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Analgesic and anti-anxiety activity of two Ayurvedic formulations (*Rasnadi ksheerapaka* and *Sahacharadi taila*) on experimental rat models

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ABSTRACT

Background: *Rasnadi ksheerapaka* and *Sahacharadi taila* are known to be effective in *shula* and *vatavyadhi* and used very frequently in conditions involving pain. **Aims and objective:** To evaluate the analgesic and anti-anxiety activity of *Rasnadi ksheerapaka* and *Sahacharadi taila matrabasti* in Charles Foster Albino rats. **Materials and method:** For evaluation of analgesic study tail flick response in tail flick test and paw licking response in Eddy's hot plate test was performed. Anti-anxiety study was conducted with elevated plus maze and open field maze. **Result and conclusion:** After both the experiments it is effectively proven that both test drugs *Rasnadi ksheera paka* and *Sahacharadi taila* have analgesics effects but they failed to exhibit anti-anxiety effect in rats.

Keywords: Tail flick test, Eddy's hot plate test, Open field behavior, Elevated plus maze.

INTRODUCTION

The use of animals in pharmacological research and education dates back to the period when humans started to look for ways to prevent and cure ailments. Most of present day's drug discoveries were possible because of the use of animals in research and scientists who advocate the relevant and judicious use of animals in research so that new discoveries can continue [1]. In this study the pharmacological study is carried out in Charles Foster Albino rats to assess the analgesic activity and anxiolytic activity of test drug *Rasnadi ksheera paka* which was administered orally and *Sahacharadi taila matra basti* which was administered rectally.

Rasnadi ksheerapaka which contains *Rasna, Gokshura* and *Vasa* is a classical preparation mentioned in the Charaka Samhita in the context of *yoni shula and yonivyapat chikitsa [2]. Sahacharadi taila* is mentioned in the Ashtanga Sangraha in the chapter on *Vatavyadhi chikitsa* [3]*.* These formulations are known to be effective in *shula* and *vatavyadhi* and used very frequently in conditions involving pain. Certain experimental studies provided various evidences for the analgesic and anti-inflammatory effects of these drugs.

MATERIAL AND METHODS

Experimental animals

After getting approval from institutional ethical committee, inbred Charles foster albino rats of either sex (150-200 gm each) were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University. The rats were housed in healthy and clean surrounding in polypropylene cage with paddy husk as bedding. All the animals had free access to pure drinking water and standard pellet laboratory animal diet. Animal room temperature was set at 24±20 ºC with controlled illumination to provide a light-dark cycle for twelve hours. The experiments were carried out in conformity with the guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission (No. Dean/2022/IAEC/3245) and care of animals were taken as per the Committee's guidelines for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

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Grouping and dose fixation

Total four groups were proposed with five animals in each group and the groups were Control, Standard, Test group A and Test group B.

The analgesic activity of the groups was evaluated by tail flick latent period test and hot plate test. Control group were given tap water orally, Diclofenac injection was administered intraperitoneally in standard group. The test group A was given *Rasnadi ksheera paka* orally for 15 days prior to the experiment and test group B were given *Sahacharadi taila* as *matrabasti* administered per rectal for 8 consecutive days. The anxiolytic activity of the groups was evaluated by open field maze and elevated plus maze. After the washout period of 21 days Diazepam injection was administered intraperitoneally in standard group and rest of the groups were given same treatment as in analgesic activity experiment.

The dose for the herbal preparations were calculated by converting human dose to animal dose on the basis of body surface area ratio by referring to the table of Paget and Barnes (1964) [4]. On the basis of these the calculated rat doses were 4.5g/ kg and 6.48g /kg for oral *Rasnadi ksheera paka* and *Sahacharadi taila matrabasti* respectively. The dose of diclofenac was 10 mg/kg and diazepam were 1 mg/kg which were administered intraperitoneally [5, 6].

Rasnadi ksheera paka administered orally for 8 consecutive days and introduced into the oral cavity by rat feeding needle with 2 mL syringe to the left side of incisor teeth in the midline. *Sahacharadi taila* was administered for 8 consecutive days through rectal routes with the help of an infant feeding tube which was sieved on 2mL plastic syringe. Single dose of diclofenac and diazepam were administered intraperitoneally.

Figure 1: Materials required for administration of *Ksheerapaka*-oral route

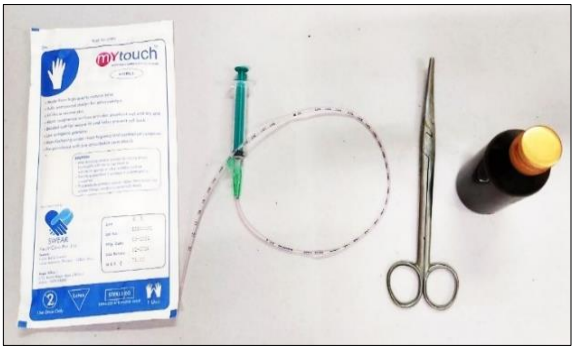

Figure 2: Materials required for administration of *Basti*- rectal route

Figure 3: Intraperitonial injection

Figure 4: Oral administration of *Ksheerapaka*

Figure 5: Per rectal administration of *Basti dravya* in rat

Tail flick test

The tail flick test performed with digital analgesiometer apparatus, involves application of a heat stimulus to the tail of mice and rats, and the time taken for the tail to "flick" or twitch is recorded. A cut-off period of 15 s is observed to avoid damage to the tail. After the

administration of the drug, the tail flick response was taken at 30 min, 60 min and 120 min [7].

Eddy's hot plate test

The hot plate test was performed at 55 °C on the paw of each rat. Animals were placed on the heated smooth surface in an analgesiometer and the latency of licking was measured. Hot plate tests were performed after 15 minutes of drug administration and response was taken at 30 min, 60 min and 120 min. To prevent the tissue injury the rats were removed from the hot plate test after 30 sec [8].

Open field behaviour test

Place the rat in the Open Field and start timer and remain still, quiet and away from the arena and field of view of the rat. Each animal was placed at 1 corner of the apparatus and for next 5 minutes, it was observed for ambulation (this was measured in terms of number of squares crossed by the animal), total period of immobility (in seconds), rearing (no of times the animal stood on hind limbs) and grooming (no of times the animal made these responses viz. grooming of face, licking/ washing and scratching the various parts of the body) [9].

Elevated plus maze

Take the rat out of the cage and place at the junction of the open and closed arms in the elevated plus maze, facing the open arm opposite to the experimenter and note the behaviour of each animal consistently for 5 min. The experimenter should simultaneously record the number of arm entries and time spent in each open arm with a timer [10].

Figure 6: Digital Analgesiometer (Tail flick)

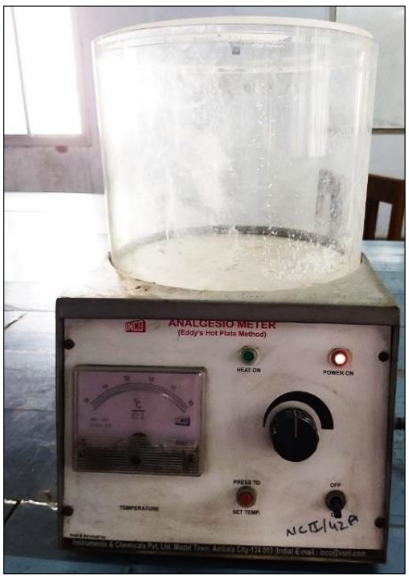

Figure 7: Analgesiometer (Eddy's Hot plate)

Figure 8: Elevated plus maze

Figure 9: Open Field maze

Figure 10: Tail Flick test

Figure 11: Eddy's Hot plate test

Figure 12: Elevated plus maze test

Figure 13: Open Field maze test

Statistical Analysis

All the data are expressed as Mean ± SEM. Statistical test was done by one-way ANOVA test followed by Post Hoc and Dunnett t test for comparison among multiple groups. A *P* value of less than 0.05 was considered significant and *P* value less than 0.01 and 0.001 were considered highly significant.

RESULTS

Table 1: Tail flick method in rats (Mean±SE)

Groups	Dose of drugs	0 minute	30 minutes	60 minutes	120 minutes
Ⅰ Control	-	7.240±0.641	5.520±0.941	6.620±0.872	6.340±0.730
Ⅱ Standard	10 mg/kg	10.600±0.912	8.080±0.809	8.980±0.355	9.820±0.827
Ⅲ Test group A	4.5 mL/kg	9.620±0.952	8.280±1.242	8.360±0.495	8.700±0.587
Ⅳ Test group B	6.48 mL/kg	9.280±0.769	9.840±1.128	9.920±0.813	9.960±0.515
Between the groups comparison one-way ANOVA	-	F=2.986 P=0.062 NS	F=2.937 P=0.065 NS	F=4.307 P=0.021 S	F=6.140 P=0.006 HS
Post Hoc test **Dunnett t test** Ⅰ - Ⅱ Ⅰ - Ⅲ Ⅰ - Ⅳ	-	-	-	 0.061 0.198 0.008	 0.006 0.063 0.004

Table 2: Eddys hot plate method in rats (Mean±SE)

Groups	Dose of drug	0 minute	30 minutes	60 minutes	120 minutes
Ⅰ Control	-	4.058±0.367	4.076±0.408	4.188±0.396	3.974±0.413
Ⅱ Standard	10mg/kg	8.438±0.439	8.908±0.381	9.052±0.377	9.342±0.283
Ⅲ Test group A	4.5 mL/kg	6.796±0.207	7.514±0.391	7.698±0.272	7.824±0.227
Ⅳ Test group B	6.48 mL/kg	6.434±0.369	5.722±0.253	6.030±0.234	6.092±0.218
Between the groups comparison one-way ANOVA	-	F=25.719 P=0.000 HS	F=33.434 P=0.000 HS	F=41.328 P=0.000 HS	F=60.837 P=0.000 HS
Post Hoc test Dunnett t test	-				
Ⅰ - Ⅱ		0.000	0.000	0.000	0.000
Ⅰ - Ⅲ		0.000	0.000	0.000	0.000
Ⅰ - Ⅳ		0.001	0.015	0.003	0.000

Table 3: Open field exploratory behavior in rats (Mean±SE)

Groups	Dose of drug	Ambulation (N)	Rearing (N)	Grooming (N)	Immobility (Seconds)
Ⅰ Control	-	36.40±1.030	7.00±0.548	6.40±0.600	163.80±11.218
Ⅱ Standard	1mg/kg	20.80±3.980	3.60±0.678	2.00±0.316	275.80±6.793
Ⅲ Test group A	4.5mL/kg	25.40±5.793	4.20±0.735	5.40±0.748	180.00±21.307
Ⅳ Test group B	6.48mL/kg	30.80±3.583	2.60±0.245	2.00±0.316	152.40±23.183
Between the groups comparison one-way ANOVA	-	F=2.875 P=0.069 NS	F=10.461 P=0.000 HS	F=18.702 P=0.000 HS	F=10.917 P=0.000 HS
Post Hoc test Dunnett t test	-	-			
Ⅰ - Ⅱ			0.002	0.000	0.001
Ⅰ - Ⅲ			0.010	0.423	0.841
Ⅰ - Ⅳ			0.000	0.000	0.934

Table 4: Elevated plus maze in rats (Mean±SE)

Groups	Entries (Number)		Time (Sec)	
	Closed Arm	Open Arm	Closed Arm	Open Arm
Ⅰ Control	7.600±0.927	4.800±0.583	234.600±13.105	65.400±13.105
Ⅱ Standard	6.000±1.140	5.800±0.800	237.800±12.265	62.200±12.265
Ⅲ Test group A	3.400±1.029	2.600±0.927	252.800±15.822	47.200±15.822
Ⅳ Test group B	5.800±0.800	3.400±0.400	241.200±5.323	58.800±5.323
Between the groups comparison one-way ANOVA	F=0.419 P=0.742 NS	F=0.419 P=0.742 NS	F=3.109 P=0.056 NS	F=4.073 P=0.025 S
Post Hoc test Dunnett t test	-	-	-	
Ⅰ - Ⅱ				0.635
Ⅰ - Ⅲ				0.105
Ⅰ - Ⅳ				0.388

Tail flick test

The *Rasnadi ksheera paka* (4.5 mL/kg p.o) did not show significant increase in latency to flick tail compared to control group (P > 0.05). The *Sahacharadi taila matra basti* (6.48mL/kg p.r) showed significant (P < 0.01) increase in latency to flick tail at 60 min and lasted up to 120 min compared to control group. The highest nociception inhibition of stimulus by *Sahacharadi taila matra basti* (6.48mL/kg p.r) was observed at 120 minutes.

Hot plate test

The *Rasnadi ksheera paka* (4.5 mL/kg p.o) showed highly significant (P < 0.001) increase in the mean basal reaction time. The highest nociception inhibition of stimulus exhibited by *Rasnadi ksheera paka* (4.5 mL/kg p.o) was observed at 120 minutes. *The Sahacharadi taila matra basti* (6.48mL/kg p.r) also showed highly significant (P < 0.001) increase in the mean basal reaction time in hot plate method compared to control group. The highest nociception inhibition of

stimulus exhibited by *Sahacharadi taila matra basti* (6.48mL/kg p.r) was observed at 0 minute.

Open field behavior test

In ambulation, *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups do not showed any increase in the ambulation and there was no significance ($P > 0.05$) as compared to control group throughout the experiment. In rearing, *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups do not showed any increase in the rearing and exhibited highly significant ($P < 0.001$) result as compared to control group in the experiment. In grooming, *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups do not showed any increase in the grooming and exhibited highly significant ($P < 0.001$) result as compared to control group in the experiment. In immobility, *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups do not showed any increase in the time of and exhibited highly significant ($P < 0.001$) result as compared to control group in the experiment.

Both drugs exhibited decrease in ambulation, rearing and grooming. Along with there was an increase in immobility period in group A compared with control group while it was decreased in group B as compared to control group.

Elevated plus maze

Rasnadi ksheera paka (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups exhibit lesser number of entries in to open arm when compared with control group and it did not show any significance ($P > 0.05$). *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) treated groups spent less time in open arm when compared with control group and it did not show any significance ($P > 0.05$). In the present study both the test drugs, *Rasnadi ksheera paka* (4.5 mL/kg p.o) and *Sahacharadi taila matra basti* (6.48mL/kg p.r) failed to exhibit anti-anxiety activity in the Elevated plus maze model. On the contrary the animals from test drug administered group exhibited the tendency of remaining longer in the closed tunnels as well as made increased number of entries into the closed tunnels.

DISCUSSION

Analgesic activity

Tail flick test

Tail flick model, which is thermal induced nociception indicates narcotic involvement which is sensitive to opioid μ receptors. Tail flick response appears to be a spinal reflex, and is regarded as a specific screening method for centrally acting analgesics [11]. In test group A treated animals, no analgesic activity was observed while in test group B treated animals, analgesia was observed at 60 min and lasted up to 120 min, this shows prolonged analgesic effect of the test drug. Presence of analgesic activity in this model indicates that the mechanism of action is central. The mechanism through which this effect is brought about may be due to modulation of opioid receptors or by release of endogenous analgesic factors such as encephalin and endorphin.

Hot plate test

The hot plate method has been found to be suitable for evaluation of centrally acting analgesics [12]. In centrally acting analgesic methods, the drug *Rasnadi ksheera paka* was found to be significantly effective. The *Sahacharadi taila matra basti* was also found to be effective in this analgesic model for evaluating centrally acting drugs.

Anti-anxiety activity

Elevated plus maze

Rasnadi ksheera paka and *Sahacharadi taila matra basti* treated groups did not show any anxiolytic effect in the present study. This activity is evaluated by employing elevated plus maze test. Normally rats are aversive to open spaces and they tend to spend more time in closed tunnels. Thus, normal control rats spend more time in the closed tunnel of the test apparatus. Drug with anti-anxiety activity drastically decrease this phobia for open space hence they spend more time in open tunnels. In the present study both the test drugs failed to exhibit anti-anxiety activity in the Elevated plus maze model. On the contrary the animals from test drug administered group exhibited the tendency of remaining longer in the closed tunnels. Since these have no statistically significant difference in this behavior in between control and drug treated group, it can be suggested that both the test drugs are devoid of anti-anxiety activity as per the Elevated plus maze stress model [13].

Open field maze

Rasnadi ksheera paka and *Sahacharadi taila matra basti* treated groups, both drugs exhibited decrease in ambulation, rearing and grooming. Along with there was an increase in immobility period in group A compared with control group while it was decreased in group B as compared to control group. Considering ambulation as a widely accepted parameter in the open field maze. Here in this study ambulation is decreased which means the anxiety level is increased.

The Open Field maze (OFM) is a simple sensorimotor test used to determine general activity levels, gross locomotor activity, and exploration habits in rodent models of CNS disorders. A number of important conventional and ethological parameters can be collected and analyzed during the performance of the OFM. These data allow the researcher to measure behaviors ranging from overall locomotor activity to anxiety-related emotional behaviors. The OFM remains one of the most widely applied techniques in rodent behavioral research. Ambulation is helpful to analyses thigmotaxis. Thigmotaxis or tendency to remain closer to wall increases as anxiety levels rise. Rearing behavior consists of subject animals standing on both hind paws in a vertical upright position. It is considered an exploratory behavior and has been used as a measure of anxiety in both the OFM and the Elevated Plus Maze [14]. However, there is no clear indication that rearing behavior is either anxiolytic or anxiogenic. Some studies indicate increased rearing is in concordance with increased anxiety levels in mice while others postulate decreased rearing behavior is indicative of increased anxiety [15].

CONCLUSION

Tail flick latent period test and Eddy's hot plate test were done for the analgesic experiments. Elevated plus maze and open field maze were done for the anti-anxiety experiments. After both the experiments it is effectively proven that both test drugs *Rasnadi ksheera paka* and *Sahacharadi taila* have analgesics effects but they failed to exhibit anti-anxiety effect in rats.

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Conflict of interest

None declared.

Financial support

None declared.

Ethical clearance

Institutional Animal Ethical Committee (IAEC) No. Dean/2022/IAEC/3245.

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REFERENCE

1. Badyal DK, Desai C. Animal use in pharmacology education and research: the changing scenario. Indian journal of pharmacology. 2014;46(3):257-265.
2. Trikamji Y. Charaka Samhita of Charaka, Chikitsasthana, chapter 30, verseno.225-26. Varanasi; Chaukhamba Orientalia; 2014, 643
3. Gupta KA. Ashtanga Sangraha of Vagbhata, Sutrasthana, chapter 28, verse no.8, Varanasi; Chaukhamba Krishnadas Academy; 2008, 207.
4. Medhi B. Practical Manual of Experimental and Clinical Pharmacology, 1st ed. Published by Jaypee brothers medical publishers (P) LTD. New Delhi, 2010.
5. Hasani A, Soljakova M, Jakupi M, Ustalar-Ozgen S. Preemptive analgesic effects of midazolam and diclofenac in rat model. Bosn J Basic Med Sci. 2011;11(2):113-8.
6. Zambotti F, Zonta N, Tammiso R, Conci F, Hafner B, Zecca L, Ferrario P, Mantegazza P. Effects of diazepam on nociception in rats. Naunyn Schmiedebergs Arch Pharmacol. 1991;344(1):84-9.
7. Deuis JR, Dvorakova LS, Vetter I. Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci*. 2017;10:284.
8. Hasani A, Soljakova M, Jakupi M, Ustalar-Ozgen S. Preemptive analgesic effects of midazolam and diclofenac in rat model. *Bosn J Basic Med Sci*. 2011;11(2):113-118.
9. Gould TD, Dao DT, Kovacsics CE. The Open Field Test. In: Gould T. (eds) Mood and Anxiety Related Phenotypes in Mice. Neuromethods, vol 42. Humana Press, Totowa, NJ. 2009.
10. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*. 2007;2(2):322-328.
11. Ganeshpurkar A, Rai G. Experimental evaluation of analgesic and anti-inflammatory potential of Oyster mushroom Pleurotus florida. Indian journal of pharmacology, 2013;45(1):66-70.
12. Ganeshpurkar A, Rai G. Experimental evaluation of analgesic and anti-inflammatory potential of Oyster mushroom Pleurotus florida. Indian journal of pharmacology, 2013;45(1):66-70.
13. Bachhav RS, Gulecha VS, Upasani C D. Analgesic and anti-inflammatory activity of Argyreia speciosa root. Indian J Pharmacol 2009; 41:158-61.
14. Griebel G, Moreau J, Jenck F, Martin JR, Misslin R. Some critical determinants of the behaviour of rats in the elevated plus-maze. Behavioural Processes. 1993;29:1-2, 37-47.
15. Seibenhener ML, Wooten MC. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp*. 2015;(96)52434

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