Research Article

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AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECT OF BASTI KARMA OVER RATS: AN EVIDENCE BASED AYURVEDA THERAPY FOR PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease. Understanding of Parkinson's disease in terms of Ayurveda can be done under the Vatavyadhi, to be specific, caused due to the Avarana of vata. By keeping all these points Madhu taillika basti was selected for study. To give preclinical support to this study Animal experimentation was carried out on Albino rats to determine the influence of Matra basti (MB) and Madhu tailika basti (MTB) on Psychoneuro-pharmacological activity profile. The present study aimed at assessing the anti-Parkinsonian activity of the test basti against well-known experimental models. Healthy Wistar albino rats of either sex weighing about 150-200 g were selected and divided into 4 groups. Group 1 Control group, Group 2 Standard group (activity specific), Group 3 Basti group (ordinary or non - Murchita tila taila) and Group 4 Test group (Madhu tailika basti). Animal experimentation revealed that both Matra basti and Madhu tailika basti have produced highly significant anti-depressant effect in behavioural 'despair' test. Both produced significant anti-anxiety activity, open field apparatus test a complex behavior pattern was observed. In the memory and learning related test both the test procedures did not affect the learning process significantly but both produced remarkable reversal of the amnesic effect of scopolamine, this indicates memory enhancing effect. Similarly, anti-amnesic effect was observed in CAR based protocol also. Both improved the performance of the rats on the rota rod. Both the test procedures produced good anti-parkinsonian activity. Animal experimentation revealed that both Matra basti and Madhu tailika basti have no effect on general gross behavior.

Key words: Parkinson's disease, Colon drug delivery, Basti karma, Ayurveda, psychoneurology, Panchakarma

INTRODUCTION

Panchakarma (Bio purification/ cleansing methods) is one among the mode of treating the disorders of *Ayurveda* (alternative medicine). Such purification allows the biological system to return to haemostasis, to rejuvenate rapidly [1] & also facilities the desired pharmaco -therapeutic effects of medicine with colon drug delivery. *Basti karma* (~therapeutic enema) is a mode of main therapy among Panchakarma. Rectal is a route of drug administration effective in musculoskeletal and neurological disorder. [2] Whereas the use of animals in scientific research has made dramatic improvements in our understanding of the human race. [3] Despite the controversies that surround this issue, without this process of testing it is certain that much of what is known today towards the quality & quantity of life would remain closed off to us. In Ayurvedic (alternative medicine) classics also use of animals for testing effect of drug or toxic food is justified. [3] The main objective of pharmacology is to provide a scientific foundation for therapeutics and to increase the resources of the art of healing. The actions of a drug can be evaluated by Carrying out experiments on healthy or diseased animals as certain animals bear on anatomical & physiological resembles to man. Basti (therapeutic enema) is the only panchakarma therapy which is indicated not only in humans but also for animals.[5] Acharya Charaka have explained specific quantities, drugs & equipment's for administration of basti to different animals. [6, 7] This justifies the use of basti therapy to assess its effect in experimental models thus to "**evaluate the efficacy of madhu tailka basti in Parkinson's disease.**"

Criteria for undertaking present study

Basti karma is a very important therapeutic procedure with the potential to manage many difficult to treat clinical conditions. However, experimental studies have not been undertaken to provide basis to the observed clinical efficacy of this procedure. [8] Hence a series of studies are being planned to explore different aspects of the therapeutic efficacy of this procedure. The present study was undertaken to assess the impact of niruha basti (Decoction enema) with madhu tailika basti (Compound preparation) on different psychoneurological parameters. There is currently no treatment to cure Parkinson's disease. [9] Many studies are looking at treatment that might improve some of the symptoms of Parkinson's disease. Madhu tailika basti is a treatment for neurology disorders by its rejuvenation facilitation. To give preclinical support this study has been selected. For this purpose, a battery of well-known psychopharmacological test was selected but with focus on its efficacy in test protocols supposed to be predictive for efficacy in the treatment of

parkinsonian disorders. Thus, current research was planned to evaluate the influence of Madhu tailika basti on Psychoneuro pharmacological activity profile by certain well known Neuropsychological tests through Animal experimentation.

MATERIALS AND METHODS

Animals: Healthy wistar albino rats of either sex weighing about 150-200 grams were selected and divided into 4 groups. The animals were obtained from the animal house attached to S.D.M centre for research in Ayurveda and allied sciences. The selected animals were maintained under prevailing husbandry conditions. They were fed pranav agro's 'Amrut' brand rat feed and water given *ad libitum*. The experiments were undertaken after obtaining permission from the institute's animal ethics committee and as per CPSEA guidelines wide SDMCAU/AE/06/12-13

Grouping: Each group had 6 albino rats and was kept in separate cages.

Group 1: Control group
Group 2: Standard group (activity specific e.g.: procyclidine for anti-parkinsonian activity) [10]
Group 3: Basti group (ordinary or non - murchita tila taila used for basti) [11]
Group 4: Test group (Madhu tailika basti)

Test drugs preparation

A] **Niruha basti with Madhu tailika basti**[12, 13]
Madhu tailika basti is selected from Sushruta samhita chikitsa sthana 39th chapter 100-101 verse. Madhu (Honey), murchitha tila tila (processed seasum oil), Erandamoola kwatha (decoction of root of *Ricinus communis)*, shatapuspha (*Anethum sowa*), saindava (Rock salt), Madhana phala (*Randia dumetorum*) these drugs quantity was taken as mentioned and mixed in order specified in the classics. Contents of test formulation madhu tailika basti were obtained from S.D.M pharmacy. Kuthpady. Udupi. The detail regarding this aspect has been given in drug review section of this thesis. Basti was prepared every day freshly just before the administration to animals.

B] **Matrabasti with murchita tila taila**[14]
Dose fixation and schedule:
The dose of the formulation was calculated by extrapolating the human dose to rat dose on the basis of body surface area ratio (conversion factor 0.018 for rats) by referring to the table of "Paget & Barnes" [15] i.e.
For rats: Humans dose x 0.018 = x g / 200g. Rat
X **x** 5 – y g / kg. Of rat

Procedure

Twenty-four albino rats of either sex was selected and assigned to 4 groups of 6 each. The 1st group served as control group. Rats were provided with normal food pellets and water ad libitum. To the second group activity specific standard drug was administered e.g.: procyclidine for antiparkinsonian activity served as standard group. Third group was administered with ordinary tila taila (without murchana) for 8 days through rectal route. To fourth group, murchita tila taila and madhu tailika basti was administered for 8 consecutive days as mentioned in classics, through rectal route.

Before giving basti (enema) each rat was subjected to procedure like abhyanga (massage) and swedhana (sudation). Lower portion of the abdomen and back of each rat was gently massaged with murchita tila taila and for swedhana warm water (38° c) was taken in plastic bags and was rolled gently over lower portion of the abdomen and back by applying slight pressure. Madhu tailika basti was given in the dose of 6.48 ml /200 g wt. On 2 nd, 4th, 6th day morning in empty stomach along with this 5 matra basti with murchita tila taila in the dose of 0.648 ml /200 g wt. On 1st,3rd ,5th ,7th ,8th day was given in the afternoon immediately after food.

The basti solution was administered with the help of an infant feeding tube which was sieved on to 10 ml plastic syringe. The rats were held by a helper in semi supine lateral position with slight inclination towards head. The infant feeding tube was inserted in to the rectum and the plunger of the syringe was slowly pressed to deliver the basti solution. The temperature of the solution administered was maintained at 37 to 38° c. During basti course (8days) rats were provided with 100 g. Of food pellets and 100 ml. of warm water (37° c). On 8th day one hour after drug administration experiments enumerated above were carried out. The following is the experimental details of the tests conducted.

Experimental models

1. Anticonvulsant activity [Supramaximal electric shock induced convulsions] [16]
2. Tremors induced by Oxetremerine. [17]
3. Animal's performance on Rota rod instrument. [18]
4. Gross Behavior. [Clara Morpurgo protocol] [19]
5. Anxiety /Anti-anxiety activity. [Elevated plus maze test] [20]
6. Antidepressant activity. [Behavioral 'despair' test] [21]
7. Spontaneous motor activity with exploratory behaviour assessment. [22]
8. Effects on conditions avoid response on Cooks pole climbing apparatus. [23]
9. Memory [Morris Maze test for learning and memory assessment] [24]
10. Anti- Reserpine test [25]

Statistical Analysis

Analysis of Data was done with one way ANOVA followed by Dunnet multiple comparison T test with Post hoc test using "Graph pad Instat software".

Table 1: Effect of Bastikarma on behavioral 'despair, in rats

Groups	Parameters recorded during 4 minutes	
	Duration of escape activity	Duration of immobility (Sec)
	mean ±SEM	mean ±SEM
Control group	106.16 ± 09.26	133.83 ± 09.26
Standard group (Mandooka parni)	155.50 ± 07.40**	84.50 ± 07.40 **
Matra basti group	154.66 ± 11.83**	85.33 ± 11.83 **
Madhutailika basti group	188.00 ± 09.46**	52.00 ± 09.46 **
** P<0.01 in comparison to the control group		

Table 2: Effect of basti procedure on open filed behavior test

Groups	Number of squares crossed by rats			Rearing
	Outer Squares	Middle squares	Inner squares	
	mean ±SEM	mean ±SEM	mean ±SEM	mean ±SEM
Control group	52.50 ± 16.36	5.00 ± 3.90	0.83 ± 0.83	09.33 ± 2.23
Standard group	84.83 ± 14.40	2.76 ± 1.97	1.66 ± 1.17	08.66 ± 1.63
Matra basti group	52.33 ± 10.59	3.33 ± 2.39	1.33 ± 0.88	15.50 ± 1.82
Madhutailika basti group	37.50 ± 14.29	0 ± 0	0 ± 0	16.83 ± 4.23

Table 3: Effect of test treatment on SMA in rats

Groups	Horizontal movements (X-axis)	Vertical movements (Y –axis)	Total count X + Y
	mean ±SEM	mean ±SEM	mean ±SEM
Control group	191.83 ± 22.937	199.5 ± 27. 055	392 ± 49.831
Standard group	168.166 ± 29.943	144.33 ± 33.699	312.5 ± 62.852
Matra basti group	192.33 ± 10.509	195 ± 16. 223	387.33 ± 26.222
Madhutailika basti group	165.33 ± 30.060	164.83 ± 31.839	331.83 ± 51.068
** P<0.01 in comparison to the control group			

Table 4: Effect of bastikarma on learning behavior in Morris water Maze

Groups	Time taken to reach the Platform (sec)			
	North	West	South	East
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	19.5 ± 4.794	15 ± 2.388	8.5 ± 1.278	9.5 ± 1.839
Standard	23.2 ± 5.78	7.6 ± 2.482	18.4 ± 5.938	15 ± 8.55
Matra basti group	24.16 ± 6.61	8 ± 2.82	12 ± 4.275	13 ± 8.02
Madhutailika basti	12.33 ± 3.05	16.66 ± 4.393	10.33 ± 4.47	13.16 ± 5.695
** P<0.01 in comparison to the control group				

Table 5: Effect of bastikarma on scopolamine induced amnesia with respect to jumping response in rats trained with Cook's pole climbing apparatus

	Latency of jumping on to centrally placed pole (sec)		
Group	Before scopolamine	After scopolamine	P values
	Mean ± SEM	Mean ± SEM	
Control	2.33±0.494	9.66 ± 2.801	0.0183*
Standard	2.33±0.33	4.66 ± 0.33	0.0005**
Matra basti	1.5±0.223	1.16±0.166@@	0.0873
Madhu tailka basti	1.166±0.421	1.833±0.401@@	0.3856
P<0.05*, p<0.01* in comparison to before scopolamine administration (Paired "t' test) @@@ P<0.01 in comparison to the control group			

Table 6: Effect of bastikarmas on establishment and retaining of Conditioned avoidance reflex (CAR) and unconditioned avoidance reflex (UR) in rats (Cook's pole climbing apparatus)

Groups	Number of rats showing blocking of CAR and UR	
	CAR block	UR block
Control (Dizepam treated)	6/6	6/6
Reference standard Plus diazepam	6/6	2/6
Matra basti Plus diazepam	2/6	0/6
Madhutailika basti Plus diazepam	2/6	0/6

Table 7: Effect of basti karmas against Supramaximal Electric shock induced convulsions in rats

Groups	Different parameters measured in Elevated plus maze test during 5 minutes				
	Latency to enter open tunnel	No of entry into closed arm in sec	No of entry into open arm in sec	Time spent in open arm in sec	Time spent in closed arm in sec
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control Group	8.00 ± 2.38	3.00 ±0.07	4.83 ±0.87	16.8 ± 2.92	278.6 ±5.43
Standard Group	9.66 ± 4.24	3.33 ± 0.76	4.33 ± 1.50	36 ± 2.273	268.1 ± 13.08
Matrabasti Group	8.16 ± 1.68	3.17 ±0.74	9.16 ±1.22*	51.6 ± 6.4**	211 ± 26.68
Madhutailika basti Group	7.16 ± 1.42	3.00 ± 0.86	5.50 ± 3.11	62.5 ± 13.7**	239.8 ± 38.92

Table 8: Effect of bastikarmas on different parameters measured in elevated plus maze

Groups	Duration of the different phases of convulsion in sec			
	Flexion	Extension	Clonus	Stupor
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control group	4.5 ± 1.232	4.66 ± 1.145	17.5 ± 7.932	350 ± 123.7
Standard group	3.33 ± 0.980	5 ± 2.295	0.0 ± 0.0 *	510 ± 167.39
Matra basti group	4.16 ± 1.076	3.83 ± 1.122	2.5 ± 0.922*	210 ± 55.317
Madhutailika basti	5 ± 1.211	4.33 ± 1.563	0.5 ± 0.500*	530 ± 117.39

*P<0.05 in comparison to the control group

Table 9: Effect of bastikarmas on the performance of rats on the rota rod (Phase- I)

Groups	Time spent	Attempts
	Mean ± SEM	Mean ± SEM
Control group	141.83± 22.21	4.33 ± 0.494
Matra basti gropup	167.83 ± 12.17	2.67 ± 0.614
Madhutailka basti group	156.00 ± 24.00	2.40 ± 1.077
*P<0.05 in comparison to the control group		

Table 10: Effect of bastikarmas on the performance of rats on the rota rod (Phase- II)

Groups	Time spent	Attempts
	Mean ± SEM	Mean ± SEM
Control group	106.33 ± 33.64	4.66 ± 0.21
Matra basti group	170.00 ± 10.00	1.66 ± 0.80*
Madhutailka basti group	168.00 ± 12.00	2.20 ± 0.97

Table 11: Effect of basti karmas on reserpine (2.5 mg/kg ip) induced ptosis, sedation and catatonia in rats

Groups	Ptosis		Sedation		Catatonia	
	Total	4th h	Total	4th h	Total	4th h
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control Group	11.16 ±0.59	1.8 ± 0.02	2.20 ±0.90	1.08 ±0.30	6.83 ± 1.38	0.92 ±0.46
Standard group	7.6±1.15**	1.50 ±0.31	3.25 ±1.68	0.50 ±0.50	3.16 ± 1.76	0.67 ±0.49
Matra basti group	0.8 ±0.21**	0.3 ±0.11**	5.66 ±0.36	1.60 ±0210	1.75 ± 0.49*	0.58 ±0.15
Madhu tailika basti group	0.7 ±0.34**	0.0 ±0.0**	3.08 ±0.78	0.833 ±028	1.1 ± 0.61**	0.25 ±0.17

*P< 0.05 , ** p<0.01* in comparison to the control group

Table 12: Effect of basti karmas on reserpine (2.5 mg/kg ip) induced hypothermia in rats

Groups	Rectal temperature measured at different time interval after reserpine injection			
	Basal	4th h	24th h	Difference in temp b/f & a/f reserpine
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control group	37.2 ± 0.16	36.68 ± 0.61	37.47 ± 0.18	0.266±0.23
Standard group	37.6 ± 0.018	37.65 ± 0.23	38.55 ± 0.23**	1.03±0.133*
Matra basti group	37.93 ±0.243	38.33 ± 0.30*	38.21 ± 0.21*	0.26±0.2813
Madhutailika basti group	38.6 ±0.26**	38.90 ± 0.27**	37.91 ± 0.16**	0.33±0.2813
P<0.05*, ** p<0.01 in comparison to the control group				

Table 13: Effect of test procedures on oxotremorine (500 µg/kg ip) induced tremors

Groups	Tremors	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	5.66 ± 0.33	1.00 ± 0.00
Standard group	4.33 ± 0.21*	0.83 ± 0.17
Matra basti group	1.83 ± 0.41**	0.00 ± 0.00**
Madhutailika basti group	3.33 ± 0.33**	0.83 ± 0.17
*p<0.05 , ** p<0.01* in comparison to the control group		

Table 14: Effect of test procedures on oxotremorine (500 µg/kg ip) induced Head twitches

Groups	Ataxia	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	5.17 ± 0.20	0.66 ± 0.21
Standard group	4.33 ± 0.67	0.33 ± 0.21
Matra basti group	2.16 ± 0.54**	0.33 ± 0.21
Madhutailika basti group	2.66 ± 0.21**	0.00 ± 0.00*
*p<0.05 , ** p<0.01* in comparison to the control group		

Table 15: Effect of test procedures on oxotremorine (500 µg/kg ip) induced Ataxia

Groups	Head twitches	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	3.83 ± 0.31	0.67 ± 0.21
Standard group	3.33 ± 0.21	0.67 ± 0.21
Matra basti group	0.50 ± 0.22**	0 ± 0*
Madhutailika basti group	0.66 ± 0.21**	0 ± 0*
*p<0.05 , ** p<0.01* in comparison to the control group		

Table 16: Effect of test procedures on oxotremorine (500 µg/kg ip) induced Lachrymation

Groups	Lachrymation	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	3.50 ± 0.22	0.66 ± 0.21
Standard group	1.66 ± 0.33**	0.33 ± 0.21
Matra basti group	0.66 ± 0.33**	0.00 ± 0.00*
Madhutailika basti group	0.50 ± 0.22**	0.16 ± 0.17
*p<0.05 , ** p<0.01* in comparison to the control group		

Table 17: Effect of test procedures on oxotremorine (500 µg/kg ip) induced Diarrhoea

Groups	Diarrhoea	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	3.66 ± 0.55	0.17 ± 0.17
Standard group	2.00 ± 0.44*	0.0 ± 0.0
Matra basti group	2.83 ± 0.17	0.0 ± 0.0
Madhutailika basti group	4.33 ± 0.55	1.33 ± 0.42**
*p<0.05 , ** p<0.01* in comparison to the control group		

Table 18: Effect of test procedures on oxotremorine (500 µg/kg ip) induced Salivation

Groups	Salivation	
	Total score for all the periods	Peak activity score 40 min
	Mean ± SEM	Mean ± SEM
Control group	5.66 ± 0.33	0.83 ± 0.17
Standard group	4.00 ± 0.37*	0.17 ± 0.17*
Matra basti group	3.66 ± 0.21**	0.00 ± 0.00**
Madhutailika basti group	3.50 ± 0.62**	0.50 ± 0.22
*p<0.05 , ** p<0.01* in comparison to the control group		

OBSERVATIONS AND RESULTS

Gross Behavior test

No change in the behavior could be observed in any animal in any of the groups included for this test. None of the parameters meant to assess CNS depression, CNS stimulation, ANS activity and analgesia were affected**.** Further the basti treatment did not produce any other type of miscellaneous or abnormal behavior during this test.

Behavioral 'despair' test

The data related to the effect of test procedures on duration of rat immobility in the behavioural despair test can be found in Table 1. Statistically significant decrease in the duration of rat immobility was observed in standard, matra basti and Madhutailika basti given group in comparison to the normal control group. The reduction in the first two groups is almost of similar magnitude (36 to 37 %). The shortening effect was higher in Madhutailika given group being 61.41 % less in comparison to the control group.

Open field behavior test

Table 2 contains data related to the effect of test procedure on the open field activity profile. In reference standard group an apparent increase in the number of outer squares crossed was observed. However, the observed increase did not reach statistically significant level. No effect could be observed on this parameter in matrabasti administered group while in madhutailika basti given group a moderate but statistically non-significant decrease was observed in comparison to the control group. The number middle squares entered was less and number of inner squares entered was more in the standard group in comparison to the control group. No effect could be observed on rearing in this group. In matrabasti administered group marginal decrease in the middle squares entered, increase in the number of inner squares and increase in the number of rearing were observed in comparison to the control group. However, none of the above-mentioned changes were found to be statistically significant. In madhutailika basti administered group the number of middle and inner squares entered were nil. Though increase in the number of rearing was observed in comparison to the control group but it was found to be statistically non-significant.

Effect on spontaneous motor activity (SMA)

Table 3 depicts data related to the effect of test treatment on SMA in rats. In reference, standard group and madhutailika basti group an apparent decrease in vertical, horizontal and total counts was observed in comparison to the control group. However, the observed decrease was found to be statistically non-significant. In matrabasti group no change in SMA could be observed in comparison to the control group.

Memory test using Morris water Maze

Table 4 contains data related to the effect of basti karmas on learning behavior of rats in Morris water Maze. The duration required to locate the hidden platform was found to be marginally prolonged in standard irrespective of positioning of the rats in north, west, south or east quadrant in comparison to the control group. Similar tendency was observed in matrabasti administered group except for the data recorded by placing the rat in the west quadrant; the latency to find the platform was found to be shortened. Marginal prolongation when placed in west, south and east quadrants was observed in madhutailika basti administered group in comparison to the control group. However, the observed differences were found to be statistically non-significant.

Table 5 provides data related to the effect of test procedures on scopolamine induced amnesia in rats. Administration of scopolamine lead to four-fold and statistically significant increase the latency of jumping response in comparison to latency recorded before scopolamine. In standard administered group two-fold increases was observed. The increase observed in this group was significantly less in comparison to the control group. In matrabasti and madhutailika basti administered groups there was no significant difference in the latency before and after scopolamine indicating complete antagonism of the scopolamine effect.

Table 6 provides data related to the effect of test basti's on establishment and retaining of conditioned and unconditioned avoidance reflex. In control, trained rats in which diazepam was administered exhibited blockade of both CAR and UR in 6/6 rats showing the efficacy of the treatment in blocking the training established memory and also severe sedative effect. In reference standard given group CAR was blocked in all the 6 rats while UR was blocked in only 2/6 rats. In matra basti and madhutailika basti administered groups only 2/6 rats exhibited blocking of CAR while UR was not blocked in any of the animals studied.

Table 7 contains data related to the effect of basti karma on convulsion profile in rats subjected to MES seizures. In standard group a marginal decrease in flexion phase, marginal increase in extension phase, complete antagonism of clonic phase and moderate increase in the duration of stupor was observed in comparison to the control group. However, except for clonus other changes were found to be statistically non-significant. In Matra basti administered group a non-significant decrease in flexion, extension phases and duration of stupor was noted in comparison to the control group. However, significant shortening of the duration of clonic seizures was noted. Madhutailika basti group a marginal increase in flexion phase marginal decrease in extension phase and a moderate increase in the duration of stupor were noted in comparison to the control group, however, none of these changes were found to be statistically significant. The duration of clonic phase was found to be markedly reduced in this group.

Table 8 depicts data related to the effect of basti karma's on different parameters measured in Elevated plus maze. The latency to enter open tunnel was remarkably shortened in reference standard and test basti given groups in comparison to the control group. However, this difference did not materialize into any statistically significant difference due to variation in the data. The number of entries in to the closed tunnels was not affected in any of the treatment groups in comparison to the control group. Numbers of entries in to the open arm were increased in Matra basti group & Madhu tailika basti group in reference to standard group & Control group, a significant increase was observed in matra basti group & Madhutailika basti group while in matrabasti given group the number of entries more than doubled. This increase was found to be statistically significant. In both basti group the duration spent in the open tunnel was found to be increased about standard group & the control group. The observed increase was found to be statistically highly significant. The time spent in closed tunnel showed a marginal non-significant decrease in treatment groups in comparison to the control group.

The effect of basti karma's on the performance of rat on rota rod during phase I training period can be found in Table 9 In both the basti karma given group the duration of stay on the rota rod was found to be moderately prolonged and the numbers of attempts were also less. However, the observed difference was found to be statistically non-significant. (Table 9)

The effect of basti karma's on the performance of rat on rota rod during phase II training period can be found in Table 10. In both the basti karma given group the duration of stay on the rota rod was found to be moderately prolonged and the number of attempts was also less. However, the observed difference was found to be statistically non-significant with respect to duration of stay on the rota rod. The decrease observed in the number of attempts required was significantly less in matrabasti given group in comparison to the control group. (Table 10)

Anti-parkinsonian activity

Anti Reserpine test

Table 11 contains data related to the effect of test procedures on reserpine induced ptosis, sedation and catatonia. In both the basti groups there was remarkable antagonism of the reserpine induced ptosis both at 4 th hour post reserpine injection period and average of total scores. The observed reversal was found to be statistically significant in comparison to the control group. Similar but less magnitude reversal of the ptosis was observed in reference standard group. Sedation was not affected to significant extent. Though decrease was observed for the 4th hour recording in reference standard and madhutailika basti administered group the decrease was found to be statistically non-significant. The catatonia score for the entire period was found to be significantly decreased in both the basti administered groups in comparison to the control group. In reference standard group a moderate but statistically non-significant decrease was observed. Decrease in catatonia was observed for 4th hour readings in all the treatment groups in comparison to the control group. However, the decrease was found to be statistically non-significant.

Oxotremorine test

Table 13 contains data related to the effect of test procedures on oxotremorine induced tremors. In reference standard group a moderate but statistically significant decrease in total tremor score was observed in comparison to the control group. In matrabasti a remarkable 66.67% statistically significant decrease in oxotremorine total tremor score was observed in comparison

to the control group. In madhutailika basti group also a moderate and statistically significant decrease in total tremor score was observed in comparison to the control group. Apparent decrease was also observed in the peak tremor score recorded at 40 min post oxotremorine injection. But only the decrease observed with matrabasti was found to be statistically significant ($p<0.01$).

Table 14 contains data related to the effect of test procedures on oxotremorine induced head twitches. In reference standard group a moderate and statistically non-significant decrease in total head twitch score was observed in comparison to the control group. In matrabasti a remarkable 96.97% and statistically highly significant decrease in oxotremorine total head twich score was observed in comparison to the control group. In madhutailika basti group also similar statistically significant decrease in total head twitch score was observed in comparison to the control group. Complete inhibition of the head twitches was observed at the 40-min observation period which is considered to be the peak score period

Table 15 contains data related to the effect of test procedures on oxotremorine induced Ataxia. In reference standard group a moderate and statistically non-significant decrease in total ataxia score was observed in comparison to the control group. In matrabasti a more than 50% and statistically highly significant decrease in oxotremorine total ataxia score was observed in comparison to the control group. In madhutailika basti group also similar statistically significant decrease in total ataxia score was observed in comparison to the control group. In reference standard and matrabasti administered groups an apparent 50% decrease in peak ataxia score recorded at 40 min observation period was observed in comparison to the control group. In madhutailika basti complete inhibition of the peak period ataxia score was observed.

Table 16 contains data related to the effect of test procedures on oxotremorine induced lachrymation. In reference standard group a moderate and statistically significant decrease in total lachrymation score was observed in comparison to the control group. In both the matrabasti and madhutailika basti group's statistically highly significant decrease in oxotremorine total lachrymation score was observed in comparison to the control group. The peak period (40 min observation period) score was found to be decreased in all the treated groups however, only the decrease observed in matrabasti treated group was found to be statistically significant.

Table 17 contains data related to the effect of test procedures on oxotremorine induced diarrhoea. In reference standard group a moderate and statistically significant decrease in total diarrhoea score was observed in comparison to the control group. In matrabasti group an apparent moderate and statistically non-significant decrease in total diarrhoea score was observed in comparison to the control group. In Madhutailika basti group a moderate and statistically non-significant increase was observed in comparison to the control group. In reference standard and matrabasti groups complete inhibition of the peak diarrhoeal score was observed in comparison to the control group. In madhutailika basti administered group a significant increase in peak diarrhoeal score was observed in comparison to the control group.

Table 18 contains data related to the effect of test procedures on oxotremorine induced Salivation. In reference standard group and the test basti administered groups a moderate and statistically significant decrease in total salivation score was observed in comparison to the control group. The peak period score was also found to be decreased in these groups. However, the observed decrease was found to be. Statistically significant only with respect to the decrease observed with reference standard and matrabasti administered groups.

DISCUSSION

The main objective of carrying out this study was to determine the influence of Matra basti (MB) and Madhu tailika basti (MTB) on Psychoneuro-pharmacological activity profile. This was done in the light of the fact that clinically panchakarma therapy is being used extensively for treating neurological and psychoneurological disorders often with good results. However, no objective data are available to support this observation. One of the neurodegenerative condition in which reasonably good effect has been observed clinically is Parkinsonian disorder. Taking this fact in to consideration the present study aimed at assessing the anti-parkinsonian activity of the test basti against well-known experimental models. Since it is the first of its kind of study it was thought worthwhile to assess the test procedures for other important psychoneuro-pharmacololgical effects as enumerated below. The discussion can be initiated by enumerating the results obtained and their implications and probable mechanisms of action.

Assessment of the data generated shows that both Matra basti and Madhu tailika basti have no effect on general gross behavior. Both MB and MTB produced highly significant anti-depressant effect in behavioural 'despair' test. Both produced significant anti-anxiety activity in elevated plus maze test. In open field apparatus test a complex behavior pattern was observed. Both the basti procedures increased rearing the sign of lessening of the anxiety but the pattern of movement in the outer and inner circles was not affected. In MTB a moderate decrease in the total number of squares crossed was observed. MB had no effect on SMA while MTB reduced both horizontal and vertical movements in activity cage. In the memory and learning related test both the test procedures did not affect the learning process significantly but both produced remarkable reversal of the amnesic effect of scopolamine- this indicates memory enhancing effect. Similarly, anti-amnesic effect was observed in CAR based protocol also. The test procedures did not possess significant anti-convulsant activity. Both improved the performance of the rats on the rota rod. Both the test procedures produced good anti-parkinsonian activity.

From the above briefing it becomes clear that the test procedures possess complex activity profile on CNS which is not easily categorizable in to any typical types like other CNS active pharmacotherapeutic agents. Another surprising point to be noted is in spite of producing complex behavioural changes in different test paradigm they did not affect general gross behavior. This implies that there will not be any untoward side effects. The above activity profile would be further discussed in detailed manner with test specific focus in the following paragraphs.

Table 19: Consolidated statement of Pharmacological activities of Matra basti & Madhu tailika basti

Sl. No	Name of the Test profile	*Matra basti group*	*Madhu tailika basti group*
General CNS Effects			
1]	Gross behaviour	No change	No change
2]	Spontaneous motor activity	No change	Statistically non-significant decrease
Anti-depression activity			
1]	Anti-depression activity	Highly significant *	Highly significant*
Anti-Anxiolytic activity			
1]	Open field behaviour test	No effect	No effect
2]	Elevated plus maze	Highly significant	Highly significant
Memory test			
6]	1]Morris Maze test	Statistically not significant	Statistically not significant
7]	2] A]Cooks pole climbing jumping response	No effect in the latency B /f & A/f Scopolamine	No effect in the latency B /f & A/f Scopolamine
	B]CAR & UR Blocked	Reversal of diazepam induced CAR blockade in 4/6 rats without and prevented diazepam induced blockade of UR	Reversal of diazepam induced CAR blockade in 4/6 rats without and prevented diazepam induced blockade of UR
8]	Anti-convulsion activity	Not significant Flexion , Extension & duration* Significant shortening of clonic phase.	Marginal Flexion Marginal Extension Moderate Duration of stupor. Markedly reduced clonic phase.
9]	Rota rod Phase – 1	Duration of stay on rota rod was found to be moderately prolonged. Number of attempt was less.	Duration of stay on rota rod was found to be moderately prolonged. Number of attempt was less.
	Phase – 2	Statistically not significant	Statistically not significant
Anti-Parkinson's activity			
1]	Oxetremorin test Head twitches Ataxia Lachyrmation Diarrhoea Salivation	 Highly significant Statistically significant Statistically significant Moderate & Statistically not significant Moderate & Statistically significant	 Highly significant Statistically significant Highly significant Moderate & Statistically not significant Moderate & Statistically significant
2]	Anti-reserpine test Catatonia, ptosis, sedation and Hypothermia	Statistically significant	Statistically significant
*compared to control group			

Effect on Gross behavior

This is the primary screening test normally employed in the initial stages of pharmacological screening, to obtain information about the type of activity that a given drug likely to produce. In this study, Gross behaviour test did not show any apparent changes in the behavioral profile in both Basti groups. None of the parameters meant to assess CNS depression, CNS stimulation, ANS activity and analgesia were affected. Further the basti treatment did not produce any other type of miscellaneous or abnormal behavior during this test. So, this study clearly indicates that Matra basti & Madhu tailika basti group is not affecting the behavioural profile. This is important considering that both procedures produce a constellation of important CNS effects. No effect in this test implies that the procedures may not produce any gross CNS related untoward effects.

Effect on tests carried out to assess anti – depression activity (Behavioral 'despair' test)

This is the primary test for the screening of anti-depressant activity in a test drug. All the classical anti-depressants produce decrease in the duration of immobility. In the present study Both Matra basti & Madhutailika basti group had shown significant anti depression activity in behavioral despair test. This clearly indicates that Both Matra basti & Madhu tailika basti possess anti-depression effect. This is a significant finding especially in considered in the background that in parkinsonian disorder some amount depression is likely to be presented.

The behavioral despair test is based on the principle of measurement of the duration of immobility when rodents are exposed to an inescapable situation. The majority of clinically used antidepressants decrease the duration of immobility. Serotonergic neurotransmitter dysfunction has been shown to be associated with the negative symptoms generally seen in schizophrenia. Atypical anti-depressants like clozapine improve these negative symptoms- this is attributed to their strong 5-HT receptor antagonism. The first generation of anti-depressants act by elevating the turnover of biogenic amines especially noradrenaline and serotonin. Next generation involved developing drugs with specific modulation of serotonin uptake inhibition. Another latest development has been the introduction of the noradrenergic and specific serotonergic antidepressant mirtazapine. Its antidepressant effect appears to be related to dual enhancement of central noradrenergic and serotonergic neurotransmission by blockade of alpha2-adrenoceptors. In addition, mirtazapine directly blocks 5-HT2 and 5-HT3 receptors, which may account for its anxiolytic and sleep-improving properties as well as its lack of adverse events that are typical of SSRIs. This is an interesting profile and it would be interesting to ascertain what would be the influence of test basti procedures on the above parameters.

Glutamate receptors especially NMDA subtype may be another candidate target for developing anti-depressant drugs. NMDA antagonists are being evaluated for possible use as anti-depressants especially through their effect on dorsal hippocampus which is the main site of action of several anti-depressants[26]. It is to be analyzed whether any gut hormones influence the above neurotransmistter systems and then we can look in to possible effect of basti treatment on the turnover of these neurotransmitters. What is beyond doubt is that there is every likely hood of the test basti's modulating the above factors. The possible role of gut-brain axis in CNS effects has been discussed below.

Effect on Anti-Anxiolytic activity
Open field behavoiur

This test procedure is employed as one of the supplementary test to assess anti-anxiety activity along with assessment CNS stimulation or depression. This protocol provides a supplementary evidence of anxiety in animals as well as anti-anxiety effect of the drug. The immobility or exploratory behavior indicated by number of squares crossed, the duration of freezing and episodes of defecation are taken as index of anxiety. No effect could be observed on this parameter in matrabasti administered group and while in madhutailika basti given group a moderate but statistically non- significant decrease was observed in comparison to the control group. In matrabasti administered group marginal decrease in the middle squares entered, increase in the number of inner squares and increase in the number of rearing were observed in comparison to the control group. However, none of the above-mentioned changes were found to be statistically significant. In madhutailika basti administered group the number of middle and inner squares entered were nil. Though increase in the number of rearing was observed in comparison to the control group but it was found to be statistically non-significant. The above activity profile indicates a complex effect on the CNS. As mentioned earlier both the basti procedures increased rearing the sign of lessening of the anxiety but the pattern of movement in the outer and inner circles was not affected. Thus, the data generated in this protocol did not provide unequivocal evidence for the presence of anti-anxiety activity.

Elevated plus maze test

Elevated plus maze test is the primary test for assessing anti-anxiety activity of any therapeutic measure. It provides a valid and reliable measure of anxiety in animals as well as anti-anxiety effect of the drug. Anxiety is represented by the tendency of the animal to avoid open tunnel and spend more time in closed tunnels and make less entries in to the open channel. If any therapeutic measure changes the above pattern and if the animal spends more time in open tunnel in comparison to control group and makes more entries, it would be considered as index of anxiety.

In the present study numbers of entries in to the open arm were increased in both Matra basti & Madhu tailika basti groups in reference to standard and control groups, a significant increase was observed in both the groups while in matrabasti given group the number of entries more than doubled. This increase was found to be statistically significant. In both basti groups the duration spent in the open tunnel was found to be increased in reference to standard and the control groups. This profile clearly indicates that both test groups possess significant anti-anxiety activity. The following can be the probable mechanism of action.

Anxiety is a complex behavior. Many neurotransmitters have been shown to influence it.GABA-A, serotonin, dopamine, nor-adrenaline, corticotropin-releasing factor (CRF), tachykinins, glutamate are some of the important neurotransmitter shown to be involved. Involvement of GABA receptors in the expression of the anxiolytic activity of benzodiazepines and barbiturates has been shown. Benzodiazepines bind to GABA (a) receptor chloride complex to bring about the hyperpolarization of the involved neurons. The barbiturates act by enhancing gamma-aminobutyric acid (GABA) activity, by binding to the barbiturate site at the GABA-receptor complex. This binding interferes with transmission of impulses from the thalamus to the cortex of the brain resulting in depressed CNS activity. Alpha1 subunits are more associated with sedation whereas Alpha2 subunits are associated with anxiolytic properties. Alpha1 subunits are expressed in cortex, cerebellum and brainstem and Alpha2 subunits are lcoated in the limbic system (hippo.parahippo) and frontal cortex. It is not known whether GABA-A is involved in the anxiolytic activity observed in the present study. Since no sedation was observed this neurotransmitter may not have a major role to play. Conceptually speaking GABA receptor mediated mechanism is one of the possibilities but no sedation or any other CNS depression could be observed with the test basti procedures hence this aspect needs to be ascertained through further studies.

Serotonin or 5-hydorxytrypamine has long been viewed as a neurotransmitter involved in regulating emotional states. Of the 14 or so mammalian serotonin receptor subtypes that have been described in the literature, at least four have been implicated in anxiety in various animal models [27,28] This was based on the observation that decreased serotonin level leads to increased anxiety. Of the four receptor subtypes 5-HT1A has been studied extensively. It is an auto receptor located pre-synaptically on the serotonergic neurons. On stimulation, this receptor inhibits synthesis and secretion of 5-HT. Busiperone which is a strong antagonist of this receptor sub-type exhibits strong anxiolytic activity [29] This effect is produced without producing sedation like benzodiazepines. Hence this has become drug of choice for the treatment of human generalized anxiety disorder. It is pertinent to point out that both the test procedures produced anti-anxiety activity without producing sedation this suggests the possibility that the test basti procedures may share similar mechanism.

Other 5-HT receptors that have been implicated in anxiety include 5-HT2A, 5-HT2C, and 5-HT3 receptor sub types. Antagonists for the 5-HT2Areceptor, like ritanserin, exhibit anxiolytic effects in some animal models. Likewise, blockade of the 5-HT2C receptor produces anxiolytic effects in some animals. 5-HT3receptor antagonist ondansetron was reported to be anxiolytic in some animal models. In the present study both the test procedures produced anti-anxiety activity and both did not affect gross behavior or spontaneous motor activity indicating that they do not have the tendency of producing sedation or CNS depression. This opens up area for further study to assess their effect on different 5-HT related receptor mechanism.

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide. It has been reported to play an important role in mediating the body's physiologic and behavioral responses to stress. A CRF-1 antagonist is believed to have good potential to be an anxiolytic however no such drug has been introduced in the market. CRF injection caused anxiety like state (John F Tallman- 2002). It would be interesting to ascertain whether the test basti procedures modulate the activity of CRF.

Glutamate receptors are classified as either ionotropic or metabotropic. Ionotropic receptors, which mediate fast synaptic transmission, are coupled to cation-specific ion channels and bind the agonists *N*-methyl-D-aspartate (NMDA), _-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA). These receptors gate both voltage-dependent and voltage-independent currents carried by Na^+, K^+ and Ca^+ ions. Antagonists of AMPA receptors have also been proposed to have anxiolytic actions in preclinical models. It needs to be ascertained what would be the effect of test basti procedures on these receptor mediated effects.

Tachykinins collectively refer to a group of small peptides that include substance P (SP), neurokinin A (NK-A), and neurokinin B (NK-B). They show affinity to three receptors known as NK-1, NK-2, and NK-3, respectively and are linked to G-protein coupled receptor family. Among the three NK-1 and NK-3 are found in the brain, whereas NK-2 is primarily localized peripherally in smooth muscle of the respiratory, urinary, and gastrointestinal tracts. Neurokinin receptors are localized in a number of different brain areas which are supposed to play major role in anxiety, including the amygdala, hypothalamus, and locus coeruleus[30]. GI tract is known to produce many brain influencing hormones. Considered from this angle it would be interesting to see what effect the basti karma has on the activity mediated by these receptor systems.

Recent studies have shown that gut hormones play important role in regulating brain functions. Relation of gut brain starts with bidirectional communication between the gut and the brain. Carriers responsible for communication process are vagal and spinal afferent neurons, immune mediators such as cytokines, gut hormones and gut microbiota-derived signaling molecules, transmit information from the gut to the brain. The influence of the basti karma on different aspects of these neuronal and neurohumoral communication systems needs to be further studied.

Relation of neuropeptide Y and peptide YY are expressed by cell systems at distinct levels of the gut–brain axis. In addition, PP and PYY signal to the brain to attenuate food intake, anxiety and depression-related behavior [31]

Thus, there is tremendous potential to study the effect of basti karma effect on the above parameters and their implications in the management of different neurodegenerative disorders through basti karma.

Another point of interest to be noted that the test basti procedures produce both anti-depressant and anxiolytic activity. There are clinical conditions in which depression and anxiety exist as co-morbidity. Fluoxetine which is initially introduced as an effective anti-depressant has been found to have both anti-depressant and anti-anxiety activity. It proved to be effective in the treatment of this kind of co-morbidity [32] In addition, mice chronically injected with fluoxetine displayed antidepressant- and anxiolytic-like behaviors [33], and suggesting depression and anxiety might share common neural substrates. Thus, the presence of both anxiolytic and anti-depressant activity in the test basti can be a major therapeutic advancement in the treatment of such co-morbidities.

Effect on learning and memory assessment
Morris Maze test

Morris Maze test is used to assess the effect of test drug on learning and memory. It can be used to assess effect of test procedures on both learning and memory. Learning is assessed by noting down how quickly the test procedure given animals learn to identify a hidden platform. Memory can be tested by causing amnesia by the administration of amnesic agent like scopolamine or using memory fading techniques. In this protocol the test procedures failed to enhance learning behavior. However, produced remarkable reversal of the scopolamine induced amnesic effect. This is evident by more time taken by scopolamine administered rats to identify the hidden platform and its reversal by the test procedure. The mechanism underlying these effects needs to be elucidated through further studies.

Cooks pole climbing jumping response

This procedure is used to separate neuroleptics from sedatives and anxiolytics. Whereas sedative compounds suppress both avoidance and escape responding at approximately the same doses, neuroleptic drugs reduce avoidance responding at lower doses than those affecting escape responding. Though the primary use of this test is to assess the anti-pscyhotic activity, it is also made use for assessing the drug effect on learning and memory. In this protocol the animals are trained to establish conditioned avoidance response. The effect of test drug on the learning pattern that is how quickly the rat's exhibit establishment of CAR can be used to assess the learning enhancement of the test procedure. Learning enhancement is reflected in the form of less trial requirement for the establishment of CAR and less latency to jump on to the pole to escape electric shock. For assessment of effect on memory the animals trained to establish CAR (escape of the animal on to the central pole on presentation of cues like buzzer sound or flashes of light) was given diazepam which produces retrograde amnesia. Diazepam treatment not only abolishes CAR it also affects the unconditioned reflex that is jumping of the animal on to the pole after receiving shock as a sequel to failure to perceive the cues.

Learning process was not influenced to significant extent by the test procedures. However, remarkable effect could be observed with regards to reversal of amnesic effect of diazepam. In diazepam administered group both CAR and UR were inhibited in 6/6 rats in the group. In both the basti given group only 2/6 rats exhibited loss of CAR and while UR was not affected in any of the animal. This clearly indicates that the test procedures have remarkable memory enhancing effect or anti-amnesic activity.

Memory establishment and retrieval is among the most complex biological phenomenon. The exact manner in which information is perceived, processed and stored as memory and retrieved is yet to be elucidated in satisfactory manner. Learning and memory form the very foundation of our ability to habitually function within our surroundings. Learning can be defined as the experience-dependent acquisition of knowledge and skills. Memory is the process of retention and retrieval of facts or events composed of experiences. Through experience animal learn and this information is stored in the form of short term and long term memories. Short term memory (STM) indicates the small limited ability to store small amounts of information with reference to small time dependent events. Long term memory (LTM) develops over a period and involves formation new proteins and RNA and the capacity for storage of such information is tremendous. Further there is process of reconsolidation to take care of instability that may arise because of period of disuse. Thus, the memory process involves acquisition of information (which can be termed as learning), consolidation (that is conversion of labile to stable memories) and retrieval (memory recall).

Many physiological phenomenon's have been suggested as the basis for memory acquisition, storage and recall. Alterations in protein synthesis, gene expression and structural properties of neurons and synapses contribute to memory consolidation, storage and retrieval. In the neuron, synaptic depolarization activates complex molecular signaling cascades that coalesce on specific gene loci, resulting in acute modulation of transcriptional efficacy. This result in formation of new proteins which are thought to produce stable alterations in cellular phenotype by influencing the structure and physiology of postsynaptic dendritic spines.[34]

Further the plasticity of the neuronal network is considered very important along with the phenomenon of Long term potentiation (LTP). Both hippocampus and non-hippocampal brain areas are found to be involved in this phenomenon. The focus is on elucidating the role of different cellular receptor systems and enzymatic process involved in this phenomenon. LTP formation has been shown to involve cascade of cellular receptors and second messenger systems. Among them NMDA and AMPA receptors have been found to play main role. Learning activated molecular signalling cascades including NMDA receptors, Ca MK II (calcium calmodulin dependent protein kinase II), PKC (Protein Kinase C), PKS- Protein Kinase A, new protein synthesis and CREB (c-AMP responsive element binding protein) mediated gene expression play major role in the structural modification that occurs as a sequel to initiation of memory process. There is also evidence for the formation of system level consolidation at neuronal network. Further NMDA receptor reactivation-mediated synaptic reentry reinforcement (SRR) process has been suggested as the unifying cellular mechanism.[35]

Recently epigenetic factors have been shown to play important role in many physiological activities. Epigenetics is the study of stably heritable molecular phenotypes that do not affect DNA sequence. Important among the epigenetic mechanisms are modification of histones and covalent modification of DNA including DNA methylation. The last phenomenon has been shown to be involved in memory persistence in the cortex. RNA interference phenomenon is another mechanism that may have a role in the synaptic plasticity.

Considering that basti treatment has shown consistently good result in the clinical management of many neurological disorders it is worth focusing its impact on the above mechanism underlying memory and neuroprotection. Many phytochemical constituents have been reported to possess nootrophic effect[36]**.** In the present study, the test basti's could reverse both scopolamine and diazepam induced memory impairment (amnesic effect). Scopolamine,is a well-known the cholinergic neurotransmission blocker This cholinergic dysfunction is responsible for cognition impairment in rats leading amnesic effect. [37] It has also been reported that memory impairment induced by scopolamine in rats is associated with altered brain oxidative stress status also hence scopolamine-induced memory deficit model is used as animal model for screening anti-dementia drugs. As mentioned earlier in the present study both test basti procedures MB and MTB produced marked reversal of scopolamine induced amnesic effect. This may be suggestive of cholinergic blocking reversal or anti-oxidant activity effect in the test procedures.

In CAR model the test procedures reversed diazepam induced amnesia in a remarkable manner. Diazepam is a GABA mimetic agent which induces memory impairment and the subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory. This reversal may be indicative of the test procedures may have influence on GABA ergic system. It has been indicated that an increase in serotonergic transmission in the median raphe of mid brain will interfere with learning acquisition and memory consolidation. The possible role of gut-brain neuro-endocrinal axis has been discussed separately (vide infra).

Effect on Anticonvulsant activity

This assay has been used primarily to evaluate antiepileptic drugs. In MB administered group a non-significant decrease in flexion, extension phases and duration of stupor was noted in comparison to the control group. However, significant shortening of the duration of clonic seizures was noted. In MTB group a marginal increase in flexion phase, marginal decrease in extension phase and a moderate increase in the duration of stupor were noted in comparison to the control group, however, none of these changes were found to be statistically significant. The duration of clonic phase was found to be markedly reduced in this group. Analysis of the above activity profile indicates that the test procedure does not possess anti-convulsant activity.

Effect on Rota rod test

The test is used to evaluate the activity of drugs interfering with motor coordination. Skeletal muscle relaxation induced by a test drug could be evaluated by testing the ability of rats to remain on a revolving rod. The increased muscle tone is a common feature of anxiety states in humans. Thus, the test formulations were tested for their effect on muscle coordination and balance in the rota-rod test. It was observed that rats of test basti group performed much better on the rota rod in comparison to the control group. They also committed fewer mistakes. The exact nature of this activity is not known. It may be due to increased alertness or reduced anxiety or both.

Anti-Parkinson's activity

According to our present knowledge the fundamental lesion in Parkinson's disease is a marked deficiency in the dopaminergic innervation of the basal ganglia owing to degeneration of neurones in the substantia nigra. Enhancement of dopaminergic transmission restores at least partially motor function. The decrease in dopaminergic activity in the basal ganglia results in a relative excess of cholinergic influence. Parkinson's disease is the most common neurodegenerative movement disorder and the second most common neurological disorder behind Alzheimer's disease today.

Cardinal features of Parkinson's disease include motor dysfunction such as rigidity, resting tremor, postural instability and bradykinesia. These debilitating symptoms manifest due to the massive loss of dopamine in the striatum, the nerve terminal region of dopamine neurons is in the substantia nigra pars compacta (SNpc). This anatomical circuit is known as the nigrostriatal pathway and plays a critical role in fine tuning motor functions. The pathogenesis of Parkinson's disease is largely unknown, mitochondrial dysfunction; oxidative stress, intracellular protein accumulation (Lewy Bodies containing α-synuclein) and abnormal protein degradation all play a key role in disease progression. Because loss of dopamine in the striatum causes motor dysfunction in Parkinson's disease, dopamine supplementation can be used to alleviate motor symptoms but this is only a temporary solution as the efficacy diminishes with age and as the disease progresses. This indicates role for additional factors.

Parkinson's disease also causes symptoms in other parts of the nervous system. Constipation and gastrointestinal (GI) problems are often some of the earliest symptoms, well before the presence of dopamine dysfunction, and post mortem studies in Parkinson's disease patients identified protein accumulation in the enteric nervous system of the GI tract. This 'Braak's hypothesis' suggests protein accumulation in enteric neurons spreads in a retrograde manner to the brain through the dorsal motor nucleus of the vagus and triggers Parkinson's disease. Braak observed in post mortem tissue that patients with presymptomatic Parkinson's disease had protein aggregation in the peripheral nervous system but not the central nervous system Protein aggregation ascended into the central nervous system and correlated with the development of motor dysfunction.

Recent evidence supports the hypothesis that GI abnormalities, which precede central nervous system changes, trigger Parkinson's disease. In one of the studies mice expressing mutant α-synuclein in gut enteric neurons exhibited extensive GI dysfunction followed by motor abnormalities indicating link between the two systems. In another study, low doses of rotenone, a compound found in pesticides that causes Parkinson's-like conditions, produced GI disturbances and enteric neuronal α- synuclein aggregates in rats before neuronal protein aggregation. These studies, taken together with the early GI disturbances in humans, clearly implicate the GI system in the pathogenesis of Parkinson's disease. The exact nature of this link needs to be elucidated and impact of test basti procedures on them studied.

In the present study two models of experimental Parkinsonism were employed. The first one is reserpine syndrome test in which effect of test drug on reserpine induced ptosis, catatonia and sedation is assessed. These syndromes occur because of the depletion of the biogenic amines from their storage site including dopamine. The second test included was oxotremorine test. The toxicant induces tremors, head twitches, ataxia, lachrymation, diarrhoea and other cholinergic hyperactivity induced symptoms.

According to our present knowledge the fundamental lesion in Parkinson's disease is a marked deficiency in the dopaminergic innervations of the basal ganglia owing to degeneration of neurones in the substantia nigra. Enhancement of dopaminergic transmission restores at least partially motor function. The decrease in dopaminergic activity in the basal ganglia results in a relative excess of cholinergic influence. The muscarinic agonist's tremorine and oxotremorine induce parkinsonism-like signs such as tremor, ataxia, spasticity, salivation, lacrimation and hypothermia. These signs are antagonized by anticholinergic drugs. The oxotremorine antagonism has been proven to be a reliable method for testing central anti cholinergic activity. Both the tests simulate majority of the symptoms observed in parkinsonian disease and are considered to have good predictability towards anti-parkinsonian activity in clinical settings. Both MB and MTB produced significant reversal of oxtremorine induced cholinergic effect and reserpine induced ptosis, catatonia and sedation. This clearly suggests that both the procedures have good anti-parkinsonian activity.

The possible mechanism underlying the observed beneficial effect requires careful analysis. Oxotremorine effect reversal may involve anti-cholinergic mechanism. However, no effect which can be linked to cholinergic activity modulation could be observed in other tests. Some other mechanism, especially gut hormone effect may be involved. The second possibility is the increase in the brain level of biogenic amines. Though the brain level were not estimated in the absence of any behavioural correlates to indicate increased biogenic amines it is difficult to infer that this mechanism is operative. Since basti karmas are used as therapeutic measures it is important discuss the role of gut-brain linkage.

Enteric nervous system (ENS) is a unique feature of GI tract. It is in sheaths of tissue lining the esophagus, stomach, small intestine and colon. It is considered as single entity has much similarity to the functioning of the brain. It comprises of large number of neurons; many neurotransmitters are synthesized and processed by it. The two-message traffic between neurons and other structures are like those found in brain. It also is made up of complex neuronal circuitry that enables it to perform as an independent entity. According to some estimates ENS contains approximately same number of neurons as that of brain. Furthermore, nearly every chemical that controls the brain in the head has been identified in the gut, including hormones and neurotransmitters.[37]

The GI tract hormones are secreted by epithelial cells lining the lumen of stomach and small intestine. The hormones are secreted in to the blood and thus circulate systemically and can influence the functioning at different organs. Many neuropeptides and neurotransmitters that GI activity are also synthesized in the brain and are sometimes referred as "brain-gut-peptides". Important among them are ghrelin, cholecystokinin, peptide YY, pancreatic peptide, amylin, glucose dependent insulinotrophic polypeptides, glucagon like peptide, oxyntomodulin etc.

Ghrelin is well-known as a key modulatory of energy homeostasis in which activation of growth hormone secretagogue receptor (GHSR) is involved. GHSR expression in many regions of the brain like hypothalamus], substantia nigra, pars compacta (SNpc) - this implies a role for this hormone in the CNS. It has been shown to increase memory retention in mice [37] It is interesting to point out that in the present study both the basti karmas produced remarkable anti-amnesic effect. It is possible that part of the effect might be due to increased formation of this hormone.

It has also been shown that ghrelin binds to dopamine neurons in the SNpc and activates them. Further it has also been shown to increase the expression of Tyrosine hydroxylase the enzyme involved in dopamine bio-synthesis in mid brain. It also increased the turnover of dopamine in the dorsal striatum. Based on this it can be suggested that the test basti karma's may be enhancing the formation of ghrelin both in the gut and brain leading to attenuation of parkinsonian like symptoms in the experimental animals[37].

Glucagon like peptide-1 (GLP-1) is another important peptide of the gut. It performs the function of satiety signal. It is also present in the brain. In the brain its activation promotes cell survival and plasticity including learning, protection from apoptotic cell death and from oxidative mechanisms. Further its activation leads to increased neurogenesis this may provide protection against degeneration of neurons seen in Parkinsonian disease. [37] The role of the other hormones in the brain is being elucidated. Thus, it seems that many of the therapeutic benefits of basti karma may be mediated through modulation of ENS and its linkage to brain.

CONCLUSION

Animal experimentation revealed that both Matra basti and Madhu tailika basti have produced highly significant anti-depressant effect in behavioural 'despair' test. Both produced

significant anti-anxiety activity in elevated plus maze test. In open field apparatus test a complex behavior pattern was observed. In the memory and learning related test both the test procedures did not affect the learning process significantly but both produced remarkable reversal of the amnesic effect of scopolamine, this indicates memory enhancing effect. Similarly, anti-amnesic effect was observed in CAR based protocol also. The test procedures did not possess significant anti-convulsant activity. Both improved the performance of the rats on the rota rod. Both the test procedures produced good anti-parkinsonian activity. Animal experimentation revealed that both Matra basti and Madhu tailika basti have no effect on general gross behavior. The effectiveness of Ayurvedic treatment goes beyond the pharmacological and incorporates the behavioral, and ultimately depends upon the internal and subtle energies of our being. While these aspects are hard to isolate and scrutinize, they can be evaluated and it is here that genuine Ayurvedic research begins.

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