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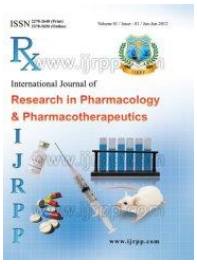

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Evaluation of alzhiemer's disease of *mimusops elengi linn*. in the experimental model of rats

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ABSTRACT

The present study has been undertaken to evaluate the possible role of *Mimusops elengi* Linn. flowers in experimental Alzheimer's disease (AD) in rats. Experimental AD in rats was produced by intra cerebro ventricular (i.c.v) administration of colchicine (Col). Various behavioural tests and biochemical analysis were performed to explore the possible role of the *Mimusops elengi* Linn. flower extract (ME)(100mg/kg & 200mg/kg doses) in AD. ME exhibited anxiolytic activity in Elevated plus maze test. In Morris' water maze test and Brightness discrimination test, ME pretreatments improved reference memory, working memory and spatial learning.ME significantly reduced the acetylcholinestarase. It reduced the Col induction increased lipid peroxidase activity, which was significantly reversed by ME (as seen from the reductions in the malondialdehyde level) and stabilized the rise in superoxide dismutase activity.ME might be effective in clinical AD by virtue of its cognition enhancement, anti-oxidant and antianxiety properties, which are the primary needs to be addressed in AD.

Keywords: Alzheimer′s disease, Anxiolytic activity, Colchicine, Rats, *Mimusops elengi* Linn.

INTRODUCTION [1-3]

The plant mimusops elengi linn(sapotacae) commonly known as bakul, madhugandha, chirapushpa, pagademara is highly reputed in traditional medicine as stomachic, astringent, ulemorrahgia. Seeds contain Saponins. The seed kernels yield 16-25% of a fatty oil, used for edible and lighting purpose.The composition of the total fatty acids of the oil is as follows: Palmitic10.97, Stearic-10.10, Behenic-0.46, Oleic-63.98, Linoleic-14.49%.Bassic acid ($C_{30}H_{46}O_5$),the characteristic Sapogenin of *sapotaceae* has been isolated from the fat-free seed meal in a yield of 2.4%.A Saponin,which on hydrolysis yields rhamnose (2mol.), arabinose(2mol.) and glucose (1mol.) has also been reported. The bark contains tannin, it is used in some parts of India for dying and tanning purposes.The bark and flowers are reported to contain a saponin and an alkaloid.

MATERIALS AND METHODS [4-10]

Collection and Authentication of Flowers

The flowers of Minusops elengi Linn. were collected from Nilgiri hills,Ooty, Tamilnadu and authentication(Voucher specimen number-PARC/2010/499) was done by Prof.P. Jayaraman, Ph.D. Plant Anatomy Research Centre, Medicinal plant Research Unit,Tambaram,Chennai-45.

Extraction of flowers of *mimusops elengi* linn

The collected flowers were cleaned, air dried at room temperature and ground to a coarse powder with an auto mix blender, passed through the sieve no:16 and stored in a deep freezer until the time for use. The powder was defatted with petroleum ether for 24 hours. Then, it was dried and cold macerated by using hydroalchoholic solvent(70% Ethanol and 30% Water) for about 5days[45].The obtained extract(ME) was concentrated under reduced pressure and controlled temperature by Rotary evaporator at 40^0c and stored in cool place

Experimental Animals

Thirty male Wistar rats, weighing 150-200 g were procured from King's Institute, Guindy. The animals were maintained in the animal house under standard laboratory conditions with natural dark and light cycle (approximately 12 h light / 12 h dark cycle) and room temperature (27 ± 1^0C) and constant humidity (60%) in accordance with Institutional Ethical Committee rules and regulations. They were fed on a standard balanced diet and provided with water *ad libitum.* The project proposal was approved by Institutional Animal Ethical committee (IAEC 75/2009).

Table No.1 Experimental Animals

Group	No. of Animals	Treatment
I	6	Normal control(Distilled water, p.o)
II	6	Control-colchicine(15µg/rat)(I.C.V.R)
III	6	Colchicine(15µg)+ ME(100mg/kg/day, p.o)
IV	6	Colchicine (15µg)+ME(200 mg/kg/day, p.o)
V	6	Colchicine(15µg)+Donepezil(1mg/kg/day,p.o)

EXPERIMENTAL DESIGN [11-18]

Induction of Alzhiemer's Disease with Colchicine

Colchicine will be administered via the intra cerebro ventricular route. The rats are anesthetised with Phenobarbital sodium and the right lateral ventricle will be cannulated and colchicine, dissolved in 5µl of artificial cerebrospinal fluid will be slowly injected into the cannulated ventricle using a 10µl Hamilton syringe. Control groups will be subjected to the same surgical procedure and received only artificial cerebrospinal fluid.

RESULTS

Preliminary Phytochemical Investigation

The revealed results of the preliminary phytochemical screening of the hydroalcoholic extract of dried flowers of *Mimusops elengi Linn.* Results were shown below. Table no: 1. The extract gave positive results for alkaloids and saponins.

Table No: 2 Preliminary phytochemical test for ME

SL.No.	Phytochemical Tests	Results	SL. No.	Phytochemical Tests	Results
1	Test for Alkaloids	+Ve	7	Test for Flavonoids	-Ve
2	Test for Carbohydrates	-Ve	8	Test for Gums and mucilage	-Ve
3	Test for Proteins	-Ve	9	Test for Glycosides	-Ve
4	Test for Steroids	-Ve	10	Test for Saponins	+Ve
5	Test for Sterols	-Ve	11	Test for Terpenes	-Ve
6	Test for Phenols	-Ve			

*+Ve: indicates the presence of compounds *-Ve: indicates the absence of compounds

Table No: 3 Effect of ME on Elevated plus maze

Group	Time spent					
	Day 7		Day 14		Day 28	
	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm
I Normal	44.2±4.02**	193.12±23.18*	47.19±2.34*	192.00±23.19*	44.12±3.45*	193.23±34.56*

II Col-control	27.12±1.69	203.18±21.23	28.76±2.38	43.12±2.56	27.34±2.45	225±24.36
III ME100mg/kg	34.67±2.37*	187.32±21.45*	37.54±3.46*	198.13±1.98*	21.35±1.67*	213.78±32.13*
IV ME200mg/kg	41.23±4.38*	201.56±21.34*	44.34+2.48*	201.67±12.78**	23.56±3.89*	203.13±22.54*
V Std-Donepezil	16.38±2.06**	239.54±23.58*	15.48±4.37*	245.12±13.67**	17.45±1.63*	248.12±43.21*

Group	Number of entries					
	Day 7		Day 14		Day 28	
	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm
I Normal	7.13±0.65**	3.21±0.79**	6.12±0.88*	4.58±0.75**	8.06±1.39**	3.00±0.73**
II Col-control	5.12±0.60	6.98±0.78	5.76±0.73	0.63±0.56	3.31±0.76	7.12±0.06
III ME100mg/kg	8.87±0.45**	2.69±0.64**	7.98±0.76*	0.14±0.04*	0.09±0.01**	3.21±0.60**
IV ME 200mg/kg	10.37±0.97**	3.42±0.42**	9.00±0.73**	0.33±0.51*	0.17±0.70**	4.10±0.49**
V Std-Donepezil	4.12±0.51*	8.02±0.42*	3.17±0.44**	9.10±0.60**	32.74±0.60**	9.83±0.06**

Values are expressed as mean±SEM of 6 animals, *P<0.05, **P<0.01 vs Col-control (group II). Comparisons were made between Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test.

Table No: 4 Effect of ME on Radial Y-maze

Group	Acquisation					
	No. of trials			Latency period(sec)		
	Day7	Day14	Day28	Day7	Day14	Day28
I Normal	7.02±0.49*	7.78±0.01*	8.32±0.19**	100.43±2.875*	102.65±5.453*	103.50±3.890*
II Col-control	8.00±0.83	8.43±0.43	9.00±0.49	105.27±1.763	106.54±4.675	107.00±4.540
III ME (100mg/kg)	7.38±0.64*	7.45±0.51**	8.38±0.34*	137.98±14.29**	139.49±14.85**	142.87±16.68**
IV ME (200mg/kg)	6.69±0.38**	6.90±0.54**	7.19±0.98**	87.12±6.324**	88.59±6.596**	90.76±7.654**
V Std-Donepezil	7.52±0.85*	7.90±0.74*	8.02+0.85**	95.87±10.87**	96.12±12.87**	98.37±12.348*

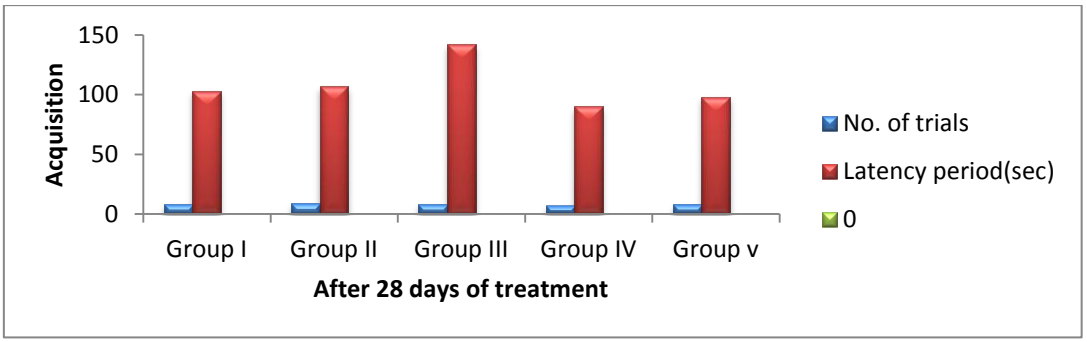

Figure -1 After 28 days of treatment

Values are expressed as mean±SEM of 6 animals, *P<0.05, **P<0.01 vs Col-control (group II). Comparisons were made between Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test

Table No: 5 Effect of ME on Conditioned avoidance response

Group		Number of escape failures		
		Day 7	Day 14	Day 28
I	Normal	4.5±0.32**	3.1±0.23**	1.9±0.31**
II	Col-control	7.5±0.15	8.4±0.41	9.3±0.15
III	ME(100mg/kg)	6.5±0.71*	5.2±0.33*	4.1±0.84**
IV	ME(200mg/kg)	5.3±0.37**	4.1±0.23**	2.5±0.51**
V	Std-Donepezil	4.9±0.53**	3.6±0.62**	2.2±0.82**

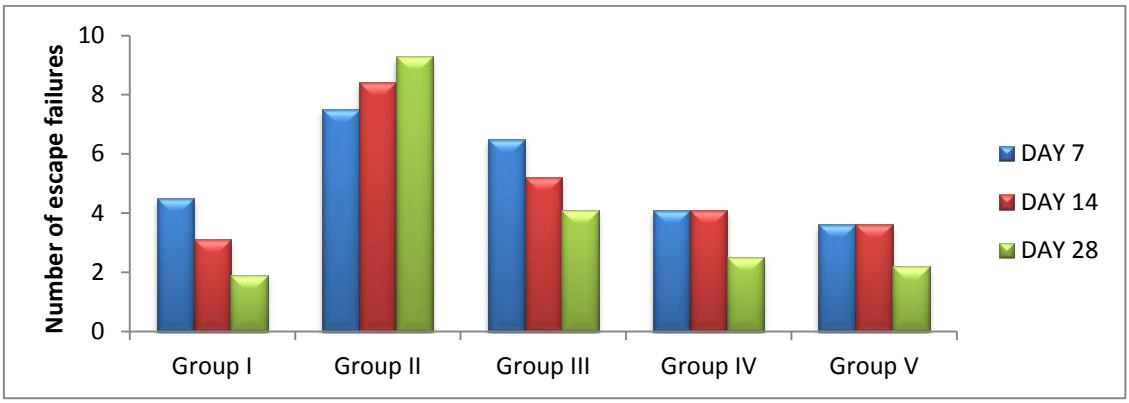

Figure -2 After 28 days of treatment

Values are expressed as mean±SEM of 6 animals, *P<0.05, **P<0.01 vs Col-control(group II). Comparisons were made between Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test.

Table No: 6 Effect of ME on Water maze

Group	Day 7			14th day			Day 28		
	E.L-1 Probe trial	E.L-2 New platform	E.L-3	E.L-1 Probe trial	E.L-2 New platform	E.L-3	E.L-1 probe trial	E.L-2 New platform	E.L-3
I Normal	54.20±1.5**	29.40±1.7**	14.50±2.1**	32.70±1.5**	15.20±1.2**	49.80±2.2**	29.20±1.7**	19.21±1.5+**	27.70±2.3**	11.10±1.3*	51.20±1.2**	31.67±2.1**	18.21±1.5**	22.40±2.5**	13.42±1.7**
II Col-control	58.40±2.1	44.50±2.6	24.67±1.2	15.42±1.7	23.42±2.1	65.33±2.1	43.17±2.8	32.19±1.3	13.25±1.9	26.70±2.8	68.20±1.2	48.37±2.4	38.77±1.4	33.40±1.9	27.22±2.4
III ME1	42.33±0.7**	18.15±1.2**	10.20±1.1**	20.40±1.6+**	10.15±1.7**	29.12±1.4**	9.77±1.2**	9.85±0.6+**	13.15±0.6**	8.20±1.2+*	24.20±0.8**	12.71±0.6**	9.40±1.2**	13.77±0.9**	9.20±1.8**
IV ME2	59.70±1.2**	27.33±1.2**	16.40±1.7**	31.17±2.1+**	16.33±1.8**	54.20±1.3**	22.33±0.9*	17.83±1.4**	26.77±0.7**	15.33±0.8*	52.20±1.2*	26.17±1.4**	21.33±2.2**	25.17±1.5**	14.22±2.6**
V Col-Doz	56.33±2.1**	24.22±1.4**	18.40±2.6**	29.17±1.3**	12.35±2.3**	55.22±2.1**	26.17±1.8**	25.40±2.6**	34.17±0.7**	15.85±1.5**	58.17±1.4**	25.77±2.1**	28.40±1.3**	24.21±1.5**	17.28±2.1**

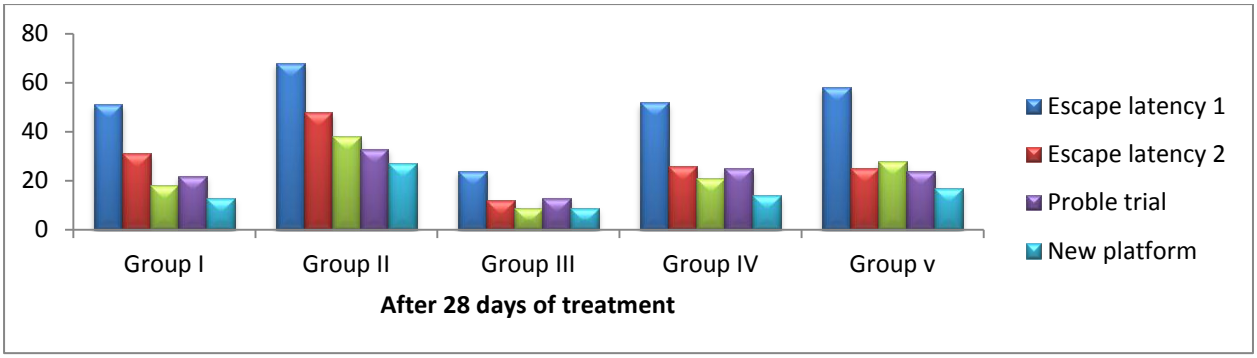

Figure -3 After 28 days of treatment

Values are expressed as mean±SEM of 6 animals, $^{*}P<0.05$ $^{**}P<0.01$ vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dennett's test.

Table No: 7 Effect of ME on acetylcholinestarase (AChE) (µ moles/min/mg protein)

Group		Reaction time(sec)			Relearning Index %		
		Day 7	Day 14	Day28	Day 7	Day14	Day28
I	Normal	6.8±0.8**	7.3±0.6**	8.8±0.4**	58.0±3.0**	70.0±5.0**	83.0±7.0**
II	Col- Control	3.2±0.6	3.8±0.3	3.9±0.3	32.0±2.0	36.0±4.0	37.0±1.0
III	ME 100mg/kg	4.3±0.2*	4.7±0.1*	4.8±0.4*	42.0±3.0**	41.0±4.0*	39.0±5.0*
IV	ME200mg/kg	5.7±0.1**	7.2±0.4**	8.0±0.1**	51.0±4.0**	48.0±4.0**	46.0±1.0**
V	Std- Donepezil	7.8±0.3**	9.6±0.6**	11.2±1.0**	56.0±5.0**	53.0±5.0**	51.0±1.0**

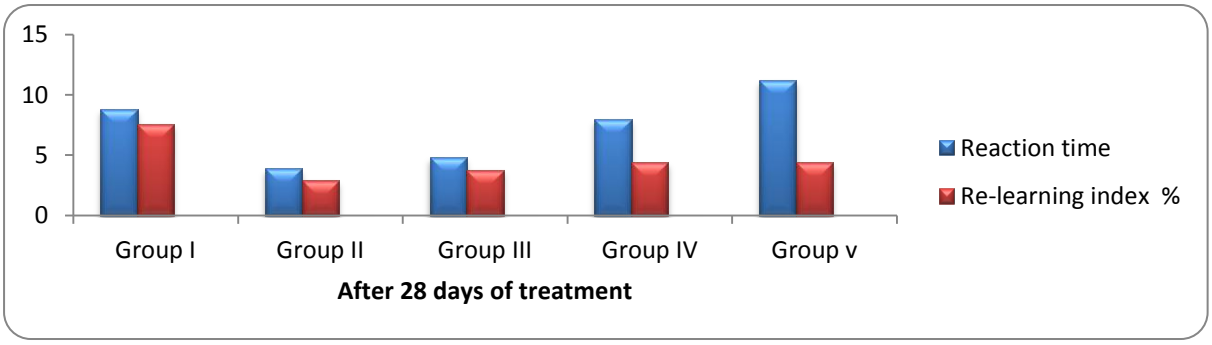

Figure -4 After 28 days of treatment

Values are expressed as mean±SEM of 6 animals $^{**}P<0.01$ vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test

Table No: 8 Effect of ME on acetyl cholinestarase (AChE) (µmoles/min/mg protein)

Group	7^{th} day	14^{th} day	28^{th} day
I Normal	35.13±3.65**	36.13±4.22**	40.09±3.12**
II Col-control	10.01±2.35	12.56±3.65	13.96±4.59
III ME(100mg/kg)	21.67±4.56**	26.34±3.89**	21.76±4.87**
IV ME (200mg/kg)	23.5±3.54**	29.38±5.43**	22.87±6.45**
V Std-Donepezil	29.44±4.21**	30.09±7.12**	27.13+5.34**

Values are expressed as mean±SEM of 6 animals**P<0.01 vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test.

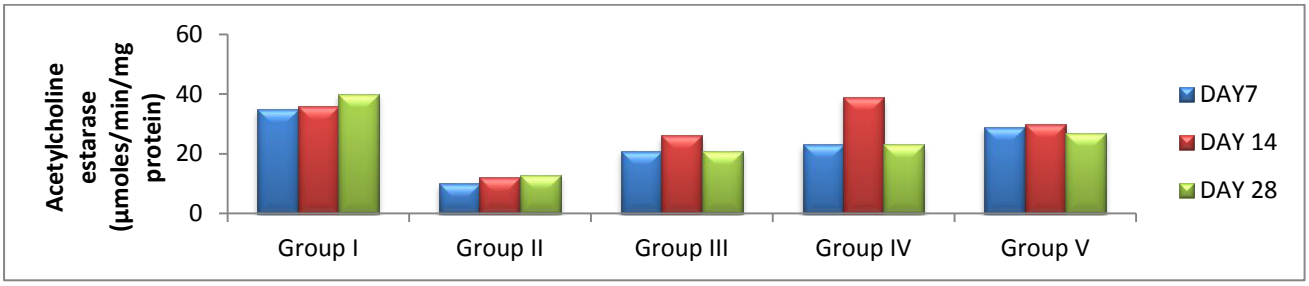

Figure –5 After 28 days of treatment

Table No: 9 Effect of ME on Lipid peroxidation (LPO) in Brain tissue (units /min /mg protein)

	Group	7th day	14th day	28th day
I	Normal	2.50±0.15**	2.1±0.301**	2.4±0.32**
II	Col-control	5.3±0.36	5.7±0.52	5.56±0.22
III	ME (100mg/kg)	4.2±0.09*	4.5±0.12*	4.33±0.21*
IV	ME (200mg/kg)	3.0±0.15*	3.2±0.22**	3.18±0.31**
V	Std-Donepezil	2.8±0.35**	2.5±0.18**	2.64±0.22**

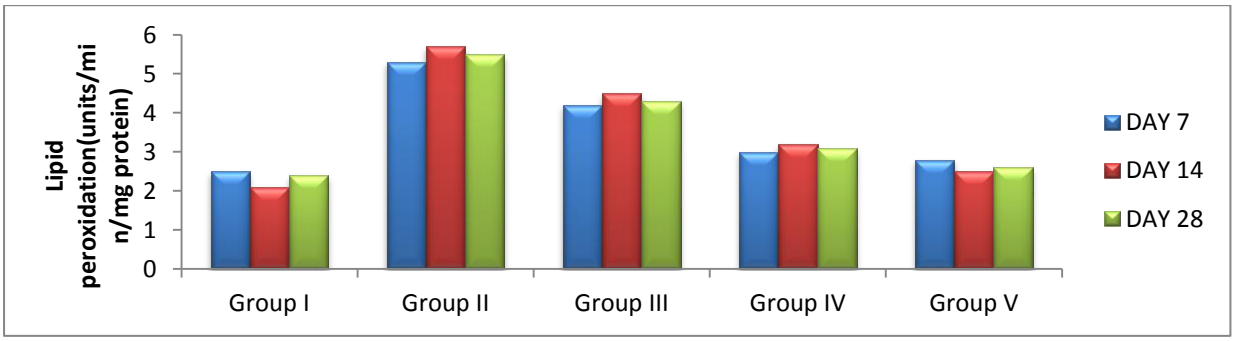

Figure -6 After 28 days of treatment

Table No: 10 Effect of ME on Superoxide dismutase (SOD) (units/min/mg protein)

	Group	7th day	14th day	28th day
I	Normal	7.39±0.03**	7.87±0.01**	8.08±0.53**
II	Col-control	4.39±0.01	4.98±0.05	5.36±0.09
III	ME (100mg/kg)	5.18±0.01*	5.91±0/02*	6.03±0.01*
IV	ME (200mg/kg)	6.72±0.04*	6.88±0.04**	7.96±0.43*
V	Std-Donepezil	7.87±0.05**	7.93±0.06**	8.36±0.07**

Values are expressed as mean±SEM of 6 animals,*P<0.05, **P<0.01 vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test.

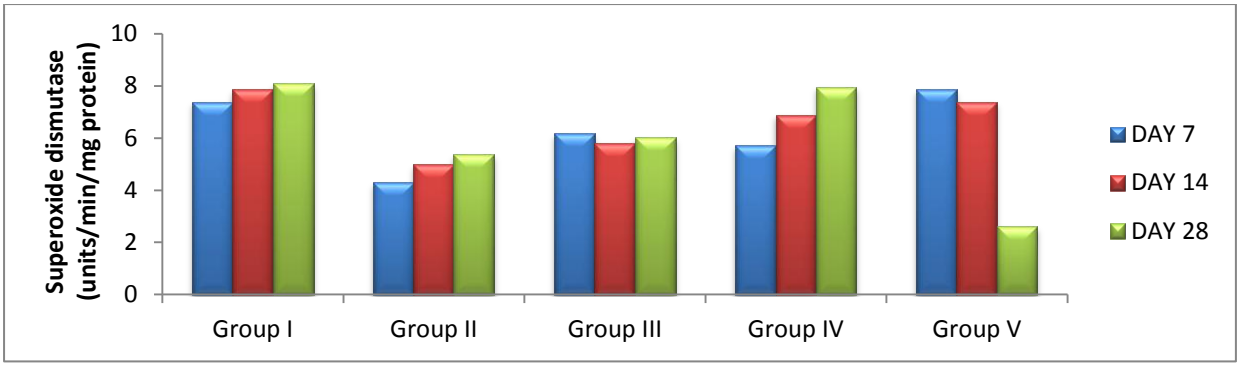

Figure -7 After 28 days of treatment

Table No: 11 Effect of ME on Glutathione peroxidase (GPx) (units/min/mg protein)

	Groups	7th day	14th day	28th day
I	Normal	34.37±1.63**	34.31±1.06**	35.15±1.01**
II	Col-control	21.81±1.28	21.11±1.34	22.21±1.07
III	ME (100mg/kg)	25.31±1.31*	25.83±1.03*	26.13±0.63*
IV	ME (200mg/kg)	30.81±1.06*	31.91±1.51*	32.31±1.08**
V	Std-Donepezil	32.66±1.91**	33.58±1.73**	34.31±1.09**

Values are expressed as mean±SEM of 6 animals, *P<0.05**P<0.01 vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200 mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dennett's test.

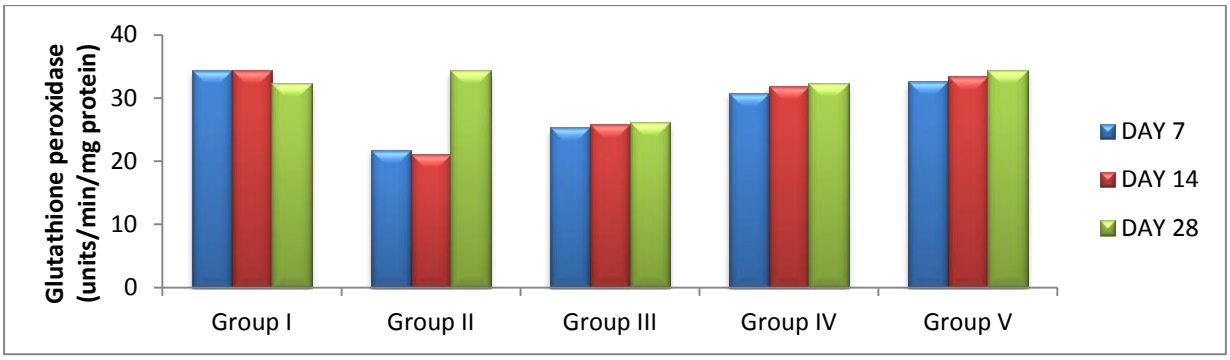

Figure -8 After 28 days of treatment

Table No: 11 Effect of ME on Reduced Glutathione (units/min/mg protein)

	Group	7th day	14th day	28th day
I	Normal	7.40±0.51**	7.03±0.63**	8.05±0.41**
II	Col-control	4.96±0.49	4.87±0.37	5.01±0.78
III	ME (100mg/kg)	6.51±0.53*	5.36±0.45*	6.03±0.03*
IV	ME (200mg/kg)	5.01±0.31*	6.82±0.26**	7.63±0.36*
V	Std-Donepezil	7.07±0.06**	7.08±0.04**	8.77±0.73**

Values are expressed as mean±SEM of 6 animals, *P<0.05, **P<0.01 vs Col-control (group II). Comparisons were made between: Col-control group with Normal, ME (100mg/kg), ME (200mg/kg) and Std-Donepezil groups. Symbol represents the statistical significance done by ANOVA, followed by Dunnett's test.

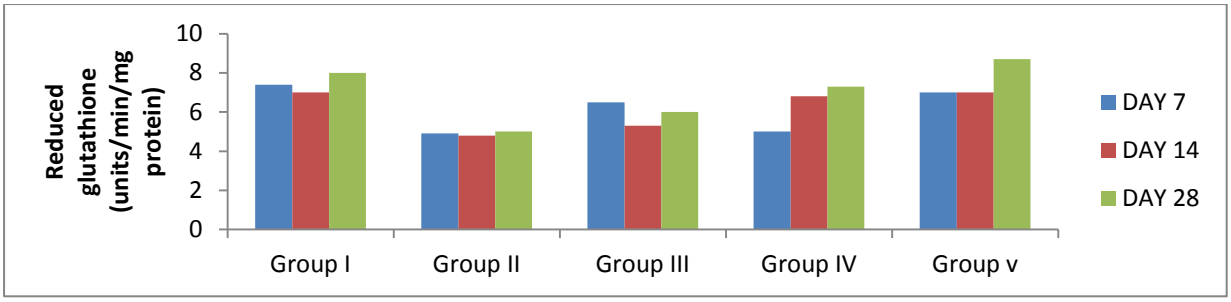

Figure -9 After 28 days of treatment

SUMMARY

The preliminary phytochemical screening of ME shows the presence of various phytochemical constituents like alkaloids, saponins, carbohydrates and tannins. The neuro protective effect of ME was assessed by Elevated plus maze, Y-maze, Conditioned avoidance response, Water maze on which it showed considerable attentive effect. In elevated plus maze test, ME significantly reversed the decrease in open arm to closed arm ration induced by colchicines indicating the anxiolytic activity. In Y-Maze test, after the injection of ME, the spontaneous alteration percentage was found to be improved in 200mg/kg treated group.ME administration improves the memory deficits in the active avoidance task as it reverses the increased escape latencies with colchicines. In Water maze test, the impaired learning by colchicines and the improvement of learning by ME after injection shows the significant property of memory retention which indicates the rejuvenation potential of the extract. The time required to escape on the platform is decreased on this task indicating the hippocampal learning ability of the extract. ME improves the memory and learning of animals in Brightness discrimination test by increasing reference time and re-learning index values. The biochemical changes responsible for the cognitive impairment were assessed by the estimation of acetylcholine esterase and antioxidant enzymes. The study against the colchicines induced alzhiemer's by the treatment of ME shows significant reduction in the activity of acetylcholine esterase. The antioxidant value of ME shows the regaining of the antioxidant enzymes SOD, GSH and GPx activity, it has been noted that the antioxidant properties of extract delays the generation of free radical and also showed the reversal of the decreased antioxidant enzyme levels after the memory impairment. There was a decrease in the Lipid peroxidation levels which were alleviated by colchicine treatment.

CONCLUSION

Mimusops elengi Linn is being used in traditional medicine as a antioxidant of CNS associated disorders, still there are some scientific evolutions to been made. Hence this study is emphasized to make the evident effect of the whole plant on memory disorder representing Alzhiemer's disease. The investigation was carried out on cognitive impairment due to colchicine induced impaired behavioural performace and oxidative stress. In Elevated plus maze test and Y-maze test, ME at both 100mg/kg and200mg/kg exhibited significant improvement than the colchicine group of animals. In conditioned avoidance response task and in Brightness discrimination test, ME at both doses indicated the improvement of memory and learning. The spatial learning in Water maze task showed the significant memory retention indicated by the decrease in escape latency at both dose levels.ME treatment had shown the significant reduction in the elevated enzyme levels of acetylcholine esterase which indicates the potential to increase cognitive function through the decreased degradation of acetyl choline. The antioxidant levels were also proved to be restored on ME treatment as there was an increase in SOD, GSH and GPx levels by decreasing the LPO level. In conclusion, the neuroprotective activity of the flowers of the plant *Mimusops elengi Linn.* On alzhiemer's type of dementia may be due to the inhibiting activity against AChE, free radical scavenging activity and they can be expected to be a pivot sense in neurotoxiciry.

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