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Research Article

FORMULATION AND EVALUATION OF HERBAL ANTIDEMENTIAL TABLETS

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ABSTRACT

Herbs and their extracts/fractions are used for the treatment of different diseases and few as a memory enhancing agent. Different plants have been used as a memory enhancer in the folkloric medicine. Present study was undertaken to prepare a combined herbal formulation of the extracts of dried root powder of *Plumbagozeylanica* and powdered seeds of *Silybummarianum* for the treatment of dementia. HPTLC quantification characterization and identification of the herbal extracts of *P. zeylanica* and *S. marianum*, was performed using the standard marker compound, plumbagin and silibinin respectively of these drugs. Through the acute oral toxicity studies the dose of the extract in the formulation and a LD^{50} and ED^{50} profile of the drug combination were established. Design & development of tablet using two doses of the extract was done and evaluation was carried out on various standard parameters of formulation including accelerated stability tests, which was in turn found satisfactory and within the specified limits. Preliminary pharmacological screening of dementia was performed on formulated tablets using EPM (Elevated plus maze) and MWM (Morris water maze), two of the animal models of dementia. Here, Piracetam (400mg/kg *p.o*) a nootropic agent was employed as a standard drug. Sodium Nitrite (75mg/kg *i.p*) was used to induce amnesia in young experimental model, and which is comparable with that of the age related amnesia in old rats. The results indicate that administration of tablets produce significant dose dependant improvement of memory and were almost similar with that of standard drug Piracetam. In the present study antidemential activity of the combined herbal formulation of *P. zeylanica* and *S. marianum* and a few standardization parameters were tried to established, which can be further analysed and proceeded to prepare an effective antidemential formulation.

Key words: *P. zeylanica, S. marianum,* Dementia, Herbal formulation, HPTLC,

INTRODUCTION

The occurrence of dementia and age related brain disorders are dramatically on the rise as life expectancy likewise increases. Dementia is the progressive deterioration in cognitive function - the ability to process thought (intelligence). Progressive means the symptoms will gradually get worse. The deterioration is more than might be expected from normal aging and is due to damage or disease. Damage could be due to a stroke, while an example of a disease might be Alzheimer's.[1] The significance of Alzheimer's disease is further compounded as the number of identified cases is esteemed to double or triple by 2050.[2] A lot of research is going on constantly to identify a moiety to improve learning and memory. Since there is lack of satisfactory drugs in allopathic system of medicine, as a consequence it is very important to further investigate multi-targeted and low toxicity antidemential drugs. Practically all major research involves the search for new antidemential formulation of synthetic origin. Very little attention has been directed towards the plant kingdom. Through the ages, many medicinal herbs have been used to improve memory and cognitive function and to treat neurodegenerative diseases in traditional medicine.[3]Hence, there is an urgent need for search of suitable plant materials from indigenous system with scientific validation which could be effectively used as memory enhancer. There are evidences to suggest that single herbs or herbal formulations may offer certain complimentary cognitive benefits to the approved drugs. Pharmacological effects of some plants have also been reported.[4] Here, two of such plants are studied for their antidemential effects, *P.zeylanica* as Chitraka, or popular names of "Lead wort-white flowered" and "Ceylon Lead wort" of the family Plumbaginaceae, is distributed as a weed in throughout the tropical and subtropical countries of the world Earlier chemical examination of this plant revealed that the root contains plumbagin, 3-chloroplumbagin, 2,3-biplumbagin, 6,6- biplumbagin, zeylinone, isozeylinone, chitranone, droserone, plumbagic acid, plumbazeylanone, glucose, fructose, enzymes as protease and invertase.[5] and *S.marianum* (L.) Gaertn.familyAstereaceae is grown commercially for seed in Europe, Egypt, China, and Argentina.Silymarin consists of four flavonolignan isomers namely-silybin, isosilybin, silydianin and silychristin. Among them, silybinin being the most active and commonly used. In addition, milk thistle contains apigenin; silybonol; myristic, oliec, palmitic and stearic acids; and betaine hydrochloride.[6, 7] These plants were selected following their traditional uses and various pharmacological activities like hepatoprotective, abortifacient, antimicrobial, CNS stimulant, anticancer; and treating leukoderma on *P.zeylanica* and antineoplastic, hepatoprotective, galantogogue, antibacterial and antidepressant effects of*S.marianum*. The present study was designed to investigate the evaluation parameters and antidemential effect of the developed combined herbal Tablet formulation.

MATERIAL AND METHODS

Powdered root of *P. zeylanica* and *S. marianum* seed powder was purchased from an authenticated Ayurvedic store of Bareilly, U.P,India.

Preparation of extracts

The preliminary phytochemical screening of the plant involves extraction of the plant material and identification of the plant active constituents.[8, 9, and 10]

500 gram seed powder of the crude drug *Silybummarianum*was taken and extracted by continuous hot percolation process using Soxhlet apparatus firstly with petroleum ether to remove the fatty material than directly with the ethanol to obtain ethanolic extract of the same, as most of the active constituents of the drug are reported in the ethanolic extract which are further responsible for the pharmacological efficacy. Similarly, Powdered root of *P. zeylanica* extracted firstly with petroleum ether using Soxhlet apparatus to remove the fatty constituents and then with the chloroform to obtain a chloroform extract, in which its maximum active constituents are present.

HPTLC quantification of the extracts

Reference standard plumbagin and silibinin were obtained from Sigma-Aldrich Chemicals Pvt Ltd., New Delhi, India. All reagents: toluene, n-Hexane, Ethyl Acetate, Formic acid, Chloroform and Ethanol (Merck Chemicals, India) were of Analytical grade. HPTLC Quantification was done by chromatography analysis.[11, 12, 13, 14]

Formulation and evaluation of tablet

The plant extracts were mixed with the excipients and compressed into tablets. The details of the composition was given Table no: 1 and 2.

The prepared tablets were evaluated for weight variation, hardness, friability, disintegration time and stability studies. In weight variation study, twenty tablets were selected at a random and average weight was calculated. Then individual tablets were weighed and weight was compared with an average weight. The

Pfizer hardness tester was used for the determination of the hardness of tablets. Tablets were placed in contact between the plungers, and the handle was pressed, the force of the fracture was recorded. The friability of tablets was determined using Roche friabilator (Cambel Electronics, Mumbai,India). Six tablets were tested from each formulation. In the disintegration time study tablets was put into 100 ml distilled water at 37 ± 2 ºc. Time required for complete dispersion of a tablet was measured with the help of digital tablet disintegration test apparatus . The stability study of the tablets was carried out according to International conference on Harmonization guidelines (ICH guidelines). The formulations were stored at 45 ± 2 ° c /75 ± 5 %RH for 1 month by storing the samples in stability chamber (Lab care Mumbai India). [15, 16, 17]

Table 1: Formulation of tablet of 500 mg

Batch no.	Quantity per tablet (mg)						Weight of tablet (mg)
	Methyl Parabe-n (%)	Lactose (mg)	*P.zeylanica* extract (mg)	*S.marianum* extract (mg)	10% HPMC sol. in ethanol	Magnesiun stearate(4% of the granule wt)	
T_1	0.1	90	330	70	q.s in ml	10	500
T_2	0.1	85	330	70	q.s in ml	15	500
T_3	0.1	80	330	70	q.s in ml	20	500

Table 2: Formulation of tablet of 1000 mg

Batch no.	Quantity per tablet (mg)						Weight of tablet (mg)
	Methyl Parabe-n (%)	Lactose (mg)	*P.zeylanica* extract (mg)	*S.marianum* extract (mg)	10% HPMC sol. in ethanol	Magnesiun stearate(4% of the granule wt)	
T_1	0.1	180	660	140	q.s in ml	20	1000
T_2	0.1	170	660	140	q.s in ml	30	1000
T_3	0.1	160	660	140	q.s in ml	40	1000

Table 3: Evaluation parameters of the granules

Parameters	Batch code. Of tablet					
	S_1	S_2	S_3	T_1	T_2	T_3
Bulk Density (gm/ml)	0.42	0.44	0.46	0.41	0.44	0.45
Tapped Density (gm/ml)	0.50	0.51	0.53	0.51	0.52	0.54
Carr's Index (%)	14	16.8	17	13.4	15.07	17.64
Huasner's Ratio	1.08	1.13	1.20	1.11	1.13	1.15
Angle of Repose (^{0})	32.20	32.00	33.80	31.79	27.47	30.96
Moisture content	3	3.5	3	3	3	3.2

Table: 4 Evaluation parameters of the tablet

Parameters	Batch code of tablet					
	S_1	S_2	S_3	T_1	T_2	T_3
Weight variation (%)	2.22	2.53	2.86	2.60	2.73	2.81
Hardness (kg/cm2)	4.40	4.50	4.30	4.10	4.30	4.20
Thickness (mm)	3.80	4.00	3.90	5.00	5.20	5.10
Friability (%)	0.46	0.44	0.43	0.48	0.45	0.44
Disintegration (minutes)	12.45	13.20	14.10	12.80	13.00	13.50

Preliminary pharmacological evaluation

Male and female Wister albino rats (150 – 200 gm) and mice obtained from the animal house of the College of Pharmacy, I.F.T.M, Moradabad (U.P) were used for the study. The animals were housed in polypropylene cages. They were maintained for 12 hours in light and dark cycle at 28 ± 2^{0}C in a well-ventilated house, with free access to tap water and laboratory diet. They were acclimatized to laboratory conditions for 10 days prior to commencement of the experiment. All the guidelines for the care and use of the animals (National Institute of Health, USA) were followed during the experiments and were approved by the Institutional Animal Ethical Committee (IAEC), Registration no: 837/ac/04/CPCSEA).

Piracetam (400 mg/kg) under the brand name "Pirament" of the Ipca laboratories Ltd. Dehradun was used as standard antidemential drug, to compare with the activity of polyherbal tablet of the two drug extracts on different learning and memory paradigms. [18]Powdered tablets, of standard and test drug were suspended in 0.3% carboxymethylcellulose (CMC) in distilled water separately in required doses and were administered orally in appropriate animal groups.

Acquisition and retrieval memory in Elevated plus maze (EPM)

The EPM apparatus used in the study was made up of plywood, consisted of two open (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) facing each other with an open roof. The maze was elevated at a height of 25 cm from the ground. Transfer Latency (TL) was recorded respectively. And the activity was performed as follows; [19, 20]

In young rats

Rats were divided into 5 groups of 6 animals each. The total treatments period was 15 days in which Group 1and 2 animals served as control and received 0.3 % CMC in distilled water (10 ml/kg, *p.o.*). Group 3 animals received dose of the suspension of tablet (*P. zeylanica* and *S. marianum*) of 500 mg/kg, and group 4 animals received dose 1000 mg/kg body weight orally for comparison. Group 5 was fed orally with Piracetam (400 mg/kg). All the groups of animals except group 1 were injected with sodium nitrite (75 mg/kg i.p.) after 30 min of the drug treatment on 15th day. The procedure, technique and end point for testing memory were followed using the parameters described as similar to the earlier investigators. The animals were placed individually 90 min after of above treatment at the end of open arm facing away from central platform and the time it took to move from open arm to either enclosed arm (TL) was recorded on the 15th day of treatment (training session). The TL was again recorded 24 hr after 1st exposure (i.e. on 16th day). The TL measure on 1st and 2nd exposure served as parameter for acquisition and retrieval memory respectively [20].

In aged rats

Rats were divided into 4 groups of 6 animals each and the rats of age more than 6 month were used in all groups except control, as reduction in memory is an age related factor. All the rats were treated for a period of 15 days in which Group 1 animals served as control and received 0.3 % CMC in distilled water (10 ml/kg, p.o.). Group 2 animals received dose of the suspension of test drug tablet (*P. zeylanica* and *S. marianum*) of 500 mg/kg, and 3 animals received dose 1000 mg/kg body weight orally for comparison. Group 4 was fed orally with standard drug Piracetam (400 mg/kg) orally as above. TL was recorded on the 15th day after 60 min of the treatment (training session). The TL was again recorded 24 hr after 1st exposure (i.e. on 16th day). The TL measure on 1st and 2nd exposure served as parameter for acquisition and retrieval memory respectively.

Spatial learning in Morris water maze (MWM)

The apparatus used is a circular plastic water tank (100 cm in diameter) filled to a depth of 30 cm with water (25^{0}C). Four points equally distributed along the perimeter of the tank served as starting locations. The tank was divided arbitrarily into four equal quadrants and a small platform (5 cm width) was located in the centre of one of the quadrants. The platform remained in the same position during the training days. And the activity on Morris water maze (MWM) apparatus was performed as follows. [18, 20]

Mice were grouped as 6 animals each in 4 groups, Group 1 animal served as control and received vehicle only. Group 2 animals received dose of the suspension of tablet (*P. zeylanica* and *S. marianum*) 500 mg/kg, and 3 animals received dose 1000 mg/kg body weight orally. Groups 4 received Piracetam (400 mg/kg) orally. The mice were released into the water and allowed 90 s to find the platform. Animals received 4 trials for the first day and 8 trials per day with 5 min inter-trial interval for 8 days until the performance was stable and the latency to find the platform was low (<10 sec). The test formulations were administered 30 min prior to the first trial daily. Time to find the hidden platform is considered as escape latency.

Statistical analysis: The data were initially analyzed by a one-way analysis of variance

(ANOVA), which was followed by the Dunnett's t-test.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical evaluation shows the presence of Triterpenes,FlavanoidsSteroids, Tannins and Phenolic compounds, in chloroform extract of*P. zeylanica.* And in ethanolicextract of*S. marianum*Glycosides, Triterpenes, Alkaloids, Tannins, Flavanoid, Phenolic compound and Steroids were found. Therefore these extracts were selected for the formulation of the tablets.

HPTLC quantification

Plumbagin was found to be 0.004836 µg/µg in the chloroform extract of powdered roots of *P. zeylanica* and silibinin was found to be 0.027685 µg/µg in the prepared ethanolic extract of the *P. zeylanica*, by performing HPTLC quantification analysis. Hence, the active constituent in the *S. marianum* extract was approximately 5.7 times of the active constituent in the *P. zeylanica.*

Acute oral toxicity studies

The aim to perform acute toxicity studies was for establishing the therapeutic index of a particular drug and to ensure the safety in-vivo. The LD_{50} determination was done in mice by OECD guideline 423. As all the doses till 4000 mg/kg were found safe and no lethal effect was observed. Therefore, any dose can be selected up to 4000 mg/kg; hence, 1/10th and 1/5th of the dose i.e. 400 mg/kg and 800mg/kg were taken as ED_{50} in the present study.

Evaluation of prepared granules and tablets

The results obtained on various parameters of preformulation studies of granules were found satisfactory. The granules obtained for the trial batches (S_1-S_3, T_1-T_3) were satisfactory. No rat holing was observed during the flow of granules from the hopper. Capping and sticking was not observed. From the compressibility index and Hausner's ratio values obtained for granules of all the batches, granules were found to have good flow properties. The prepared tablets were spherical, brownish coloured with smooth surface having acceptable elegance. (Table-3) The maximum weight variation of the tablets was ± 2.86%, which falls within the acceptable weight variation range of ± 5%, hence the tablets of all batch passed the weight variation test. Hardness for tablets of all batches was in the range of 4.1 to 4.5 kg/cm^2, which falls above the limit of not less than 3.0 kg/cm^2. Friability value for tablets of none of the batch was more than 0.48%. The thickness of the tablets of all the batches was found in the range of 3.8 - 4.0 mm indicating fairly acceptable tablets. Disintegration time is an important parameter of tablet. As an ideal tablet should disintegrate within 15 min, the tablets of all the batches disintegrated within 14 minutes 10 seconds (Table-4). Stability studies carried out on the final formulation T_1 show no significant change in the physical parameters. There was a marginal increase of moisture content and hardness, while no change in the friability was found, showing that these changes were within the specified limits (Table-5).

Pharmacological evaluation of the tablet

Acquisition and retrieval memory in elevated plus maze.

Sodium Nitrite produced a significant ($p < 0.01$) increase in TL on day 1st compare to control indicating impairment of memory. Sodium Nitrite induced increased in TL was however significantly ($p < 0.01$) reversed by dose dependant 15 days prior administration with extract which were comparable to standard drug Piracetam (400 mg/kg) indicating that it improved the learning and memory of rats (Table 6, 7 and Figure 1, 2).

Spatial learning in water- maze.

In this learning task, Escape latency (EL) was significantly reduced during 8 days trials after the test drug administration compared to control, and was comparable with that of the standard, indicating the tablet improve the learning and memory of rats in a dose dependent manner (Figure 3), though the effect was not significant statistically in 500 mg tablet and 1000 mg tablet treated group on 6th, 7th, 8th day and on 7th, 8th day in Piracetam treated group. (Table 8, Figure 4,5).

CONCLUSION

Herbal products may contain a single herb or combination of several herbs believed to have complimentary and/or synergistic effects. Herbal products are sold as either raw plants or extracts of portion the plant.

The present study was an attempt to investigate the antidemential effects of the plants *P. zeylanica* and *S. marianum*. It deals with the formulation and evaluation of the tablet made from chloroform extract of roots of *P. zeylanica* and ethanolic extract of seeds of *S. marianum* drugs with synergistic activity and reduced side effects. Three batches of the tablets were prepared of the both the two doses of drug. From these, S_1 batch of lower dose and T_1 batch of higher dose was found to be the best formulation in terms of disintegration time taken. And therefore this formulation was selected for performing the anidemential activity. Hence this investigation gave a support on the selected medicinal plants which ascertain its folklore uses and interplay with dementia biology and pharmacology, lead to rapid development in dementia treatment. Thus, this holds great promise for future research for the formulation of potent antidemential drug from these plants.

Table: 5 Accelerated stability studies of the prepared tablets

Storage condition	Description		Weight variation (%)	Hardness (kg/cm2)	Friability (%)	Disintigration time (min)
	Colour	Odour				
Initial	Dark brown	Characteristic	2.60	4.10	0.48	12.80
1 month at 45⁰C/75% RH	Dark brown	Characteristic	2.60	4.30	0.48	13.40
1 month at 45⁰C/75% RH	Dark brown	Characteristic	2.60	4.85	0.50	13.60

Table 6: TL of young rats on EPM

Treatment group	No: of rats(n)	Transfer latency (Mean± SEM)	
		Day 1	Day 2
Control	6	33.0± 0.816	22.5± 0.846
Sod. Nitrite	6	60.5± 1.871**	34.5± 0.763**
Test drug (500mg Tab)	6	36.0± 0.894*	28.0± 0.516**
Test drug (1000mg Tab)	6	34.0± 1.673	27.0± 0.577**
Piracetam	6	30.3± 1.802*	25.5± 0.6708*

n = 6 in each group. **P<0.01, *P<0.05 are significant, compared to control (One-way ANOVA followed by Dunnett's test)

Table 7: TL of old rats on EPM

Treatment group	No: of rats(n)	Transfer latency (Mean± SEM)	
		Day 1	Day 2
Control	6	58.5± 0.763	37.0± 0.683
Test drug (500mg Tab)	6	34.0± 0.577**	29.0± 0.683**
Test drug (1000mg Tab)	6	32.5± 0.763**	26.3± 0.843**
Piracetam	6	31.0± 0.730**	24.5± 0.763**

n = 6 in each group. **P<0.01 is significant, compared to control (One-way ANOVA followed by Dunnett's test)

Table 8: EL in MWM test

Treatment Group/Day of trial	Escape latency				No: of rats(n)
	Control	Ext. 400mg	Ext. 800mg	Piracetam	
1st	78.0±0.856	76.3±0.494	75.0±0.577**	75.5±0.428*	6
2nd	62.5±0.764	58.0±0.516**	56.0±0.730**	54.0±0.856**	6
3rd	50.6±0.667	45.0±0.577**	38.5±0.562**	34.8±0.600**	6
4th	38.8±0.601	26.0±0.730**	24.5±0.764**	20.5±0.428**	6
5th	20.5±0.563	17.0±0.577**	13.8±0.601**	15.0±0.577**	6
6th	13.0±0.577	12.0±0.856	10.8±0.601	10.0± 0.577*	6
7th	10.0±0.577	10.0±0.730	10.0±0.683	10.0± 0.577	6
8th	8.8±0.601	8.0±0.5774	8.0± 0.516	7.8±0.792	6

n = 6 in each group. **P<0.01, *P<0.05 are significant and P>0.05 is non–significant compared to control (One-way ANOVA followed by Dunnett's test).

Figure: 1

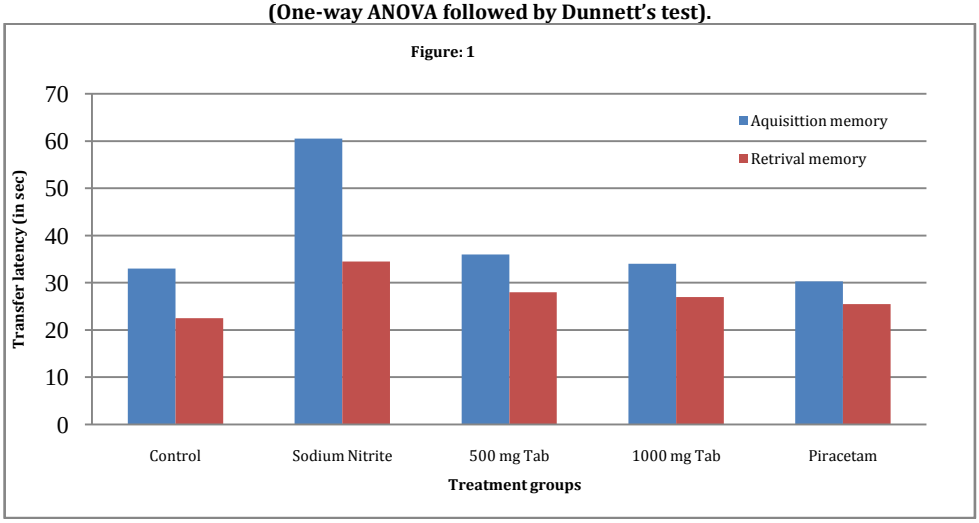

Figure1: EPM test in young rats Effect of test drugs and standard drug (Piracetam) onacquisition and retrival memory of young rats.

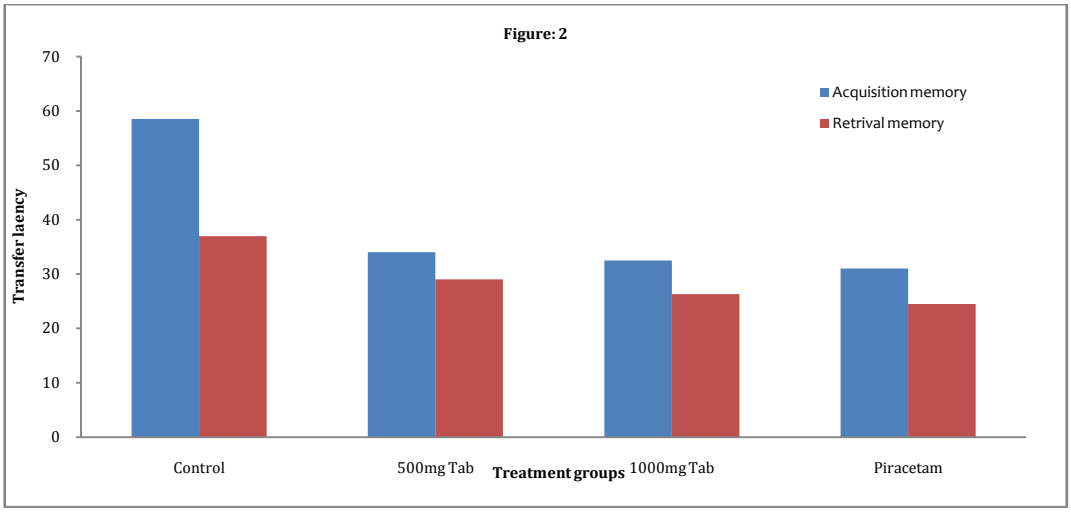

Figure: 2 EPM test on old rats Effect of test drugs and standard drug (Piracetam) on acquisitionand retrieval memory of old rats.

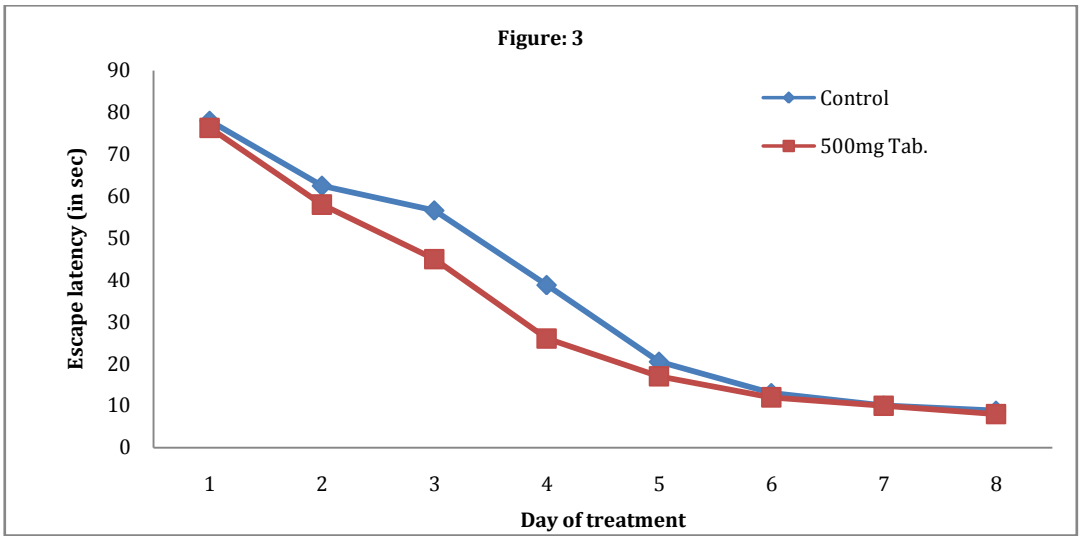

Fig.3: Effect of test drug (500 mg Tab) compared with the standard drug (Piracetam) on spatialmemory in rats.

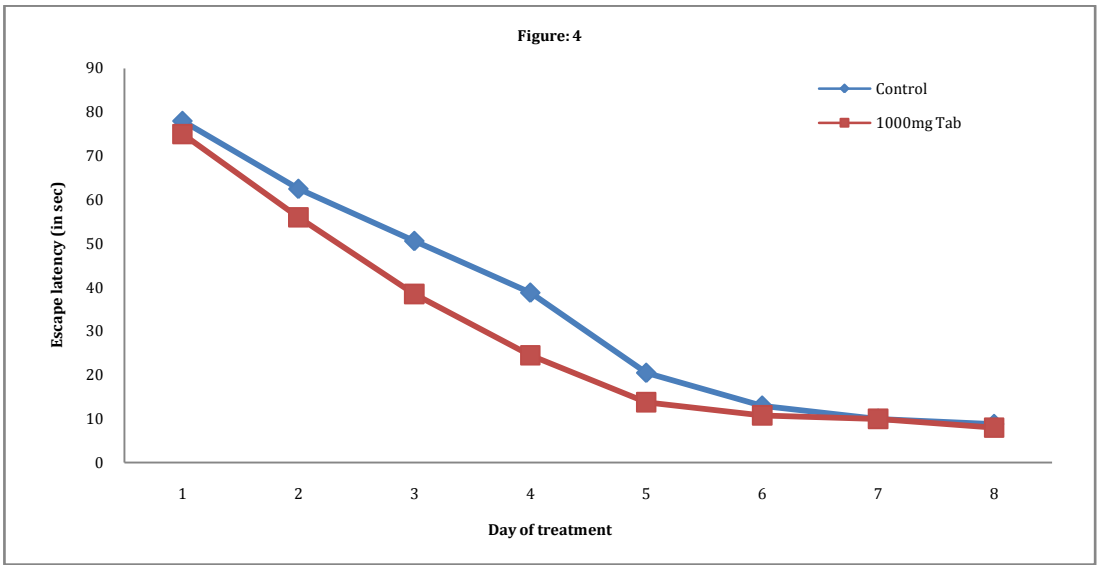

Fig. 4: Effect of test drug (1000 mg Tab) compared with the standard drug (Piracetam) on spatialmemory in rats.

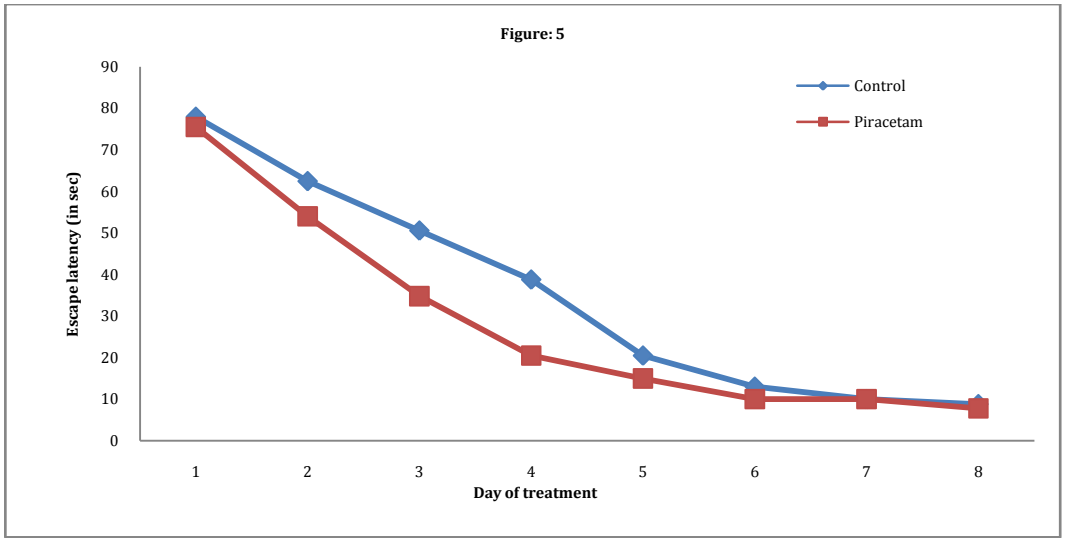

Fig.5: Effect on control (vehicle only) group, compared with the standard drug (Piracetam) group onspatial memory(MWM test) in rats.

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