Phytochemical and pharmacological investigation of hot aqueous extract of Piper betle Linn. leaves and its combination with coffee powder prepared by Bhavana process on the central nervous system of the rats.

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ABSTRACT: The aim of the present study was to evaluate the pharmacological effects of the hot aqueous extract of the leaves of Piper betle Linn. and its combination with coffee powder prepared by the Bhavana process on the central nervous system of rats. This study also involved preliminary screening of the phyto-constituents present in both the extract and the preparation.

Different animal models were used to evaluate the pharmacological activity of the extract and the preparation which was administered continuously for seven days to the rats by the oral route. Elevated plus maze was used to test the ant-anxiety activity whereas Hot plate analgesiometer was used to determine the analgesic activity. The effect on the skeletal muscle co-ordination was assessed using rotamex instrument whereas the effect on learning and memory was assessed using Morris water maze. Diazepam (4 mg/kg) and Pentazocine (5 mg/kg) were used as positive control. The readings were recorded on day 1, day 4 and day 7 at 1hour, 4hour and at 8hour interval.

The readings were statistical analysed by one-way Anova followed by Dunnett's test. It was found that none of the administered test compounds showed anti-anxiety activity. The hot aqueous extract of Piper betle showed marginal whereas Bhavana preparation showed dose dependent significant analgesic activity. None of the test compounds showed skeletal muscle relaxation activity. In case of learning and memory all test compounds that is coffee extract, hot aqueous extract of Piper betle and the Bhavana preparation at both the doses showed significant activity.

KEYWORDS: Bhavana, Piper betle Linn., Elevated Plus maze, Water maze, Hot plate

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INTRODUCTION

It is universally accepted fact that good health plays an important role in human development. The contribution of Traditional systems of Medicine (TSM) for global health care is well recognized. The traditional systems of India, which are now called Indian System of Medicine (ISM), have a very strong conceptual base and have been practiced uninterruptedly since ancient times. Ayurveda, Siddha & Unani are three

important traditional systems practiced in India. Ayurveda is the oldest & the most widely practiced system among the three.[1]

Plants have been used in medicine since ancient times and have continued to occupy an important role in traditional as well as modern systems of medicine. The Indian system of Medicine (ISM) predominantly use plant based raw materials in most of their preparations and formulations.[2]

In Ayurveda medicinal plants are processed prior to its administration. Various dosage forms are prepared based on the sound processing techniques. According to Ayurvedic texts shodhana vidhi is an important process which enhances the biological activity of a compound and at the same time reduces its toxicity.[3] Besides shodhana there is one more procedure called Bhavana vidhi which aims at potentiating the action of original herb. In Bhavana vidhi multiple coats of one medicine are given to the core medicine with the aim to obtain a product with enhanced properties. Besides processing of the drug, the duration of administration of the drug is of great importance in Ayurvedic therapy. It is claimed in Ayurveda that dug produce effect on repeated administration rather than acute administration.

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In this study we attempted to elucidate the scientific principles involved in the process of Bhavana as well as the duration of administration of the drug on its effect. In this process a drug or mixture of drugs in powdered form is triturated with a liquid extract of appropriate herb. This paste is dried and the process is repeated several times. This unique process mentioned in Ayurveda mixes the drugs, thereby augmenting the potency of the medicine many folds.[4] In this study the hot extract of *Piper betle* Linn. leaves was used to give Bhavana to coffee powder.

Piper betle Linn. is a shade-loving, perennial ever-green climber of tropical origin. It is generally known as 'Paan' in the Indian subcontinent.[5] It belongs to the family Piperaceae. Chewing betel leaf is supposed to prevent bad breath (halitosis), improve the vocalization, harden gums, conserve teeth and sweeten the breath. The infusions prepared from the leaves and stems are supposed to be useful in treating indigestion, bronchitis, constipation, congestion, cough and asthma. The Essential oil which is

isolated from the leaves is supposed to be useful in treating respiratory catarrhs and as an anti-septic.[6]

The coffee powder is obtained from the roasted beans of *Coffea arabica* plant. The main effect of coffee is CNS stimulation and diuretic action which is due to caffeine present in it. It is also used to combat the toxic effects of CNS depressant drugs and as a specific analgesic in migraine along with ergotamine tartarate to potentiate its action.[7]

Literature review revealed that no comparative study till now has been performed on the hot aqueous extract of *Piper betle* L. leaves and its combination with powdered coffee prepared by the process of Bhavana. Therefore it was decided to investigate and compare the pharmacological activities of both the above mentioned extract and its preparation on the central nervous system of the rats.

MATERIALS AND METHODS

PLANT MATERIAL

Fresh leaves of *Piper betle* Linn. were obtained from the market.

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PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of the hot aqueous extract of *Piper betle* Linn and the Bhavana preparation test was carried out by performing various standard phytochemical tests.

ANIMALS

Female Wistar rats weighing about 300-350g were used in this study. They were housed in polypropylene cages under standard laboratory conditions (temperature 25 ± 2 ° C, relative humidity 55 ± 10 % and 12 h. light : 12 h. dark cycle) . The animals had free access to standard pellets of rat feed and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment (CPCSEA guidelines). All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to commencement of the experiment (Ref. No.: GCP/IAEC/l3/02).

METHODS

Preparation of Hot Aqueous Extract of *Piper betle* Linn. (HAE):

After collection and identification, the fresh leaves *Piper betle* Linn. were dried for 3 days in shade and cut into small pieces. 20grams of these pieces were boiled in 100ml of distilled water for 2 hours. The above solution was cooled and with filtered using muslin cloth to obtain the extract which was used for feeding of animals and for phytochemical studies. The remaining extract was stored in the refrigerator until use.

Preparation of Aqueous Extract of Coffee Powder:

The instant coffee powder was further grinded manually using a mortar and pestle to get a fine powder. The powdered coffee (1.5g) was added to distilled water (50ml) and boiled until it forms a solution. The above solution was cooled and used for further studies. The remaining solution was stored in the refrigerator until use.

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Preparation of Combination of coffee powder and aqueous extract of *piper betle* Linn. leaf by Bhavana process:

The combinational preparation was prepared by the process of "Bhavana" mentioned in Ayurveda. First the hot aqueous extract of *Piper betle* leaves was prepared as mentioned above. Powdered coffee was triturated with hot aqueous extract till all the extract was completely absorbed. The paste obtained was dried in the sunlight and overnight. This procedure was repeated for seven times on seven consecutive days to complete one Bhavana. The product obtained was reconstituted with distilled water and used for further study.

EXPERIMENTAL PROCEDURE

The rats were divided into 7 groups; each group consisted of 6 rats:

- Group I – water, served as control
- Group II -received hot aqueous extract of *Piper betle* Linn. leaves (10ml/kg)- HAE

- Group III - received aqueous extract of coffee powder (100mg/kg)- Coffee 100mg/kg
- Group IV - received combination preparation of coffee powder and hot aqueous extract of *Piper betle* Linn. Leaves (100mg/kg) –Bhavana 100mg/kg
- Group V – received combination preparation of coffee powder and hot aqueous extract of *Piper betle* Linn. Leaves (200mg/kg)- Bhavana 200mg/kg
- Group VI-received diazepam (4mg/kg) which served as positive control for anti-anxiety and skeletal muscle relaxation activity.
- Group VII- received pentazocine (5 mg/kg) which served as positive control for analgesic activity.

ADMINISTRATION OF DRUG

The hot aqueous extract of *Piper betle* Linn leaves, aqueous extract of coffee powder and the combination preparation after cooling to room temperature were administered to rats orally. Pentazocine and diazepam were administered to the rats by intra-peritoneal injection.

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SCREENING METHODS

The following screening methods were used in our study:

SCREENING METHOD FOR ANTI-ANXIETY ACTIVITY:

ELEVATED PLUS MAZE METHOD

EPM consists of two open arms and two enclosed arms. It is based on the apparent natural aversion of rodents to open and high spaces, and used for the measurement of anxiety as well as short term memory. Because of the disliking for the open arms, the animal spends more time in the enclosed than the open arms and this aversion quality becomes apparent only when the animal enters them.[8]

For this test, the rats after 1hr of administration of the test solutions were individually placed in the centre of the maze facing one of the open. During a 5min test period the following measures were taken: the number of entries into and time spent in the open

arms. The above mentioned parameters were recorded using a video tracking camera and Smart software. This data was used to calculate percentage of open arm entries (%OAE) and percentage of time spent in open arm (%TSOA). Test was subsequently performed at 4hr and 8hr. All the groups were administered with the respective test solutions for a continuous period of 7 days and the readings were also recorded on the 4th and 7th day.

SCREENING FOR ANALGESIC ACTIVITY:

HOT PLATE METHOD

The hot plate method has been used by many investigators and has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The paws of mice and rat are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws.[9]

For this test, the rats after 1hr of administration of the test solutions were individually placed on the electrically heated surface of the Hot plate analgesiometer maintained at 55±0.5 °C. The time until the paw licking or jumping response occurred was recorded. This was noted as the reaction time. The cut off time of 15 sec was maintained in order to avoid any damage to the paws of the rats. Test was subsequently performed at 4hr and 8hr. All the groups were administered with the respective test solutions for a continuous period of 7 days and the readings were also recorded on the 4th and 7th day.

SCREENING FOR MUSCLE RELAXANT PROPERTY:

ROTAROD METHOD

The rotarod assembly is immensely useful for screening of drugs affecting motor coordination. It consists of a rotating rod with gradually increasing speed. Latency of the animal to fall of the rotating rod is considered as the index for skeletal muscle relaxation activity. Only those animals which have demonstrated their ability to remain on the revolving rod for at least 1 minute are used for the test.[9]

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For this test, the rats after 1hr of administration of the test solution were placed on the rotating rod of the Rotamex apparatus. The time taken for the rats to fall from the rotating rod was noted. Test was subsequently performed at 4hr and 8hr. All the groups were administered with the respective test solutions for a continuous period of 7 days and the readings were also recorded on the 4th and 7th day.

SCREENING FOR LEARNING AND MEMORY

MORRIS WATER MAZE METHOD

In this test a mouse or a rat is placed into a circular tank containing water, and has to find an escape platform in a fixed place just beneath the surface. After swimming around for a certain time, the animal will eventually come across the platform and climb onto it to escape from the water. When placed again in the water on subsequent occasions, the animal will generally find the platform with increasing rapidity, indicating that it has learned the position of the platform. The Morris maze for rats consists of a circular water tank (150 cm in diameter) filled with water and maintained at 27 °C with an escape platform (15 cm in diameter) 18 cm from the perimeter always in the same position 2 cm beneath the surface of the water. The principal measure taken is the escape latency at each trial. Decreases in the escape latency from trial to trial indicate learning.[9]

For this study rats were given a single training session. The good swimmers were segregated. Only those rats that could find the escape platform within 120secs were used for the study. The rats after 1hr of administration of the test solution were individually placed in the water tank containing water in quadrant labelled as quadrant number 1 (Q1). The latency, time spent on platform (TOP), time in platform's quadrant (TPQ) and time in other quadrants (TOQ) were recorded using the video tracking camera and duration of trial was of 60secs. Following this, the rat was placed in quadrants labelled as Q2, Q3 and Q4 and all the above mentioned parameters were recorded automatically. Activity was noted after 1 hour, 4 hours and 8 hours of administration of test compound. The above procedure was followed to obtain the

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fourth and seventh day readings also. The smart video tracking software was used to record the readings.

STATISTICAL ANALYSIS

Values are presented as mean ± SEM. Statistical analysis was by done by one-way ANOVA followed by Dunnett's test. Differences between means were considered to be statistically significant at $p< 0.05$ and $p<0.01$.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the hot aqueous extract of *Piper betle* Linn and the Bhavana preparation test revealed the presence of carbohydrates, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins.

Anti-anxiety study

In the anti-anxiety model (EPM) %TSOA and %OAE are the parameters which were accessed as indices for this activity. Even after continuous administration for seven days, none of the groups showed any anti-anxiety activity except the standard (group VI). The results obtained at the various time intervals and on the various days are as tabulated in Table No.1.

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Analgesic study

The results obtained at the various time intervals and on the various days are as tabulated in Table No.2. The graphical representations of the results are shown in Fig. No: 1, 2, 3. Group II i.e. HAE showed significant increase in the reaction time only at 4hr on day 4 and at 8hr on day 7 indicating that it has marginal analgesic activity. Group IV i.e. Bhavana 100mg/kg and group V i.e. Bhavana 200mg/kg showed significant increase in reaction time at various degrees at different time intervals during all three days indicating that this groups have significant analgesic activity.

Motor co-ordination study

The effect on motor coordination of rats was measured using Rotamex apparatus. The results obtained at the various time intervals and on the various days are as tabulated in Table No.3.

In this study it was observed that in spite of continuous administration none of the groups showed significant decrease in the time of fall of the rats in comparison to the control. Group IV showed an increase in time of fall on day1, 8hr which was statistically at $P<0.01$ in comparison to the control. Group V showed an increase in the time of fall on day 1 at 4hr and 8hr which was statistically significant at $P<0.01$ and on day 4 at 8hr which was significant at $P<0.05$.

Learning and memory

The results obtained at different time interval on the on three different days are tabulated quadrant wise for each group in Table No.4-9. The graphical representations of the result are shown in Fig.4-39. The Table No.4 tabulates the latency and time on platform at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 1 when the rat was placed in Q1, Q2, Q3 and Q4. The Table No.5 provides with the time in platform's quadrant and time in other quadrants at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 1 when the rat is placed on Q1, Q2, Q3 and Q4. The graphical presentation of the latency, time on platform and time in other quadrants for day 1 are shown in figure (4-7), (8-11) and (12-15) respectively. The Table No.6 tabulates the latency and time on platform at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 4 when the rat was placed in Q1, Q2, Q3 and Q4. The Table No.7 provides with the time in platform's quadrant and time in other quadrants at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 4 when the rat is placed on Q1, Q2, Q3 and Q4. The graphical presentation of the latency, time on platform and time in other quadrants for day 4 are shown in figure (16-19), (20-23) and (24-27) respectively. The Table No.8 tabulates the latency and time on platform at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 7 when the rat was placed in Q1, Q2, Q3 and Q4. The Table No.9 provides with the time in platform's quadrant and time in other quadrants at 1hr, 4hr and 8hr for Group I, II, III, IV and V on day 7 when the rat is placed on Q1, Q2, Q3 and Q4. The graphical presentation of the latency, time on platform and time in other quadrants for day 7 are shown in figure (28-31), (32-35) and (36-39) respectively.

In this study it was seen that there was a significant decrease in latency and TOQ and significant increase in TOP at various time intervals on different days of analysis for

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all the groups i.e HAE (group II), coffee (group III), Bhavana 100mg/kg and Bhavana 200mg/kg (group V) as compared to the control (group I). TPQ was found to significant only on few instances in comparison to other parameters.

Discussions

The main aim of the present study was to elucidate the scientific principles involved in the Ayurvedic processing technique known as "Bhavana" and to study its effect on the pharmacological properties of the drugs. In this study the hot aqueous extract of the *Piper betle* Linn. leaves was subjected to Bhavana treatment with instant coffee powder to obtain a combination preparation and the pharmacological investigation was carried out to evaluate its effects as well as the effects of its individual constituents on the central nervous system of rats. The impact of duration of administration of the drug on its effect was also evaluated.

It was seen that none of the groups showed significant anti-anxiety activity. HAE group showed only marginal analgesic activity which could be due to chronic administration. The coffee group did not show any analgesic effect however the Bhavana group at both the doses i.e. 100mg/kg and 200mg/kg showed significant analgesic activity at various degrees at different time intervals during all three days. It was also found that the analgesic effect shown by Bhavana preparation was dose dependent i.e. the overall significance showed at 200mg/kg dose was more as compared to the 100mg/kg dose. So it can be said that coffee itself do not show any analgesic effect but on coating by Bhavana treatment with HAE it gives a preparation with enhanced analgesic activity. Theoretically it is seen that coffee has a tendency to cause hyperalgesia on chronic administration.[10] So we can say that this effect is antagonized when it is coated with the hot aqueous extract of *Piper betle* Linn by Bhavana treatment.

All the test groups do not show any skeletal muscle relaxation activity when compared with the control. In case of learning and memory both the extract and the preparation (at both the doses) showed significant effect. It was also observed that the decrease in latency shown at 4hr on each experimental day was more than that at 1hr and that

shown at 8hr was more than that shown at 4hr on many occasions for all the test groups indicating the inducement of spatial memory in the rats. All the groups show significant improvement in learning and memory of rats. The decrease in latency shown by HAE and coffee group was lesser as compared to that shown by the Bhavana groups at both the doses. This effect shown by the Bhavana group was not dose dependent. Based on this finding we conclude that the Bhavana treatment potentiates the effect of the individual drugs on learning and memory however we did not find any significant change on chronic administration. Thus the claims of Ayurveda are substantially proved in our study but it requires further scientific investigation to find out the basis for such effect and substantiate this claims.

CONCLUSION

It can be concluded that none of the administered test compounds show anti-anxiety activity. The hot aqueous extract of *Piper betle* shows marginal whereas Bhavana preparation shows a dose dependent significant analgesic activity. None of the test compounds show skeletal muscle relaxation activity. In case of learning and memory all test compounds that is coffee extract, hot aqueous extract of *Piper betle* and the Bhavana preparation at both the doses show significant activity.

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REFERENCES

1. Mishra LC. Scientific basis for Ayurvedic Therapies. Florida: CRC Press; 2010. P. 1-2.

2. Singh K, Jhakar ML, Singh D. Medicinal herb and spices —Scientific ecofarming and technology. Jaipur: Aavishkar Publishers, Distributors; 2008. P. 3-5.
3. Paul A, Gajjar U, Donga J. Effects of avurvedic shodhana (processing) on dried tuberous aconite (*Aconitum napellus Linn*.) root. Indonesian J. Pharm [Internet]. 2013; 24(1):40 – 46. Accessed through Google scholar.
4. Verma D, Gupta P, Singh AK. Sanjeevanivati in therapeutics with special reference to Samprapti Bhang. IJRAP [Internet]. 2011; 2(6):1642-1644. Available from http://www.ijrap.net
5. Kumar N, Misra P, Dube A, Bhattacharya S, Dikshit M, Ranade S. *Piper betle* Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. Current science [Internet]. 2010 October;99(7):922-924. Available from http://www.ias.ac.in/currsci/10oct2010/922.pdfias.ac.in
6. Rai MP, Thilakchand KR, Palatty PL, Rao P, Rao S, Bhat HP, Baliga MS. *Piper Betel* Linn (Betel Vine), the Maligned Southeast Asian Medicinal Plant Possesses Cancer Preventive Effects: Time to Reconsider the Wronged Opinion. Asian Pacific J Cancer Prev [Internet].12:2149-2156. Accessed through Google scholar.
7. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 44th ed. Nirali Prakashan; 2009.
8. Verma A, Kulkarni SK. Effect of Herbal Psychotropic Preparation, BR-16A (Mentat), on Performance of Mice on Elevated Plus-Maze. Indian Journal of Experimental Biology. [Internet] 1991; 29:1120-1129. Availablefrom:http://himalayacentroamericana.com/himalaya_researchpapers/pdf_files/mentat004.pdf

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9. Vogel GH, editor. Drug discovery and Evaluation: Pharmacological Assays. 2nd ed. New York: Springer- Verlag Berlin Heidelberg; 2002.

10. Wua WP, Haoa JX, Fredholmb BB, Hallina ZW, Xua XJ. Effect of acute and chronic administration of caffeine on pain-like behaviors in rats with partial sciatic nerve injury. Neuroscience Letters. 2006 July;402(1-2):164-166. doi: 10.1016/j.neulet.2006.03.065.

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Table No.1: Elevated Plus Maze - Day 1, 4 and 7

Treatment	Time in hours	Day1		Day4		Day7	
		%TSOA	%OAE	%TSOA	%OAE	%TSOA	%OAE
Control (Group-I)	1	3.692±1.196	25.726±7.352	15.553±6.098	22.585±21.843	2.615±1.100	16.455±8.975
	4	7.023±3.941	23.010±7.539	2.280±1.106	21.843±7.581	7.765±2.068	28.683±12.318
	8	6.087±1.767	24.468±7.943	8.103±4.128	37.935±17.361	17.358±6.771	37.332±6.370
Diazepam (Group-VI)	1	29.880±10.534*	58.637±5.116	50.570±16.501*	66.473±3.281*	69.948±4.321**	71.863±2.633**
	4	32.155±9.957*	59.255±10.003	63.963±8.449**	65.257±3.672**	73.058±4.946**	80.263±5.962*
	8	35.480±15.707*	67.810±12.836**	72.190±13.634**	72.817±5.707*	63.730±10.285**	79.830±8.348
HAE 10ml/kg (Group-II)	1	9.023±5.555	41.362±11.156	12.042±5.424	51.690±11.568	13.708±2.803	59.187±11.917**
	4	5.938±3.280	57.437±6.984	16.995±6.288	55.585±6.537*	12.600±4.464	28.967±13.133
	8	2.688±1.084	40.673±9.171	2.417±0.6239	39.118±17.533	19.645±13.359	40.005±13.111
Coffee oral 100mg/kg (Group-III)	1	13.975±5.854	32.113±7.004	21.272±7.453	55.852±7.961	18.455±8.958	37.625±9.078
	4	13.340±4.482	36.560±11.224	13.868±7.151	44.073±9.859	9.617±2.256	39.445±12.202
	8	12.968±4.440	50.062±10.489	9.997±3.947	31.983±8.816	36.245±10.270	53.563±7.443
Bhavana 100mg/kg (Group-IV)	1	12.093±6.777	35.847±10.340	2.782±1.200	21.603±10.128	2.070±0.4258	18.750±8.606
	4	6.702±3.681	20.215±14.779	3.087±1.149	17.953±7.137	8.225±4.257	18.733±11.086
	8	4.162±1.521	33.882±10.812	11.097±4.964	25.252±9.337	3.380±1.358	33.577±14.024
Bhavana 200mg/kg (Group-V)	1	15.123±6.650	29.850±13.225	2.255±0.5045	31.350±12.526	3.473±1.349	31.263±2.778
	4	9.803±3.988	22.720±8.834	8.867±3.873	41.120±9.151	4.192±2.131	18.055±8.718
	8	3.125±1.084	40.772±7.653	9.090±4.131	27.895±5.466	10.693±4.748	24.293±14.117

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control.

Table No.2: Hot plate analgesiometer - Day 1, 4 and 7

Treatment	Time in hours	Day1	Day4	Day7
		Reaction time (secs)	Reaction time (secs)	Reaction time (secs)
Control (Group-I)	1	0.9333±0.1256	1.283±0.1138	1.050±0.1335
	4	1.117±0.1046	1.017±0.1424	1.300±0.1528
	8	0.9167±0.04773	0.9000±0.1461	1.133±0.1308
Pentazocine (Group-VII)	1	3.733±0.2512**	4.033±0.1430**	4.250±0.1962**
	4	3.633±0.2813**	4.133±0.1498**	4.467±0.3818**
	8	3.7667±0.1892**	4.20±0.2422**	4.683±0.3790**
HAE 10ml/kg (Group-II)	1	1.067±0.04944	1.317±0.1778	1.550±0.1875
	4	1.333±0.1054	1.583±0.1447*	1.917±0.2040
	8	1.383±0.1973	1.517±0.1195	1.900±0.1732*
Coffee oral 100mg/kg (Group-III)	1	0.9333±0.09888	1.200±0.07303	1.033±0.06667
	4	0.8833±0.08333	0.8500±0.09574	1.183±0.1537
	8	1.100±0.06325	1.117±0.07491	1.017±0.06540
Bhavana 100mg/kg (Group-IV)	1	1.450±0.1335	1.400±0.2066	1.617±0.1833
	4	1.833±0.09189*	1.733±0.1909**	2.067±0.1054
	8	1.517±0.2600	1.633±0.1687*	1.983±0.1195*
Bhavana 200mg/kg (Group-V)	1	1.483±0.1493	1.467±0.2261	1.800±0.3578
	4	2.100±0.2098**	2.533±0.06146**	2.283±0.2242*
	8	1.717±0.2676*	1.383±0.1579	2.450±0.2094**

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Contro1

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Table No.3: Rotamex - Day 1, 4 and 7

Treatment	Time in hours	Day1	Day4	Day7
		Time of fall (secs)	Time of fall (secs)	Time of fall (secs)
Control (Group-I)	1	330.78±31.840	246.53±42.584	324.62±41.058
	4	303.77±31.477	298.90±33.736	278.88±34.633
	8	234.47±31.248	269.03±40.609	282.73±29.782
Diazepam (Group-VI)	1	83.80±16.806**	80.433±12.927*	53.633±10.012**
	4	92.183±5.085**	81.367±15.023*	65.933±11.356*
	8	81.133±12.004	74.183±13.233**	60.483±7.785*
HAE 10ml/kg (Group-II)	1	442.98±34.351	310.22±78.544	340.10±70.794
	4	347.57±51.896	355.70±32.987	294.80±78.039
	8	370.78±71.535	311.70±66.557	395.45±91.717
Coffee oral 100mg/kg (Group-III)	1	371.53±46.958	390.77±31.420	291.07±42.456
	4	291.33±48.044	281.01±45.039	313.00±46.616
	8	355.30±48.628	240.97±52.514	312.35±53.442
Bhavana 100mg/kg (Group-IV)	1	293.98±41.083	351.48±68.109	355.73±75.240
	4	441.38±60.840	304.02±101.46	391.53±90.314
	8	438.33±65.950**	338.15±75.097	312.38±66.726
Bhavana 200mg/kg (Group-V)	1	456.45±88.733	358.02±57.119	315.77±102.40
	4	499.48±42.618**	417.87±81.251	430.53±86.267
	8	482.67±34.294**	433.95±57.963*	395.30±57.368

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control.

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Treatment group	Time in hours	Latency (Time to reach the platform in secs)				Time spent on platform- TOP(secs)			
(when rat placed in individual quadrants)		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Control (Group I)	1	13.748±0.964	13.905±1.568	12.572±1.001	12.840±1.543	35.505±4.688	32.912±5.086	38.193±2.674	43.457±1.473
	4	11.843±1.622	11.208±1.893	10.540±2.470	9.405±1.058	46.078±1.764	44.307±2.782	45.105±2.313	46.265±1.696

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Table No.4: Latency and time on platform using Morris Water Maze -Day1

	8	9.977±0.775	12.258±1.568	9.405±1.680	8.528±2.536	44.617±1.402	35.275±4.831	40.725±4.040	48.790±2.668
HAE (Group-II)	1	8.333±4.523	8.135±2.271	9.323±3.177	10.147±3.529	50.165±4.374*	51.283±2.532**	48.523±4.056*	48.770±4.367
	4	10.397±4.553	4.665±0.649*	4.147±0.551	8.135±2.671	49.572±4.583	52.530±2.117*	54.968±1.207**	51.542±2.612
	8	5.490±1.310	8.945±2.199	9.113±2.769	1.738±0.794	54.385±1.333*	48.718±3.699*	53.550±2.551	57.907±0.792
Coffee oral (Group-III)	1	7.365±1.599	3.530±0.677**	4.300±1.092*	6.238±1.916	52.290±1.504**	56.458±0.682**	55.312±1.031**	52.528±1.545
	4	4.832±1.704	2.738±0.801**	3.342±0.546	5.467±1.557	55.758±1.849	55.113±0.878**	56.238±0.5750**	54.528±1.556
	8	3.717±1.412	3.435±0.6030*	2.322±0.4703	11.520±7.623	56.280±1.412**	51.272±5.240*	57.665±0.4675**	46.582±7.415
Bhavana 100mg/kg (Group IV)	1	5.178±2.265	6.927±2.692	6.063±1.940	2.405±0.247**	54.113±2.290**	54.237±2.814**	53.935±1.939**	50.397±6.147
	4	9.135±2.724	3.905±1.181**	9.510±1.912	3.135±0.726**	50.362±2.544	54.780±1.531**	52.228±1.591*	52.208±3.218
	8	11.883±2.866	6.237±2.334	9.532±3.139	5.063±1.197	46.803±3.541	50.488±2.386*	39.842±6.264	49.405±3.447
Bhavana 200mg/kg (Group-V)	1	7.905±3.814	8.175±3.403	3.790±1.949*	6.853±4.988	48.260±3.884*	49.242±3.952**	53.862±1.336**	49.915±3.349
	4	8.238±2.594	8.238±2.594	7.385±4.080	3.132±1.107**	47.395±3.288	51.992±1.911	54.230±1.942**	49.710±6.292
	8	7.780±2.886	5.227±2.446	3.927±0.792	7.760±2.711	49.270±3.161	51.715±6.805*	54.792±0.7122	49.887±2.600

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control.

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Treatment group	**Time in hours**	**Time spent in platform's quadrant- TPQ(secs) (Q4)**				**Time spent in other quadrants- TOQ(secs) (Q1+Q2+Q3)**			
(when rat placed in individual quadrants)		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Control (Group I)	1	5.632±3.127	8.352±4.625	2.554±0.827	1.002±0.245	17.672±1.403	17.540±1.682	16.912±1.397	15.285±1.239
	4	0.6140±0.212	0.922±0.402	2.058±0.528	1.726±1.654	12.967±1.746	14.333±2.392	12.243±2.254	10.872±1.146
	8	6.028±2.525	6.501±4.126	2.068±0.654	1.082±0.328	20.460±3.441	20.328±4.322	14.652±2.373	9.545±2.610

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Table No.5: Time in platform's quadrant and time in other quadrants using Morris Water maze-Day1

Treatment group	Time in hours	Latency (Time to reach the platform in secs)				Time spent on platform- TOP(secs)			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
HAE (Group-II)	1	4.562±1.972	1.508±0.378	2.247±0.768	2.342±0.704	5.270±3.081**	7.040±2.527**	9.020±3.816	8.885±4.120
	4	3.677±1.273	3.603±2.330	1.885±1.057	1.967±0.569	6.750±3.371	3.863±0.449**	3.143±0.437*	6.488±3.041
	8	2.612±1.458	3.687±0.861	1.967±0.614	1.062±0.460	2.998±0.799**	7.495±2.890**	7.290±2.656	1.030±0.5069
Coffee oral (Group-III)	1	2.487±0.799	0.915±0.154	2.020±0.413	2.687±0.666	5.217±0.996**	2.623±0.545**	2.667±0.786**	4.778±1.283*
	4	2.008±0.737	1.895±0.512	1.040±0.348	2.572±0.903	4.207±1.593	2.985±0.404**	2.317±0.499*	2.895±0.751**
	8	1.402±0.703	1.572±0.290	0.708±0.0749	6.842±3.535	2.312±0.728	2.040±0.442**	1.625±0.488**	6.573±4.146
Bhavana 100mg/kg (Group-IV)	1	2.895±0.871	2.197±1.160	1.980±0.691	4.633±2.592	2.985±1.595**	8.123±2.710*	4.085±1.469**	4.968±3.664*
	4	10.665±1.432	9.473±2.204	7.172±1.478	7.823±1.699	40.935±2.981	44.458±2.663	48.408±2.631	44.638±5.621
	8	2.780±0.687	1.853±1.328	2.262±0.939	1.823±0.386	6.833±2.060	3.365±1.120**	3.490±1.061	5.633±2.639
Control		8.783±2.160	7.312±1.775	9.302±2.646	7.247±1.904	43.038±2.938	46.870±2.415	40.840±4.154	39.152±7.876
		5.177±1.980	3.863±1.087	11.553±4.811*	5.738±2.984	7.998±3.145**	5.643±1.345**	8.603±3.940	4.835±1.665
Bhavana 200mg/kg (Group-V)	1	5.145±2.115	6.103±3.432	3.123±1.176	3.717±2.487	6.590±3.003**	8.133±3.753*	3.005±0.6473**	6.360±2.145
	4	5.488±2.297*	1.425±0.330	2.555±1.488	2.298±0.809	6.997±3.139	6.582±1.899**	7.213±4.457	2.548±0.799**
	8	3.447±1.326	3.885±1.822	2.028±0.584	2.708±0.764	7.198±3.045**	4.395±2.137**	3.172±0.738**	7.405±2.974

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control.

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Table No.6: Latency and time spent platform using Morris Water maze -Day4

Treatment Group	Time in hours	Time spent in platform's quadrant-TPQ(secs) (Q4)				Time spent in other quadrants- TOQ(secs) (Q1+Q2+Q3)			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
(Group I)	8	8.897±1.851	9.643±2.449	7.935±1.548	7.583±1.614	47.825±1.843	45.347±3.297	47.822±1.608	46.713±3.385
Haldi (Group-II) (when rat placed in individual quadrants)	1	6.457±1.875	3.862±0.993*	3.913±0.903	2.613±1.511	50.790±3.817	55.187±1.339*	55.248±0.900*	51.490±4.321
	4	5.552±1.632	4.947±1.039	3.968±1.511	5.685±2.143	54.417±1.640*	54.978±1.047*	54.290±1.995*	54.177±2.162
	8	5.178±1.535	6.092±0.836	6.460±1.409	6.768±3.190	52.395±2.520	49.695±4.104	46.800±6.792	49.713±3.390
Coffee oral (Group-III)	1	2.593±0.634**	4.448±1.476	3.342±0.689*	4.175±0.721	57.383±0.620**	52.635±2.794	54.032±1.245	50.312±1.711
	4	6.238±1.240	4.781±1.176	2.607±0.657*	4.027±0.841	51.083±2.864	53.800±1.753	54.284±1.527	53.760±2.018
	8	5.742±1.728	4.080±0.730*	5.388±0.940	3.680±0.518	52.348±2.305	55.687±1.659	54.213±1.274	54.371±1.716
Control (Group-III)	1	6.655±2.299	4.448±0.843	3.425±0.445*	2.863±1.302	52.738±2.256*	54.802±0.926*	53.708±1.525	52.490±3.995
Bhavana 100mg/kg (Group-IV)	4	4.572±2.003	3.685±1.046	4.752±1.216	1.458±0.384*	55.342±1.977*	56.310±1.045**	52.020±3.228	52.050±3.215
	8	3.740±0.706*	3.020±0.869**	3.615±1.529*	3.657±0.727	56.113±0.739*	56.903±0.822*	50.708±3.193	52.425±3.195
Bhavana 200mg/kg (Group-V)	1	3.572±0.8436*	2.778±0.686**	2.872±0.671**	4.477±1.390	55.060±0.984**	51.873±1.840	54.397±0.779*	52.822±1.807
	4	4.427±3.026	6.062±1.682	2.478±0.455*	3.240±0.362	45.448±4.377	49.227±1.633	50.175±2.914	52.008±2.504
	8	3.550±0.393*	3.677±0.625*	2.958±0.399*	2.353±0.548	52.698±2.341	55.647±1.026*	54.042±2.063	54.583±2.199

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control.

Table No.7: Time in platform's quadrant and time in other quadrants using Morris Water maze -Day4

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(Group I)	8	1.273±0.467	1.932±0.597	1.223±0.569	3.538±2.003	10.883±1.972	12.327±3.238	10.223±1.452	9.618±2.276
HAE (Group-II)	1	2.729±1.059	2.208±1.0369	2.405±1.062	6.458±3.189	4.383±1.279**	4.383±1.279**	2.342±0.789**	2.050±1.306*
	4	1.873±0.414	1.270±0.233	2.458±1.132	2.457±0.844	3.707±1.434**	3.748±1.011**	3.248±0.900*	3.362±1.500*
	8	4.250±2.216	6.633±4.319	9.113±6.640	3.937±1.309	3.353±1.117**	3.665±0.675**	4.082±0.773**	6.520±2.134
Coffee oral (Group-III)	1	0.4883±0.115	3.927±2.259	3.508±1.078	5.633±2.023	2.122±0.682**	3.433±0.930**	2.457±0.531**	4.050±1.044
	4	3.062±1.429	3.145±1.671	5.155±1.977	1.633±0.469	4.728±1.861**	4.048±0.893**	8.287±5.761	6.600±1.596
	8	5.217±2.135	4.667±1.532	5.163±1.447	1.572±0.977	3.955±0.675**	4.643±1.035*	3.693±0.801**	1.707±0.577*
Bhavana 100mg/kg (Group-IV)	1	3.355±1.078	2.352±0.725	2.687±1.377	3.707±2.167	3.907±1.241**	2.840±0.415**	3.603±0.907**	3.798±1.865
	4	1.653±0.736	0.7483±0.0607	3.937±2.018	2.768±1.237	2.998±1.392**	2.935±1.007**	4.038±1.690	5.177±2.069
	8	1.260±0.381	1.052±0.315	4.123±2.095	1.530±0.697	2.623±0.493**	2.040±0.560**	5.163±1.641*	6.040±2.561
Bhavana 200mg/kg (Group-V)	1	1.957±0.486	5.677±1.654	3.042±0.996	3.093±1.063	2.977±0.834**	2.443±0.636**	2.562±0.683**	4.083±1.345
	4	10.520±3.720**	6.030±3.896**	7.778±2.698	5.695±2.376	4.028±1.202**	4.735±1.408*	2.038±0.447*	2.292±0.206*
	8	2.238±1.558	1.197±0.6903	1.352±0.780	1.883±0.969	5.057±1.493**	3.153±0.618**	4.602±1.618**	3.532±1.284

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Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control

Table No.8: Latency and time on platform using Morris Water maze -Day7

Treatment group	Time in hours	Latency (Time to reach the platform in secs)				Time spent on platform- TOP(secs)			
(when rat placed in individual quadrants)		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Control (Group I)	1	11.793±1.710	10.803±1.879	11.975±1.799	12.740±2.424	45.467±1.9277	46.672±2.595	46.090±2.029	45.322±2.615
	4	12.373±1.536	10.720±1.546	10.102±1.527	7.777±1.856	43.173±1.929	42.190±1.938	46.568±1.811	44.807±3.148
	8	8.618±2.026	9.737±1.932	8.113±2.331	10.923±2.774	48.858±2.492	46.485±2.285	46.197±2.777	44.568±2.791
HAE (Group-II)	1	11.487±4.793	6.677±2.319	6.800±1.809	5.935±2.885	48.458±4.813	53.323±2.319	52.248±2.377	53.855±2.872
	4	5.042±1.501*	6.635±1.254	5.353±0.657	2.635±1.141*	54.697±1.416**	53.175± 1.205**	47.155±6.814	54.042±2.987
	8	4.125±1.555	8.145±2.258	3.978±1.012	7.562±3.185	55.875±1.555*	49.822±3.733	55.248± 0.925*	52.343±3.162
Coffee oral (Group-III)	1	9.637±2.593	5.437±0.807	9.012±2.604	4.302±1.704*	45.967±2.733	50.520±2.839	48.330±3.326	50.010±3.139
	4	3.998±0.569**	5.435±1.009	7.415±2.924	4.532±1.631	53.488±2.543*	54.293± 0.913**	51.570±3.426	53.342±2.709
	8	3.893±0.894*	7.677±2.781	5.105±1.198	4.385±1.164	53.063±2.861	50.562±3.097	53.112±1.855	52.145±2.828
Bhavana 100mg/kg (Group-IV)	1	4.178±1.778	3.812±1.243*	2.823±0.247**	5.428±1.548	53.728±2.697	54.550±2.551	56.135± 0.882*	54.572±1.548
	4	5.268±1.029*	5.718±2.429	2.760±0.942*	3.457±0.481	52.843±2.025*	54.155± 2.552**	57.238±0.941	55.427± 1.539*
	8	3.688±1.082*	2.978±0.471*	2.355±0.552*	4.750±1.633	56.238±1.066*	50.458±6.113	54.385±2.00*	53.137±1.657
Bhavana 200mg/kg (Group-V)	1	3.590±0.857	4.408±1.871	3.965±1.733*	4.165±0.755*	54.437±0.883	53.320±2.087	51.332±3.159	48.220±4.287
	4	5.250±2.354*	3.040±0.775**	3.362±0.413*	6.123±1.522	54.530±2.314**	56.302± 1.099**	56.637±0.414	53.093±1.450
	8	3.967±0.854*	2.790±0.384*	3.020±0.874*	3.508±1.464	54.290±1.359	55.707±1.282	56.637± 1.042**	55.895± 1.681*

Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control

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Table No.9: Time in platform's quadrant and time in other quadrants using Morris Water maze-Day7

Treatment group	**Time in hours**	**Time spent in platform's quadrant-TPQ(secs) (Q4)**				**Time spent in other quadrants- TOQ(secs) (Q1+Q2+Q3)**			
(when rat placed in individual quadrants)		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Control (Group I)	1	0.6140±0.202	0.2518±0.101	0.9912±0.352	1.342±0.599	13.653±1.972	12.268±2.242	12.755±1.941	14.995±2.451
	4	1.692±0.646	3.572±1.436	1.315±0.421	2.372±1.756	14.267±1.397	15.530±1.983	10.423±2.013	11.758±2.452
	8	0.9721±0.344	1.092±0.474	0.5140±0.102	1.528±0.541	9.987±2.338	12.253±1.926	10.505±2.785	12.902±2.361
HAE	1	2.520±1.441	1.593±0.774	2.750±0.991	3.862±1.621	9.017±3.432	5.082±1.616	5.000±1.766*	2.282±1.305**

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(Group-II)	4	1.530±0.425	2.248±0.534	8.935±6.711	2.218±1.051	3.768±1.093**	4.572±1.006**	3.903±0.327*	3.740±1.955*
	8	1.268±0.817	2.312±1.003	2.102±0.722	2.653±1.272	2.853±0.758*	5.832±1.806	2.643±0.525**	4.998±2.075*
Coffee oral (Group-III)	1	5.772±2.462	1.748±0.540	2.582±1.235	3.260±1.488	8.257±2.425	7.727±2.669	9.082±2.208	6.725±2.000
	4	1.782±0.393	1.343.±0.366	2.163±1.304	4.300±2.595	4.723±2.514**	4.362±1.074**	6.258±2.299	2.353±0.857**
	8	1.718±0.652	2.852±1.417	2.122±0.484	1.040±0.218	5.215±2.376	6.577±1.905	4.758±1.475*	6.810±2.669
Bhavana 100mg/kg (Group-IV)	1	1.885±1.023	2.717±2.044	1.710±0.994	2.500±0.5304	4.387±2.003*	2.728±0.778*	2.153±0.332**	2.925±1.221**
	4	2.488±1.457	1.625±1.062	0.6350±0.265	1.198±0.368	4.662±1.072**	4.215±1.520**	2.125±0.703**	2.373±1.554*
	8	0.7500±0.318	3.893±2.647	3.042±1.857	1.712±0.379	3.008±0.974*	5.645±3.517	2.572±0.298**	5.157±1.454
Bhavana 200mg/kg (Group-V)	1	2.510±0.942	3.215±1.325	3.903±3.097	4.655±1.543	3.048±0.913*	3.457±1.519*	4.767±1.751*	7.125±4.027
	4	0.9683±0.387	0.8617±0.680	0.2900±0.106	1.718±0.614	4.498±2.016**	2.832±0.585**	3.072±0.336**	5.185±1.504
	8	2.218±0.785	1.895±0.926	0.7800±0.400	1.197±0.435	3.483±0.939*	2.397±0.373**	2.677±0.730**	2.905±1.441**

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Values expressed as mean± SEM (n=6).*P<0.05, **P<0.01 vs. Control

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