PRELIMINARY ,PHYTOCHEMICAL SCREENING AND MEMORY ENHANCEMENTACTIVITY OF AQUEOUS EXTRACT OF FICUS RACEMOSA LINN ON RAT

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ABSTRACT

Objective of the study

Ficus racemosa is a medicinal plant it also having some religious aspect. It having antioxidant and anti-inflammatory property due to presence of glycoside racemosic acid and phenolic compound which leads to raise Ach level in brain by inhibiting Acetylcholinestrase.

The aim of the study is to elevate the Ach level in brain, responsible for memory and learning activity.

Materials and methods

The memory enhancement activity of the aqueous extract of leaves of *Ficus racemosa* were estimated on male albino wistar rats, memory loss induced by Scopolamine (1 mg/kg p.o) by evaluation of Ach level in brain by using Pole climbing apparatus .The Ach was determined by using Phosphate buffer, DTNB,& Acetylthiocholine by decapitation of brain after last day of dosing .From decapitated brain isolation of hippocampus ,cerebral cortex, and brain septum are homogenized and by using these chemicals absorbance is determined in photoelectric colorimeter.

Result

From the various phytochemical testing we obtained many chemical compounds like phenolic compounds such as quercetin, kaempherol , friedelin psoralen alkaloids tannins , and glycosides .They exhibits antioxidant activity act as inhibitor of Acetylcholinestrase , therefore causes elevation of Ach level in brain.

Conclusion

From the deep study of plant *ficus racemosa* we found that it having some religious as well as medicinal aspect it is used in various disease, moreover it having memory enhancement activity due to presence of phenolic compound and racemosic acid .

Index terms : Acetylthiocholine, antioxidant , memory , Traditional medicine , decapitation.

1. INTRODUCTION

The German psychiatrist and neuropathologist Dr. Alois Alzheimer's described first time about dementia it further turned into Alzheimer's disease. Alzheimer's disease is neurodegerative disease which becomes worsen over time. AD is distinguished by the memory loss and behavioral changes in the patient. Several Neuropathological changes occurs during this disease including cerebral atrophy, neurotic plaques, and neurofibrillary tangles. According to many studies shows the major neurotransmitter (Acetylcholine) which is responsible for memory & learning decreased their condition in case of disease in the cerebral cortex and

hippocampus .There are various synthetic drugs are available for raising the level of acetylcholine with the use of acetylcholinestrase inhibitors produce improvement in cognitive function in patients. Some commonly used AChE inhibitors are Donepezil, Rivastigmine, and Galantamine etc.

2.　　MATERIAL AND METHODS

2.1　　Plant collection

The leaves of *Ficus racemosa* family Moraceae were collectd in the june and july 2017 from the local area of Phaphamau ,Allahabad Uttarpradesh , India .The plant was taxonomically identified by Dr.G.P.Sinha botanical survey of India ,CRC,10 Chatham line Allahabad 211002 the voucher specimen AS-1 is retained in the herbarium of BSI ,Allahabad for future reference.

2.1 Drugs and Chemicals

Piracetam (standard drug, 200mg/kg), Scopolamine as inducing agent and extracts of different dose of extract. Acetylthiocholine is required for estimation of level of Acetylcholinestrase. All drugs were dissolved in distilled water and administered orally**.**

2.3 Animal

Healthy, Male Albino wistar rats of 150-180 gm. weight are taken for experimentation. Animals are housed in polypropylene cages, and kept in room by providing proper condition (12 h day/light cycle; temperature 23 ± 2 ^{0}C, 50 ± 5 %, relative humidity), rats were fed with standard rat pellets .The Institutional Animal Ethical Committee (SIP-IAEC/006/05/17) approved this study .

2.4 Analysis of Physicochemical parameter

The physicochemical testing was performed to check the Ash value, extractive values, loss on drying on the powdered leaves of *Ficus racemosa*.

Table no. 2 **Physicochemical analysis of leaves of *Ficus racemosa* Linn**

S. No.	Parameters	Percentage (%w/w)
1.	**Ash values**	
	Total ash value	21.66 %w/w
	Acid insoluble ash value	18.98 % w/w
	Water soluble ash value	20.67 % w/w
2.	**Extractive values**	
	Alcohol soluble extractive	7.98 % w/w
	Water soluble extractive	9.62 % w/w
3.	**Loss on drying**	10.94 %w/w
4.	**P^H**	6.88

2.5 Determination of Percentage yields

The leaves of *Ficus racemosa* were extracted with various solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and distilled water by cold maceration method.

After performing extraction procedure, highest percentage yield was found aqueous extracts of leaves of *Ficus racemosa*

Table 1. **Percentage yield of extracts of various extracts of leaves of Ficus racemosa**

S. No.	Solvent	% of extract obtained
1.	Pt ether	0.11%
2.	Ethyl acetate	0.23 %

3.	Chloroform	0.48 %
4.	Ethanol	0.63 %
5.	Aqueous extract	1.403 %

2.6 Preliminary Phytochemical analysis of different extract of *Ficus racemosa* leaves.

Preliminary testing's carried out for assessment of various chemicals like Flavonoids, steroids, quinones, Tannins, Phenol, and Glycoside etc.

Table 3. Preliminary Phytochemical screening of the leaves of *Ficus racemosa*

Phytochemicals	Extract				
	Pt. Ether	Methanol	Ethanol	Water	Ethyl acetate
Flavonoids	+	+	+	+	+
Quinones	-	+	+	+	_
Steroids	+	+	+	+	+
Tannins	+	+	+	+	_
saponins	+	+	_	-	_
Phenol	+	+	+	+	+
Glycoside	+	+	+	+	_

3.   Acute Toxicity

Animal studies were complying as per rule and regulation & guideline of CPCSEA with registration number 34140/Re Bi 5/99/CPCSEA. The study has been done for duration of 14 days for estimation of *Ficus racemosa* linn. Memory enhancement activity. Acute toxicity testing is carried out for checking the mortality in rats from different doses of extracts .

4.   MODEL USED (Pole climbing apparatus)

Pole climbing apparatus is used to check the memory & learning capacity of animals its experimental chamber (25×25×25 cm) measuring, with floor grid in a soundproof enclosure. It having a pole, 2.5 cm in diameter at the center of chamber associated with upper lid of chamber. (Cook L, Weidley E,1957)

Four rats were divided in to six groups each 150-180 gm, animals were kept in fasting condition overnight before the test but water was given.

Group of adult male albino rats 100-150 g each consisting of 6 animals was divided in to six group and animals are fasted overnight prior to the test but water was supplied. Before starting dosing schedule all groups of rats were trained so, they can fulfill active avoidance response.(**Vogel Gerhard H, Vogel Wolfgang H, 2002**) Active avoidance response was defined as climbing response <10 sec and escape response >10 sec 0.5 . The pole climbing apparatus having three types schemes causing stimulus.

❖   Light
❖   Buzzer
❖   Electric shock

Training of rats were done by placing the individual rats in chamber kept animal in chamber for 45 sec for being familiar with chamber, then after 45 sec starts to give stimulus .Firstly turn on the light for 2 sec followed by buzzer (conditional stimulus, CS) was sounded for 2 sec & electric shock (Unconditional stimulus, UCS 30 v, 0.5sec) over the floor grid . After that 180 sec rest was allowed.

 The procedure for giving conditional and unconditional stimulus should repeated until animals become familiar and respond for active avoidance response. Daily 10 trials should done for proper learning .The rats when served with scopolamine they take longer time to respond and more number of shocks while administration of herbal extract and standard drug Piracetam   increases the Ach level in brain results less time to respond and reduce number of shock.

Figure No.1 Pole climbing apparatus .

5.   Dosing schedule

Ficus racemosa extract were suspended in distilled water & Scopolamine and Piracetam were also freshly suspended in distilled water prior to administration .All the drugs were given by using oral cannula.

❖   Alzheimer's disease is induced by Scopolamine (1.0mg/kg p.o.) daily for 14 days.

❖   Different doses of herbal extract and Piracetam for 7 days i.e. on 8 [th] to 14 [th] days and by using pole climbing apparatus variables like number of shock and time spent in shock zone was noted .

❖   **Group 1**   : Rats were treated with Scopolamine alone (1.0 mg/kg p.o) daily for 14 days.

❖   **Group 2**   : Rats were treated with Piracetam (200 mg/kg, p.o) which served as standard from 8 [th] day.

❖   **Group 3**   : Rats were treated with aqueous extracts of *ficus racemosa* (150 mg/kg p.o) from 8 [th] day.

❖   **Group 4 :** Rats were treated with aqueous extracts of *ficus racemosa* (300 mg/kg p.o) from 8 [th] day.

❖   **Group 5**   : Rats were treated with aqueous extracts of *ficus racemosa* (400 mg/kg p.o). from 8 [th] day

❖   **Group 6**   : Normal group treated with distilled water till 14 days

6.   Determination of Acetylcholinestrase activity in rats

Decapitation of brain of rats of various six groups were done after 60 minutes of treatment of doses, brain are quickly placed in ice cold saline and brain parts are isolated such as frontal cortex , hippocampus and septum .

The tissue were homogenized in 0.1 M phosphate buffer (pH 8), 0.4 ml of homogenate added in cuvette containing 2.6 ml of phosphate buffer and 100 µml of DTNB mix it properly and take absorbance by photo electric colorimeter (H2) at 412 nm wavelength.20 ml of Acetylthiocholine is added and and change in absorbance is noted for period of 10 minute at interval of 2 minutes. (Milind Parle and Mani Vasudevan, 2007)

Calculations

The enzyme activity is calculated using the following formula

R= 5.74×10-4×A/CO

Where,

R= Rate in moles of substrate hydrolyzed /minutes/gm tissue

A=Change in absorbance /minutes

CO=original concentration of the tissue (mg/ml)

7.   Statistical Analysis

Memory enhancement activity

Statistical analysis by one way ANOVA followed by Dennett's multiple comparision tests using Graph pad instat 3.0 software $P<0.05*$, $P<0.01$.

Table no.4 Effect of *Ficus racemosa* various extract on Active Avoidance Learning and Retention in Rats (Mean ± SEM)

TREATMENT	NUMBER OF SHOCK			TIME SPENT IN SHOCK ZONE (IN SEC)		
	Learning (acquisition) 1st day	Relearning 15th day	Retaining 16th day	Learning (acquisition) 1st day	Relearnig 15th day	Retaining 16th day
Scopolamine (1mg/kg)	7.5 ±1.049	8.16 ±1.329	7.16 ±1.722	15.33 ±2.066	13.833 ±2.317	10.16 ±1.722
Piracetam 200 (mg/kg)	6.83 ±1.472	1.5 ±1.049	0.66 ±0.8165	12.166 ±2.401	4.33 ±1.506	1.833 ±0.7528
Lower dose (150 mg/kg)	1.66 ±0.8165	2.166 ±1.169	1.33 ±0.5164	4.166 ±1.472	5.166 ±1.169	3.33 ±1.366
Medium dose 300 (mg/kg)	1.0 ±0.00	2.0 ±0.8944	1.166 ±0.4082	4.66 ±1.751	3.5 ±1.378	3.0 ±1.414
Higher dose(400 mg/kg)	5.33 ±2.338	0.833 ±0.4082	0.5 ±0.5477	11.5 ±2.429	5.0 ±0.8944	1.66 ±0.8165
Normal Group (treated with Distilled water)	7.166 ±1.722	1.833 ±1.472	0.833 ±0.752	12.333 ±2.066	4.666 ±1.366	1.666 ±0.816

8.   RESULT

Result of Aqueous extract of Ficus racemosa leaves on Active Learning and Retention in Rat.

Through the study of various Aq. extracts of *F.racemosa* leaves & standard doses (Piracetam) of drug in different groups we obtained that successive reduction of time spent in shock zone and number of error.

9.   DISCUSSION

Alzheimer's disease is discriminated by gradual deterioration of brain cells and neurons which leads dropping of Ach level in various reasons of brain such as hippocampus, brain stem, cerebral cortex etc. Acetylcholine is the important neurotransmitter present in the brain due to lack of it causes memory loss .In AD condition the level of Ach reduces from normal level .Through the experimentation it have been shown that the plant which have antioxidant property, anti-inflammatory & anticholinestrase activity. After 100 % training of rats against acting for Conditional and unconditional stimulus helps to produce active avoidances response in animals. The impairment of learning and memory caused by scopolamine (1.0 mg/kg) an anti-

cholinergic agent was showed increase number of shocks and duration of time in shock zone while in other case extract of *Ficus racemosa* increases the level of acetylcholine by considerable reduction of cholinesterase activity in rat's brain and improve mood and learning capacity.

10. CONCLUSION

Alzheimer's disease is a dynamic neurodegenerative disease causing impairment of memory .The plants which having antioxidant property and anti-inflammatory property preferred for treatment of Alzheimer's disease. Hence *Ficus racemosa* is potent antioxidant and anti-inflammatory plant, its aqueous extract is responsible for increases the level of Ach and help in learning and memory improvement.

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