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Pharmacological and behavioral comparative study of *allium cepa* linn. Bulb and coffee with Bhavana treatment in rats

Abstract

Objective: To evaluate and compare the effects of *Allium cepa* Linn. bulb and coffee with Bhavana treatment on the central nervous system of the rats.

Materials and methods: Female Wistar Albino rats weighing 300-350gm were used to study the effect of *Allium cepa* Linn. juice and coffee with bhavana treatment on anxiety, pain, muscle co-ordination and learning and memory. The following methods were used elevated plus maze model of anxiety, hot plate analgesiometer method for evaluating analgesic activity, Rotarod test for evaluating effect on muscle coordination while learning and memory was evaluated by using Morris water maze test.

Results: Combination of coffee and *Allium cepa* Linn. juice by Bhavana treatment showed an increase in the reaction time on the hot-plate method on repeated administration and was found to be a similar to the positive control. It also showed a variable and marginal effect on learning and memory of the rats.

Conclusion: Comparative analysis showed a significant difference between the activities of *Allium cepa* Linn. juice and coffee individually and its combination by Bhavana treatment.

Keywords: *allium cepa* linn, coffee, bhavana, analgesia, learning and memory

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Abbreviations: TOP, time spent on the platform; TPQ, time spent on platforms quadrant; TOQ, time in other quadrants: % OAE, percentage of open arm entries; % TSOA, percentage of total time spent on open arms; Q1, quadrant 1; IAEC, institutional animal ethical committee

Introduction

Herbs are nature's gift to mankind and herbal renaissance is blooming across the world. Medicinal plants have proved to be of utmost importance to the human race.[1] With its base in medicinal plants and its origins in ancient Indian history, Ayurveda is recognised as one of the major systems of alternative medicine. Today, after a century has elapsed, we have come to realize the limitations of the allopathic system of medicine hence scientists have started concentrating on drugs of plant origin again.[2] In this study, the juice of onion was used to give Bhavana to coffee powder. A unique process used in Ayurveda is the process known as "Bhavana" (Impregnation/ Trituration). In this process, a drug or mixture of drugs in powdered form is triturated with a liquid extract of appropriate herb. The wet extract is dried and the process is repeated several times. This process mixes the drugs completely, breaks the complicated chemical molecules into easily absorbable simpler ones thus augmenting the potency of medicines to many folds.[3] 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 drug produces effect on repeated administration rather than acute administration.

The objective of the present study was the evaluation of the principals involved in Ayurvedic processing as well as the duration of administration on its effects. Nasal therapy, also called "NASYA KARMA", has been recognized form of treatment in the Ayurvedic system of medicine. Nasyas are advised to the people suffering from diseases of the head and other organs situated above the shoulder.[4] The plant used in this study is *Allium cepa* Linn, commonly known as onion belonging to the family *Liliaceae*. The bulbs are useful in haemorroids, dysentery, flatulence, dyspepsia, bronchitis, ophthalmia, vomiting, otalgia, pharyngodynia, malarial fever, lumbago, epilepsy, tumours, wounds, paralysis, arthralgia, leucoderma, asthma and skin diseases.[5] The plants *Allium cepa* are proved to show the analgesic,[6] antidiabetic,[7] antioxidant,[8] antidepressant,[9] aphrodisiac,[10] antihyperlipidemic.[11] Coffee powder is obtained from the roasted beans of *Coffea Arabica* plant. The main effect is CNS stimulation and diuretic action due to the presence of caffeine which is the major pharmacologically active purine present in coffee. The seeds of coffee are bitter, stimulant, diuretic, antipyretic and aromatic. It stimulates the flow of digestive juices and intestinal peristalsis.[12] A literature review has revealed that no comparative study till now has been performed on the *Allium cepa* Linn along with powdered coffee prepared by the process of Bhavana.

Materials and methods

Animals

Female adult Wistar Albino rats weighing of 300-350gm, obtained from the animal house of Department of Pharmacology, Goa College of Pharmacy were used for this study. The animals were housed in polypropylene cages in groups and maintained under standard conditions (temperature 25±2°C, relative humidity 55±10% and

12h. light:12h. dark cycle) and had a free access to standard pelleted rat feed and water *ad libitum*. All the animals were acclimatized to the laboratory conditions for a week before commencement of experiment (CPCSEA guidelines). All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the commencement of the experiment. (Ref. No.: GCP/IAEC/13/01).

Plant materials

The bulbs of *Allium cepa* Linn. were procured from the local market.

Preparation of test compounds

Preparation of onion juice: Fresh onions (*A. cepa.*) were purchased from the market. On the day of experiments the onions were pealed, weighed and crushed and minced well in an electrical mixer to obtain 20ml of the onion juice sufficient enough to feed the rats on that day. The crushed product was filtered using a muslin cloth. The transparent liquid obtained was used freshly within 2hrs after preparation to carry out the experiments. The obtained juice was used for feeding of animals and phytochemical studies. The juice was freshly prepared for 7days.

Preparation of aqueous extract of coffee: Instant soluble Coffee (1.5gms), obtained from the local market was solubilised with 50ml of water until a uniform solution is obtained. The obtained aqueous extract was used for feeding of animals. For animal studies extract was freshly prepared every day.

Preparation of combination: Instant soluble coffee powder was triturated with the fresh juice of onion bulbs till all the juice was completely absorbed. The combination prepared was subjected to seven cycles of trituration with the juice and was dried seven times on seven consecutive days in the day light and overnight.[13] Then 1.25 gms of this combination was re dissolved and reconstituted with 50ml of water to obtain the combination by Bhavana treatment.

Preparation of the onion and coffee paste for inhalation: The onion bulb was crushed and minced in mixer to obtain a paste of required thickness. Similarly instant soluble coffee powder was mixed with a minimum quantity of water and a paste of required thickness was obtained.

Inhalation apparatus

Set up for "inhalation apparatus": Inhalation apparatus consisted of polypropylene cage having dimension 24×17×15cms, covered with metal lid. Husk was used as bedding material. In case of coffee paste muslin cloth was cut into rectangular shape (3×4cm). This rectangular cloth piece was tied to a thread and then tied to the lid of rat cage at the height of 9cm from the bottom of the cage. Four pieces of muslin cloth were tied. In case of onion paste a bulk of the paste was wrapped in muslin cloth and suspended in the centre and two ends of the cage to potentiate the aroma. The metal lid was then covered with aluminum foil (slight perforation for ventilation) to form a closed chamber. This formed the "*inhalation assembly*". For simulated control group cloth pieces were simply tied without paste application. The rats were allowed to freely inhale this for seven days continuously for a period of 24hours, the paste being replenished every day, after the readings the suspended paste was removed.

Preliminary phytochemical investigations

The test solutions were subjected to chemical tests qualitatively for identification of different phytoconstituents like glycosides, saponins, carbohydrates, sterols, alkaloids, flavonoids, tannins, proteins, triterpenoids by using Distilled water, Diazepam, Pentazocine, 2N Hydrochloric acid, Alcoholic α-naphthol solution, Concentrated sulphuric acid solution (conc.H_2SO_4), Fehling's A and B solution, Benedict's reagents, Barfoed's reagent, Bial's reagent, Cobalt Chloride, Iodine solution, 20% Tannic acid, 4% Sodium hydroxide solution, 1% Copper sulphate solution, Millon's reagent, 40% NaOH, Ninhydrin solution, 10% Lead acetate solution, Chloroform, Acetic anhydride, Picric acid, Pyridine , alkaline Sodium nitroprusside solution, Glacial acetic acid, Dichloromethane, Ammonia, 5% Aqueous $FeCl_3$, Benzene, Magnesium turnings, Zinc dust and Conc. Hydrochloric acid, Dragendorff's reagent (Potassium bismuth iodide solution), Mayer's reagent (Potassium mercuric iodide solution) , Wagner's reagent (Iodine potassium iodide solution), Gelatin solution, Bromine water, Potassium permanganate ($KMnO_4$), dilute nitric acid. All the chemicals and solvents used were of analytical grade. Biochemical reagents used were freshly prepared.

Drug and test solutions administration

The test solutions were administered to the rats orally using oral feeding needle. The positive controls (Pentazocine and Diazepam) were administered to the rats by intra-peritoneal injection. Distilled water was given as the control which was also given orally using oral feeding needle.

Experimental procedures

The rats were randomly divided into nine groups; each group consisted of six animals.

i. **Group I** - Water, served as control (p.o.).

ii. **Group II (OJO)** - Received 2.5ml of onion juice (.p.o.).

iii. **Group III (CEO)** - Received 1ml of aqueous extract of coffee powder (p.o.).

iv. **Group IV (Bhavana)** - Received 1ml aqueous extract of combination preparation of coffee powder with onion juice (p.o.).

v. **Group V**- Received control (simulation control).

vi. **Group VI (OPI)** - Received the aroma of paste of bulbs of *Allium cepa* Linn. through inhalational route.

vii. **Group VII (CPI)** - Received the aroma of paste of coffee powder through inhalational route**.**

viii. **Group VIII** - Received pentazocine (5mg/kg), which served as standard for analgesic activity (i.p.).

ix. **Group IX**- received diazepam (4mg/kg), which served as standard for skeletal muscle relaxant and antianxiety activity (i.p.).

The compounds were administered for 7 days continuously to the respective groups.

Assessment of antianxiety activity

Elevated plus maze: The rats were individually weighed and numbered. After 1 hour of administration of the positive control (diazepam 4mg/kg, i.p.), onion juice (p.o.), coffee extract (p.o.), combination of onion juice and coffee (p.o.), pastes (inhalational) and the controls (distilled water and placebo) the rats were individually placed in the centre of the maze, facing one of the enclosed arms.

During a 5minutes test period the following parameters were monitored:

i. Number of entries into each open and closed arms.

ii. Time spent on each open and closed arms.

From the above mentioned parameters, the percentage of open arm entries (% OAE) and the percentage of time spent by the rat on the open arm (% TSOA) were calculated and taken as the indices to evaluate the anxiolytic-like effect of the tested solution.[14] The experiment was repeated again on the 4th and 7thday and the data were noted.

Assessment of analgesic activity using hot plate analgesiometer

Rats were individually weighed and numbered. The temperature of the electrically heated surface of the hot plate analgesiometer was maintained at 55±0.5°C. After 1hour of administration of the standard (pentazocine, i.p.), onion juice (p.o), aqueous extract of combination of coffee with onion juice (p.o.), pastes (inhalationally) and controls (distilled water and placebo) the rats were individually placed on the hot plate. The time until the paw licking or jumping response occurred was recorded. This noted as the reaction time. The readings were recorded after 1 hour, 4 hours and 8 hours following oral administration of the compound. The experiment was repeated and readings were noted again on 4th and 7thday.

Assessment of muscle relaxant activity using rotarod

Rats were pretested on the apparatus. Only those rats which had demonstrated their ability to remain on the revolving rod for at least 1minute were used for the test. Rats were individually weighed and numbered. After 1hour of administration of the control, standard and test solutions the rats were placed on the rotating rod of the Rotamex apparatus. The time taken for the rats to fall from the rotating rod was noted. The activity was monitored and readings were noted after 1hour, 4hours and 8hours of treatment. The experiment was repeated on the 4th and 7thday in the similar manner and the readings obtained were noted.

Assessment of learning and memory activity using morris water maze

Rats were given training on one day. The good swimmers were segregated. Only those rats that could find the escape platform within 120sec were used for the study. The test and control compounds were administered to rats 1hour before the experimentation. The treated and the control groups of rats were placed first in quadrant 1 (Q1)of the maze and the latency, time spent on the platform (TOP), time spent on platforms quadrant (TPQ) and time in other quadrants (TOQ) were recorded using the video tracking camera and duration of trial was of 60sec. Subsequently, it was placed in Q2, Q3 and Q4 and the same parameters were recorded automatically. Activity was noted after 1hour, 4hours and 8hours of administration of test compound and the procedure was followed to obtain the fourth and seventh day readings. The smart video tracking software was used to record the readings.

Statistical analysis

Data was analysed using one way ANOVA followed by Dunnett'stest. Statistical significance difference was set at $P<0.05$ and $P<0.01$ and the data are expressed as mean±S.E.M.

Results

Qualitative phytochemical investigations

The preliminary phytochemical tests of onion juice and Bhavana preparation revealed the presence of carbohydrates, proteins, glycosides, flavanoids, alkaloids and tannins by using the appropriate reagents.

Pharmacological investigations

Elevated plus maze test: The anxiolytic-like effect of the extract was evaluated using the Elevated Plus Maze (Columbus instruments). Percentage of total time spent on open arms (% TSOA) and the percentage of open arm entries (% OAE) were the parameters used to assess the anti-anxiety activity in the maze. Neither of the test compounds showed any significant increase in the above parameters as shown in Table 1.

Hot plate analgesiometer: The OJO and Bhavana groups showed a significant increase in the reaction time at $P<0.01$ compared to the control. Peak effect was seen on day 4 and day 7 as seen in Table 2 and Figure 1-3. No such significant increase in reaction time was shown by CEO and CPI. The OPI showed effect on Day 7 at 8thhour at $P<0.01$.

Table 1 Data of elevated plus maze test on Day 1, Day 4 and Day 7

Treatment (mg/kg)	Time in Hours	Day 1		Day 4		Day 7	
		% TSOA	% OAE	% TSOA	% OAE	% TSOA	% OAE
Control (Group-I)	1	3.692±1.196	25.767±7.352	15.553±6.098	22.585±10.654	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 4mg/kg (Group-IX)	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**
OJO 10ml/kg (Group-II)	1	3.580±1.982	18.645±10.167	6.425±2.090	51.537±13.865	5.848±3.218	18.590±11.851
	4	1.968±0.9809	2.892±2.078	1.927±1.565	23.310±14.801	4.578±1.440	35.607±11.845
	8	3.413±1.726	35.312±9.496	4.095±1.721	44.922±15.247	3.607±1.942	15.368±10.723

Table Continued..

Treatment (mg/kg)	Time in Hours	Day 1		Day 4		Day 7	
		% TSOA	% OAE	% TSOA	% OAE	% TSOA	% OAE
CEO 100mg/kg (Group-III)	1	13.975±5.854	32.115±7.004	21.272±7.453	55.855±7.961	18.455±8.958	37.630±9.079
	4	13.340±4.482	36.563±11.225	13.868±7.151	44.077±9.859	9.617±2.256	39.447±12.202
	8	12.968±4.440	50.063±10.490	9.997±3.947	31.987±8.817	36.245±10.270	53.567±7.442
Bhavana 100mg/kg (Group-IV)	1	1.423±0.7496	12.637±10.055	2.077±0.8028	18.000±8.948	1.083±0.8527	4.127±3.268
	4	4.513±2.848	18.923±8.366	2.125±0.6266	15.172±7.048	13.207±12.281	18.427±10.916
	8	4.088±2.710	24.840±11.534	1.910±0.4978	31.410±16.391	13.158±12.291	25.927±11.624
Simulation Control (Group-V)	1	26.212±16.408	42.383±16.126	3.135±1.260	17.313±10.542	10.058±4.313	38.188±14.994
	4	36.158±20.169	63.135±13.927	4.187±2.251	19.123±10.101	2.027±0.9948	33.095±13.554
	8	24.737±16.847	29.242±13.947	1.923±0.9722	11.947±5.939	8.052±1.945	21.712±3.710
OPI (Group VI)	1	3.118±1.247	10.107±6.352	6.293±2.485	36.843±12.819	29.167±16.959	41.457±17.444
	4	2.077±1.040	7.778±4.994	2.703±1.718	19.375±9.073	18.795±16.330	11.112±11.112
	8	0.5583±0.3668	8.890±5.880	3.348±1.332	34.213±13.986	7.053±3.342	35.578±17.486
CPI (Group VII)	1	4.862±2.009	31.320±9.483	7.078±6.889	14.092±9.930	0.7917±0.4518	3.738±2.427
	4	5.150±1.935	38.243±5.456	0.06167±0.02257	9.068±5.764	4.022±0.9155	46.165±9.151
	8	3.778±1.232	28.960±7.134	9.647±6.698	30.378±13.829	18.933±16.232	50.035±16.209

% OAE- Percentage of open arm entries; % TSOA- Percentage of Time spent on open arm.

Values are expressed as mean±SEM (n=6) *P<0.05 **P<0.01 ***P<0.001 vs. Control.

Table 2 Data of the hot plate analgesiometer on Day 1, Day 4 and Day 7

Treatments	Time in Hours	Day 1	Day 4	Day 7
		Reaction Time (secs)	Reaction Time (secs)	Reaction Time (secs)
Control (Group-I)	1	1.217±0.07032	1.467±0.1838	1.483±0.1078
	4	1.017±0.07491	1.633±0.09545	1.217±0.1222
	8	1.183±0.07923	1.667±0.05578	1.283±0.1778
Pentazosine 5mg/kg (Group-VIII)	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	3.767±0.1892**	4.200±0.2422**	4.683±0.3790**
OJO 10ml/kg (Group-II)	1	1.500±0.1983	2.067±0.1909*	2.317±0.3468*
	4	1.583±0.1579	2.033±0.2216	2.733±0.1333**
	8	1.700±0.1693	2.800±0.05774**	2.783±1.600**
CEO 100mg/kg (Group-III)	1	0.9333±0.0988	1.200±0.07303	0.9833±0.07491
	4	0.8833±0.08333	1.200±0.1915	0.9667±0.1202
	8	1.100±0.06325	1.417±0.1701	1.000±0.05774

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Table Continued..

Treatments	Time in Hours	Day 1	Day 4	Day 7
		Reaction Time (secs)	Reaction Time (secs)	Reaction Time (secs)
Bhavana 100mg/kg (Group-IV)	1	1.833±0.1585	2.117±0.1014*	2.767±0.2319**
	4	1.800±0.1549*	2.133±0.2044	3.250±0.09916**
	8	1.883±0.1701*	2.583±0.1721**	3.233±0.1333**
Simulation Control (Group-V)	1	1.250±0.1088	1.283±0.1327	1.333±0.1667
	4	1.283±0.1167	1.250±0.1176	1.367±0.1145
	8	1.300±0.08165	1.367±0.09888	1.350±0.1310
OPI (Group VI)	1	0.9833±0.1046	1.067±0.04216	1.817±0.2212
	4	0.8833±0.1138	1.717±0.1078*	2.450±0.1688*
	8	0.8833±0.08333	2.150±0.2717	2.717±0.2007**
CPI (Group VII)	1	0.9167±0.09098	1.000±0.6831	0.8833±0.8333
	4	0.8667±0.08819	1.000±0.05774	1.000±0.05774
	8	0.9833±0.09458	1.083±0.07923	0.9667±0.06667

Significantly different by *p< 0.05 and **p< 0.01.

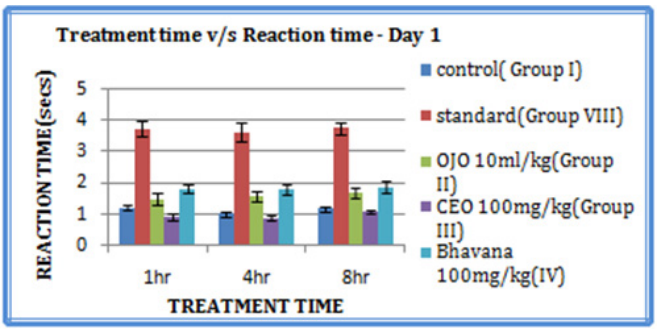

Figure 1 Reaction time of the Group I, Group II, Group III, Group IV, and Group VIII using Hot Plate Analgesiometer- on Day 1.

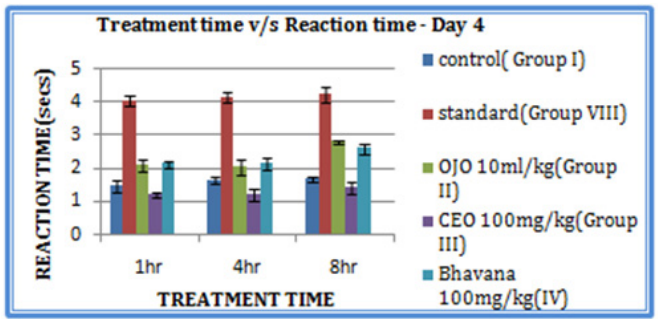

Figure 2 Reaction time of Group I, Group II, Group III, Group IV, and Group VIII using Hot Plate Analgesiometer-on Day 4.

Rotarod test

The motor in co-ordination of the test compounds was evaluated using the Rotamex instrument. A decrease in the time of fall from the rotating rod is taken as indices for motor in coordination. Neither of the test compounds showed any significant decrease in the time of fall.

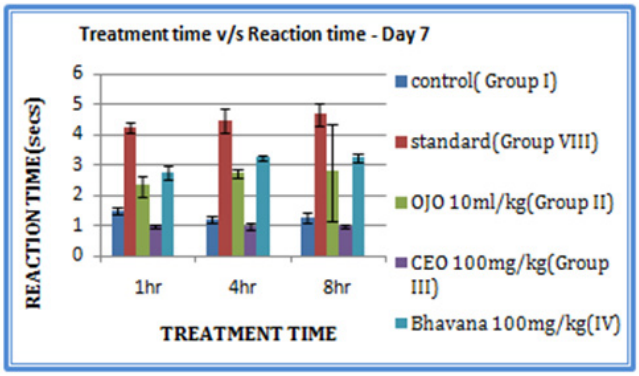

Figure 3 Reaction time of Group I, Group II, Group III, Group IV, and Group VIII using Hot Plate Analgesiometer- on Day 7.

Discussion

This study revealed no significant anxiolytic effect either pure of drug or of Bhavana treated drug. Route of administration also did not make any effect on the anti-anxiety of the drugs. As far as analgesic activity is concerned; OJO (group II) showed a significant

increase in reaction time as already evident in the literature. The difference in single and repeated administration effects was clearly seen. Onion juice on repeated administration showed significant analgesic activity. Coffee which has caffeine as its active constituent produces hyperalgesia on chronic administration.[15] This effect is in fact antagonized by the repeated administration of onion juice. This may be the reason why there is a significant analgesic activity shown on the 7thday of administration. The analgesic activity shown may be either due to the processing of the drug or just the additive effect of repeated administration. On repeated administration of the combination of onion juice and coffee by Bhavana process the hyperalgesic effect of coffee is masked and in fact it produces significant analgesic effect. It is contended that Ayurvedic drugs takes longer time to produce the effect. In this study, we also have found that onion juice on chronic administration produces significant analgesic effect compared to its single administration. OPI (group VI) showed significant analgesic activity. This may suggest the effects of the inhalational route administration. The present results revealed no effect on motor in coordination of rats which was evident from no change in time of fall as compared to control. In Morris Water Maze improvement in learning and memory is shown when there is a decrease in latency time, increase in time on the platform (TOP), increase in time spent on platform's quadrant (TPQ) and a decrease in time spent on other quadrants (TOQ). All these parameters showed significance for OJO (group II), CEO (group III) and Bhavana (group IV) at different time intervals on different days after continuous administration and the maximum significant effect was seen on the 7thday of administration. On the 7thday, OPI (group VI) and CPI (group VII) which were administered through inhalational route also showed marginal improvement of activity. It was also observed that the values for latency for most of the groups that showed positive effect decreased over the period of time (1hr, 4hr and 8hr) on specified day showing the improvement of spatial learning and memory. Literature survey also reveals that coffee individually doesn't show any analgesic activity in fact in some cases it produces hyperalgesia however first time it is found that on combination with a drug that already has analgesic activity it antagonises its effect. Learning and memory effect shown by coffee and marginal effect shown by onion juice and its combination may be due to the stimulant effect on the CNS which is seen by onion and also in coffee due to the presence of caffeine. The administration of onion juice with NSAIDs is worth examining. We find two possibilities in such studies, there could be additive analgesic effect and we also see a possibility of drug interaction. This indicates that coffee shows significant improvement in learning and memory of rats whereas onion juice and Bhavana show effect on repeated administration thus confirming the Ayurvedic principles however further studies are required to substantiate these claims. On comparing the routes of administration, it was found that the groups that were administered orally produced better analgesic and learning and memory than paste inhalation groups.

Conclusion

This study suggests that onion juice and its combination with coffee by Bhavana process has a significant analgesic effect after repeated administration on CNS of the rats. The effect for Bhavana group is cumulative. It can also be concluded that coffee shows significant improvement in learning and memory of rats whereas onion juice and Bhavana show effect on chronic administration thus confirming the Ayurvedic principles however further studies are required to substantiate these claims.

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None.

Conflict of interest

The author declares no conflict of interest.

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