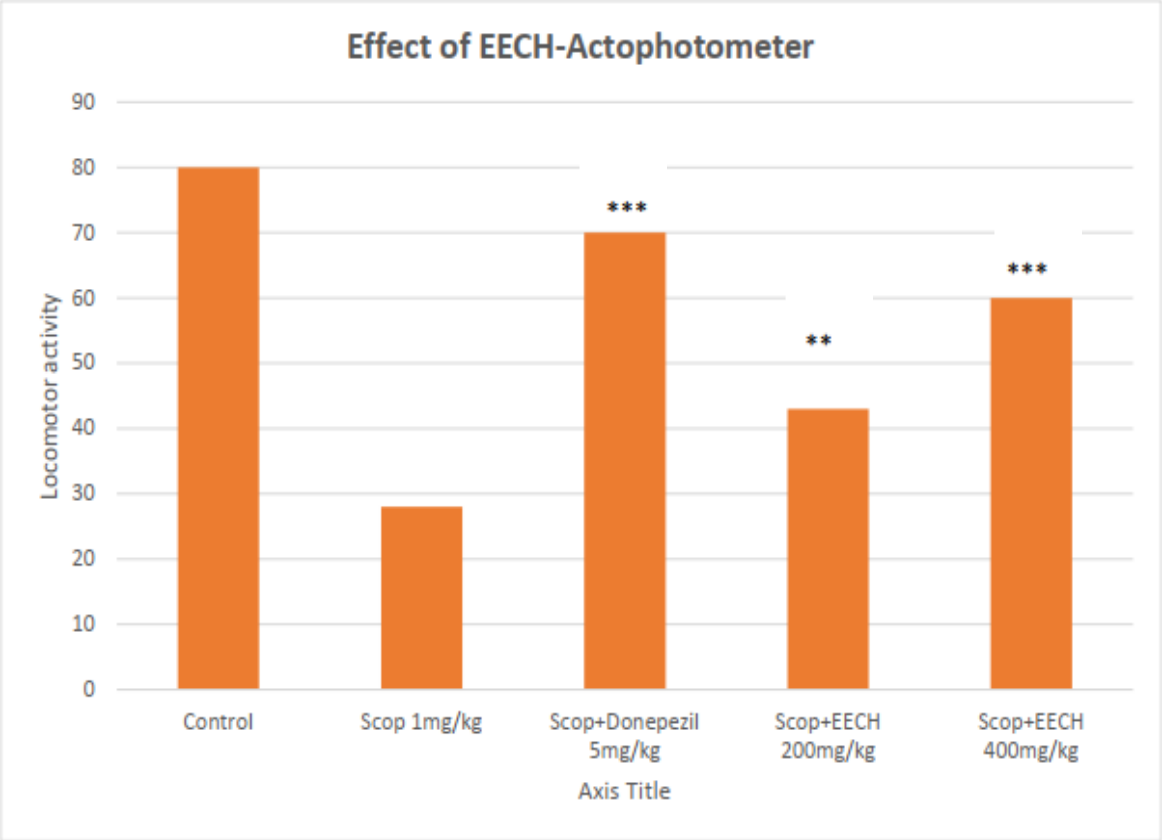


Fig no: 4–Effect EECH- Actophotometertest

Table No.5.Effect of ethanolic extract of *croton hirtus* on Forced swim test

GROUP	**IMMOBILITYTIME(s)**
CONTROL	20±2.5
SCOPOLAMINE(1mg/kg,i.p)	61±2.3
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	28±1.8 ***
SCOPOLAMINE+EECH(200mg/kg,i.p)	48±2.6 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	31±2.2 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001 The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group

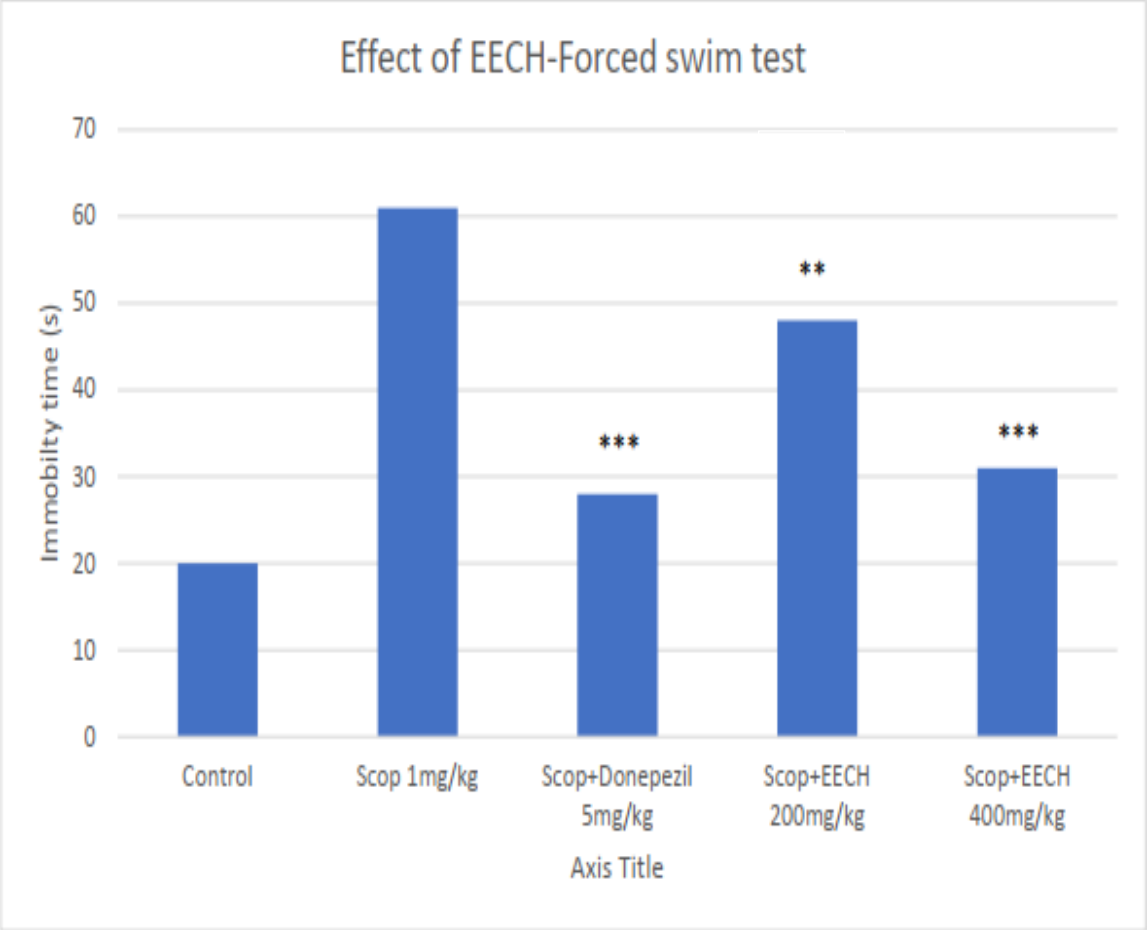
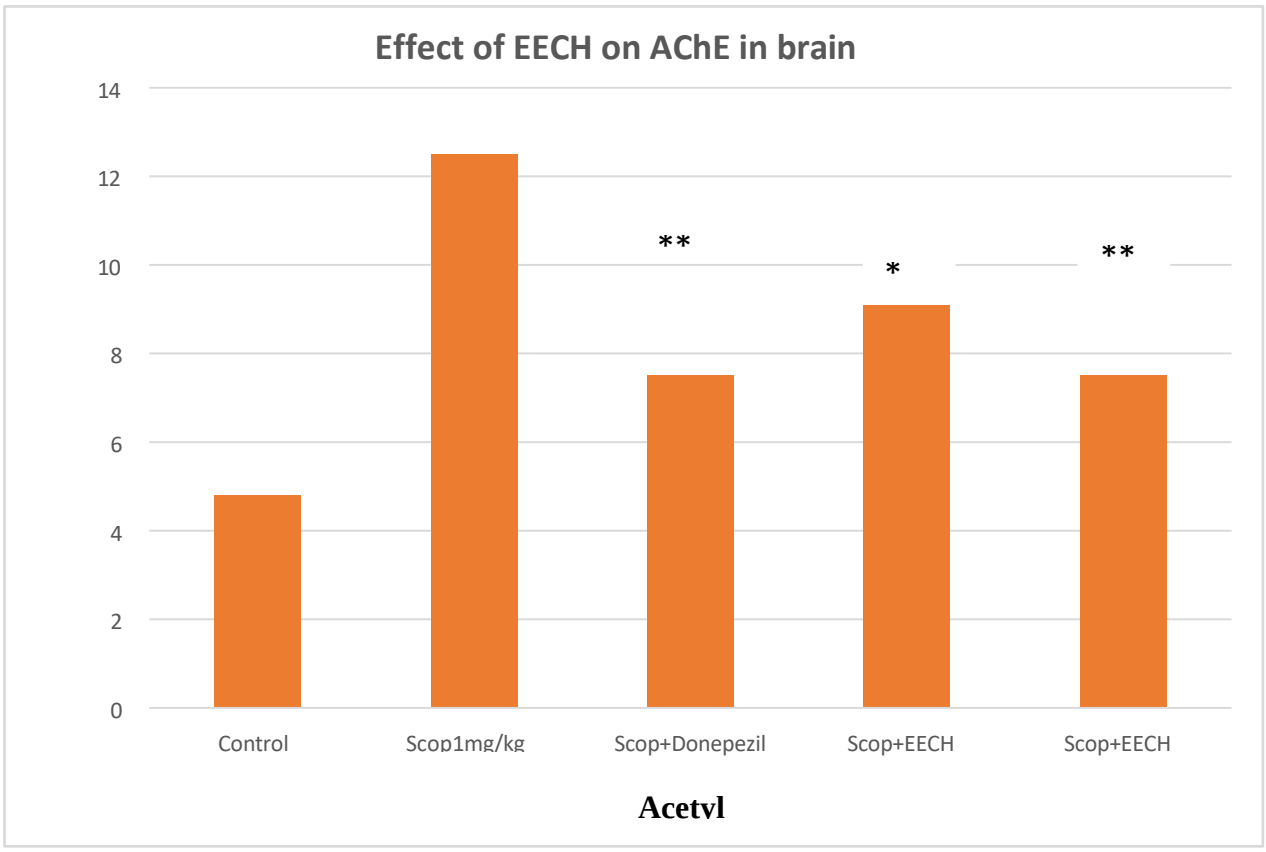


Fig no: 5– Effect EECH-Forced swim test

Table No. 6. Effect of ethanolic extract of *croton hirtus* by Acetyl cholinesterase Enzyme in Brain

GROUP	**AChELEVEL (µmol/min/mg tissue)**
CONTROL	4.8±0.2
SCOPOLAMINE(1mg/kg,i.p)	12.5±0.12
SCOPOLAMINE+DONEPZIL(5mg/kg,i.p)	7.5±0.36 ***
SCOPOLAMINE+EECH(200mg/kg,i.p)	9.1±0.47 **
SCOPOLAMINE+EECH(400mg/kg,i.p)	7.1±0.28 ***

All the treated groups are compared against the disease group. *P<0.05;**P<0.01;***P<0.001 The values are expressed as mean ± SEM (n=6) Normal group is compared against disease group



Reference:

1. Bent S. Herbal medicine in the United States: Review of efficacy, safety, and regulation. Journal of General Internal Medicine. 2008;23(6):854-859
2. Kessler RC, Davis RB, Foster DF, Van Rompay MI, Walters EE, Wilkey SA, Kaptchuk TJ, Eisenberg DM. Long-term trends in the use of complementary and alternative medical therapies in the United States. Annals of Internal Medicine. 2001;135(4):262-268
3. Bodeker G, Burford G. Traditional, complementary and alternative medicine: Policy and public health perspectives. Bulletin of the World Health Organization. 2007;86(1):77-78
4. Fasinu PS, Bouic PJ, Rosenkranz B.An overview of the evidence and mechanisms of herb–drug interactions. Frontiers in Pharmacology. 2012;3:69. DOI: 10.3389/fphar.2012.00069
5. Bensoussan A, Myers SP, Wu S, O'Connor K. Naturopathic and western herbal medicine practice in Australia: A workforce survey. Complementary Therapies in Medicine. 2004;12(1):17-27
6. Van-Wyk BE, deWet H, Van-Heerden FR. An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. South African Journal of Botany. 2008;74:696-704
7. WHOTraditionalMedicineStrategy2002-2005. Geneva:WHO.p.2002

8. Barrett B, Kiefer D, Rabago D. Assessing the risks and benefits of herbal medicine: An overview of scientific evidence. Alternative Therapies in Health and Medicine. 1999;5:40-49
9. Medically reviewed by Kerry Boyle D.Ac., M.S., L.Ac., Dipl. Ac., CYT — Writtenby Oladimeji Ewumi on October 6, 2022
10. AntonioSalatino Universidade de São Paulo, Instituto de Biociências , Departamento de Botânica, São Paulo, São Paulo, Brazi 2007,https://doi.org/10.1590/S0103-50532007000100002
11. .*Crotonglandulosus*L.,Syst.(ed.10)2:1275.1795,var.*hirtus*(L'Herit)Muell.-Arg.inDC.,Prodr.15:684.1866;N.P.Balakr.&Chakrab.,Fam.EuphorbiaceaeIndia213.2007.
12. *Crotonhirtus*L'Herit.,Strip.Nov.1:17t.9.1785;Ramach.etal.,IndianJ.For.15:
13. 183.1992; Sunil&Sivadasan,Fl.AlappuzhaDist. 625. 2009.V.S.Ramachandran,K.Ravikumar&V.Balasubramanian,1992
14. ReitzC., BrayneC., Mayeux R.EpidemiologyofAlzheimerdisease. Nat Rev Neurol. 2011;7:137. doi: 10.1038/nrneurol.2011.2.
15. Association, Alzheimer Association 2014 Alzheimer's disease facts and figures. Alzheimers Dement. 2014;10:e47–e92. doi: 10.1016/j.jalz.2014.02.001
16. Association, Alzheimer Association 2017 Alzheimer's disease facts and figures. Alzheimers Dement. 2017;13:325–373.
17. LiX.-L.,HuN.,TanM.-S.,YuJ.-T.,TanL.Behaviouralandpsychologicalsymptoms in Alzheimer's disease. Biomed Res Int. 2014;2014:927804. doi: 10.1155/2014/927804. [DOI] [PMC free article]
18. Alzheimer’s Association. Stages of Alzheimer’s (https://www.alz.org/alzheimers-dementia/stages). Accessed 12/10/2022.
19. Alzheimer’s Association. What is Alzheimer’s disease?
20. (https://www.alz.org/alzheimers-dementia /what-is-alzheimers) Accessed 12/10/2022.
21. Stages of Alzheimer's disease. Alzheimer'sAssociation. http://www.alz.org/alzheimers_disease_stages_of_alzheimers.asp. Accessed March 17, 2023.
22. Jameson JL, et al., eds.Alzheimer's disease. In: Harrison's Manual of Medicine. 20th ed. McGraw Hill; 2020. https://accessmedicine.mhmedical.com.Accessed March 17, 2023.
23. What are the signs of Alzheimer's disease? National Institute on Aging. https://www.nia.nih.gov/health/what-are-signs-alzheimers-disease. Accessed March 17, 2023.
24. Abraham CR, Selkoe DJ, Potter H 1988. Immunochemical identification of the serine protease inhibitor, α1-antichymotrypsin in the brain amyloid deposits ofAlzheimer’s disease. Cell 52: 487–501
25. Povova J, Ambroz P, Bar M, Pavukova V, Sery O, Tomaskova H, et al. Epidemiological of and risk factors for Alzheimer’s disease: a review. Biomed Pap Med FacUnivPalacky Olomouc Czech Repub. 2012;156:108–114. doi: 10.5507/bp.2012.055
26. Qabaha K, Hijawi T, Mahamid A, Mansour H, Naeem A, Abbadi J, Al-Rimawi F. Anti-inflammatory and Antioxidant Activities of *Teucrium polium* Leaf Extract and its Phenolic and Flavonoids Content.
27. Keerthana G, Kalaivani MK, Sumathy A. In-vitro alpha amylase inhibitory and anti-

oxidant activities of ethanolic leaf extract of *Croton bonplandianum*. Asian J Pharm Clin Res. 2013 Sep 1;6(4):32-6.

28. Jin X, Wang J, Xia ZM, Shang CH, Chao QL, Liu YR, Fan HY, Chen DQ, Qiu F, Zhao F. Anti-inflammatory and anti-oxidative activities of paeonol and its metabolites through blocking MAPK/ERK/p38 signaling pathway. Inflammation. 2016 Feb;39:434-46.
29. Fan HJ, Xie ZP, Lu ZW, Tan ZB, Bi YM, Xie LP, Wu YT, Zhang WT, Liu-Kot K, Liu B, Zhou YC. Anti-inflammatory and immune response regulation of Si-Ni-San in 2, 4-dinitrochlorobenzene-induced atopic dermatitis-like skin dysfunction. Journal of Ethnopharmacology. 2018 Aug 10;222:1-0