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A comparative pharmacological study on the effect of Tagara *(Valeriana wallichii)* AND Jatamansi *(Nardostachys jatamansi)* in the management of anidra w.s.r to primary insomnia

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Abstract

The present study was undertaken to evaluate Tagara and Jatamansi for certain CNS activities to ascertain the basis for their use as a sedative and hypnotics. The drugs were evaluated for hypnotic and sedative, anti anxiety, anti depressant and anti psychotic activities using standard experimental protocol. The data generated suggests that the test drugs Tagara and Jatamansi have complex CNS activity profile which is not easy to categorize under general CNS activity profile. Both possess significant anti-anxiety activity without significant sedative-hypnotic activity. This increases their utility for the patients suffering from anxiety due to sleep disturbances.

Keywords: Tagara, Jatamansi, Hypnotic and sedative activity, Anti anxiety, Anti depressant.

Introduction

Sleep is a complex physiologic process that is influenced by many internal and environmental factors, and problems with sleep are often related to specific personal circumstances or are based on subjective reports from the affected person. Insomnia has emerged as an important condition afflicting human population. Primary insomnia is the difficulty in initiating sleep, difficulty in maintaining sleep and not related to any mental disorders or physical conditions[1]. Primary insomnia is estimated to occur in 25% of all chronic insomnia patients[2]. Although human subjects are used widely in the study of sleep and sleep disorders, the study of animals has been invaluable in developing our understanding about the physiology of sleep and the underlying mechanisms of sleep disorders. In the present study, the aim is to undertake a comparative pharmacological evaluation of the test drugs, to determine the basis for their use as sedative-hypnotics in therapeutic settings.

Materials and Methods

Collection of Plant Material

The powder of *Valeriana wallichii*and *Nardostachys jatamansi* DC of family Valerianceae were prepared in the pharmacy attached to SDM College Ayurveda, Udupi, from authenticated plant material (authentication by Pharmacognosy Lab). The powder obtained from a single batch was used throughout the experimental study. The drug samples were subjected to chemical profiling as per the protocol of Ayurvedic Pharmacopoeia[3].

Physico-chemical analysis

The drugs were subjected to physical examination, thin layer chromatography (HPTLC), microscopic examination and powder microscopy[3]

Animals

Wistar strain albino rats of either sex weighing between 200±50g and Swiss albino mice of either sex weighing between 20 – 30 g were used for experimental study. The animals were obtained from the animal house attached to the Pharmacology Laboratory of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature and humidity. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum.* Animals were acclimatized to

laboratory conditions one week prior to initiation of experiments.The experiments were carried out after obtaining IAEC committee permission (SDMCAU/IAEC 2011-12/HSN01) as per CPCSEA guidelines.

Experimental Design

The selected animals were grouped as follow as

Group I administered with tap water at a dose5 ml/kg, considered as control group.

Group-II administered with drug diazepam at a dose- 2mg/kg or *Centellaasiatica* / or test specific reference standard.

Group III- *Tagarachurna* - The aqueous solution of *Tagarachurna* was given. Dose -1080mg/kg.

Group-IV- *Jatamansichurna- Jatamansichurna* was prepared as a suspension using carboxy methyl cellulose -Dose-1080mg/kg.

The human dose for *Tagara and Jatamansi* was given as 12 g per day. Considering the adult human dose of both the drugs, the dose for the experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio [4].

Rat dose= human dose X 0.018 X 5

= 12g X 0.018 X5

= 1.08g/kg body weight

The test drugs were administered according to the body weight of the animals by the oral route, with the help of No 3 gastric catheter sleeved on to a syringe.

Gross Behavior test

The rats were placed one by one in the centre of three concentric circles. The profiles measured were grouped in to the following types of parameters: CNS Depression- Hypo activity, Passivity, Relaxation, Narcosis, Ataxia. ANS Effects- Ptosis, Exophthalamus. CNS stimulation- Hyper activity, Irritability, Stereotypy, Tremors, Straub tail, Analgesia. The activities were recorded before and at 60, 120, 180, 240 min after drug administration[5].

Hypnotic Potentiating test

The grouping was similar to the one described above. On the fifth day, one hour after the administration of the test drug, Pentobarbitone (45 mg /kg) was injected intra peritoneally. The duration of sleep was measured as the duration between loss and regaining of the righting reflex. Effect on latency of onset of sleep, duration of sleep and mortality, if any, were noted down [6].

Effect of test drug on spontaneous motor activity

On the fifth day, one hour after the administration of the test drug, each animal was gently placed in activity meter and observed for a period of 5 minutes. Number of horizontal movements, number of vertical movements and total number of activity were noted down [7].

Test for muscle tone and balance by using Rotating Rod.

Untreated rats were placed on a horizontal iron rod rotating at the rate of 24 revolutions per minute. Animals that remain on the rod for 2 or more minutes in four trials carried out in two days divided into morning and evening two sessions were selected and divided into different groups. Vehicle and test drugs were administered by oral route according to the grouping and placed on rod at hourly intervals for 4 hours after the administration. In the modification of the above procedure- the trained rats from different groups were administered with diazepam (4 mg/kg) and their performance on the rota rod was recorded one hour after the administration of diazepam[8].

Effect on mice performance in zero maze

This is a task designed to monitor the level of anxiety. Each mouse is placed on the maze for 5 minutes and the time it spends in the open and closed section is measured. One hour after the administration of the test drugs on 5th day the animals were placed just inside the closed arm. The behavior of the mouse was carefully observed and the following parameters were recorded for the duration of five minutes. The number of entry in to open tunnel, frequency of entry in to open tunnel, number of head dips in the closed tunnel, number of head dips in the open tunnel and number of times the mouse crossed from one section to the other section of the zero maze were noted down [9].

Behavioral 'despair' test in Rat

On the fifth day, one hour after the administration of the test drug, each rat was gently placed into a glass cylinder, about 40 cm. long, 18 cm. in diameter, filled up to30 cm. with water. Observations were made for 6 minutes. First two minutes were not considered for recording the drug effect and were taken as stabilizing time. The limb movements and the effort of the rat to get out of the cylinder in the next 4 minutes was noted and subtracted later from total time (4 min) to find the duration of immobility. This was considered as index of depression [10].

Effect of test drug on d-amphetamine induced stereotypy in mice

The test drugs were administered for five consecutive days and on the fifth day one hour after drug administration d-amphetamine (5 mg/kg.) was injected intra peritoneally. The stereotype behavior was assessed before and at the intervals of 10, 20, 40, 60 and 80 minutes after the injection of d-amphetamine. The following behavioral patterns were noted: Rearing, Licking, Grooming, Sneezing and Sniffing, Gnawing [11].

Statistical Analysis: The data obtained were presented as Mean ± SEM. The difference among different groups was determined by employing one way ANOVA with Dunnet'smultiple't' test as post hoc test. A $p<0.05$ was considered to be statistically significant.

Results

Gross behavior

In *Tagara* administered group none of the parameters which are indicative of CNS depression, CNS stimulation or ANS activity modulation were observed. The only prominent change observed was presence of straub tail in 4/6 mice at 60 min post drug. In *Jatamansi* administered group hyperactivity was observed at 60 min 2/6 mice and 4/6 exhibited straub tail.

Hypnotic potentiation

In reference standard administered group a moderate but statistically non-significant decrease in the duration of pentobarbitone sleeping time was observed in comparison to control group, 5/6 mice lost their righting reflex in this group. In test drug administered groups no significant effect could be observed on the pentobarbitone induced sleeping time and all the animals lost their righting reflex. The latency of onset of sleep was found to be moderately increased in reference standard (*Centellaasiatica*) given group, marginal increase was observed in *Tagara* administered group and marginal decrease was observed in *Jatamansi* administered groups. However, the observed changes were found to be statistically non-significant in comparison to control group (Table-1).

Table1: Effect of test drug on the Pentobarbitone sleeping time

Groups	Dose	Latencyof onset ofsleep(sec)	Duration of sleep (min)	Number of losing righting reflex
Group I Control (normal water)	5ml/kg	210 ± 040	65.83 ± 15.13	6/6
Group II*Centella asiatica*	20mg/kg	400 ± 190	46.33±13.743	5/6
Group IIITagara (*Valeriana wallichii)*	1080mg/kg	240 ± 049	63.17 ± 24.87	6/6
Group IVJatamansi*(Nardostachys jatamansi)*	1080mg/kg	198± 27	69.17 ± 21.99	6/6

Each value represented in Mean± SEM

Effect on SMA

In control group rats almost equal number of horizontal and vertical movements could be observed. In group B rats (diazepam) an apparent moderate decrease in both horizontal and vertical movements was observed in comparison to control group. The total count was also found to be decreased. However, the observed decrease was found to be statistically non-significant. In *Tagara* suspension administered group an apparent moderate increase in horizontal, vertical and total counts was observed in comparison to control group. However, the observed increase was also found to be statistically non-significant.In *Jatamansi* treated group also an apparent decrease in all the three counts was observed. The decrease observed with respect to horizontal count was near significant statistically however, p was found to be >0.05 hence, theoretically to be considered as non-significant. The data related to the effect of test drugs on SMA has been represented in Table-2.

Table2: Effect of test drug on spontaneous activity measuredUsing actophotometer

Group	Dose	Horizontal movements in 5 min duration	Vertical movements in 5 min duration	Total activity count
Group I Control	5ml/kg	191.83±22.94	199.5±27.1	391.33 ±49.52
Group IIDiazepam	4mg/kg	168.16 ±29.9	144.33 ±33.97	312.5±62.83
Group IIITagara(*Valeriana wallichii)*	1080mg/kg	234.83 ±15.4	234.50 ±15.50	469.33 ±26.85
Group IVJatamansi *(Nardostachys jatamansi)*	1080mg/kg	139.16 ±20.6 *	124.66±19.46	263.83 ±37.25

Each value represented in Mean± SEM* P<0.05 in comparison to control group

Effect on muscle tone and balance

Administration of diazepam lead to marked effect on the muscle tone and balanceof the trained rats. All the six animals in this group failed to complete the specified 120 sec duration on the rotating rod.In control group and test drug administered different groups the performance of the rats on rota rod was not affected. In the second part of the test diazepam (10 mg/kg) was administered after pre-treating the animals with test drugs to ascertain whether the test drug modulate the drastic central relaxant effect produced by diazepam or not.The diazepam treated group could remain on the rota rod for an average of 20.00 ± 07.21 sec duration on the rota rod. In *Tagarachurna* treated diazepam administered rats the performance was found to be better in that the rat were able to remain on the rota rod for an average duration of 58.17 ± 16.74 sec. Though apparent increase in the rota rod performance was observed in this group it was found to be statistically non-significant in comparison to the control. This is mainly due to variation in the acquired data.In *Jatamansi* pretreated rats the average duration of stay was found to be 26.00 ± 06. 80. Result has been represented in table 3.

Table 3: Effect of test drug on muscle relaxant property in rat using Rota rod.

Group	Dose	Fall off time in sec
Group I Control(normal water)	5ml/kg	80.5±18.90
Group II Reference standard (diazepam)	4mg/kg	20.00 ± 07.21
Group IIITagara (*Valeriana wallichii)*	1080mg/kg	58.17 ± 16.74
Group IV Jatamamsi(*Nardostachys jatamansi)*	1080mg/kg	26.00 ± 06. 80.

Each value represented in Mean± SEM

Effect on d-amphetamine stereotypy

Stereotypy values presented as total values for all the time intervals at which the syndromes were recorded and presented in table 4. The stereotype score in *Centellaasiatica* exhibited significant increase for three syndromes (except licking) and marginal increase for four values.In *Tagara* treated group the total stereotypy values were marginally less. In *Jatamansi* treated group the total stereotypy values were moderately less in comparison to control groups, with rearing syndrome being significantly less in this group in comparison to control group.

Table 4: Effect of test drug on d- amphetamine induced stereotypy

Groupsmg/kg	Dose	Effect on totalsyndromescore at various time intervals afterd-amphetamine injection				
		Rearing	Sniffing	Gnawing	Licking	Total scoreaverage
Group I Control	5ml/kg	1.73 ± 0.19	1.77 ±0.17	1.50 ± 0.20	0.93 ± 0.44	1.48 ± 0.25
Group II Centella asiatica	20mg/kg	2.23 ± 0.18	2.23 ±0.17	1.77 ± 0. 24	0.00. ± 0.00	2. 08 ± 0.20 (3) @1.56 ± 0.15 (4)[&]
Group III Tagara(*Valeriana wallichii)*	1080mg/kg	1.60 ± 0.16	1.60 ±0.12	1.33 ± 0.09	0.80 ± 0.16	1.33 ± 0.13
Group IVJatamansi (*Nardostachys jatamansi)*	1080mg/kg	0.89 ± 0.16 **	1.50 ±0.10	1.50 ± 0.09	0.67 ± 0.15	1.14 ± 0.13

Each value represented in Mean± SEM @ for three values which showed increase** p<0.01; [&] average of four which did not show elevation

Effect on mice performance in zero mice

Effect of test drug on the performance of mice in zero maze has been represented in table 5. The latency of exploration that is movement from the closed tunnel to open tunnel was found to be moderately decreased in reference standard group, in *Tagara* administered group it was marginally decreased and in *Jatamansi* administered group a moderate increase was observed. However, none of the observed changes were found to be statistically significant.The duration of stay in open tunnel was found to be apparently increased by more than 200% in reference standard and *Tagara* administered groups where as it was found to be doubled in *Jatamansi* administered group**.** The difference observed in test drug administered group was found to be statistically significant where as the difference observed in reference standard group was found to be very highly significant. Conversely the time spent in closed tunnel was found to be significantly less in these groups in comparison to control group.

Table 5: Effect of test drug on the mice behaviour in zero mice

Groups mg/kg	Latencyof onset of exploration (sec)	Duration of stay in open tunnel (Sec)	Duration of stay in closed tunnel (sec)	No. open head dipsduring the observation period	No. closedhead dipsduring the observation period	No times the section were crossed
Group I Control(normal water 5ml/kg)	08.50 ± 2.2	43.50±6.08	153 ±30.8	11.16 ±1.3	19.60 ± 2.6	08.50 ±3.6
Group IIreference standard (diazepam 2mg/kg)	05.33 ± 2.9	131.6 ± 2.6 ***	42.33±15.2	8.66 ± 05.8	03.17 ± 1.2 **	22.33 ± 8.7
Group IIITagara1080mg/kg	8.17 ±2.5	135.3 ±33.4 *	154.5±21.3	37.00 ±7**	24.67 ±5.6	5.33 ±2.5
Group IVJatamansi(1080mg/kg)	10.33 ± 2.2	97.33±23.6 *	157.17 ± 23.1	46.50±13.5 *	35.00 ±3.5**	03.52±1.4

Each value represented in Mean± SEM *p<0.05; *** p<0.001 in comparison to control group

The number of open head drops was found to be moderately decreased in reference standard group and the difference was found to be statistically non-significant. In test formulation administered group it was found to be increased by 3 and four fold and the observed difference was found to be statistically significant.The number of head dips was found to be significantly less in reference standard group in comparison to control group. In test formulation administered group it was found to be apparently increased. However, only the increase observed in *Jatamansi* group was found to be statistically significant.

Effect on behavioral 'despair' test in rat

The test drugs failed to alter the duration of mice immobility (behavioral 'despair'). The duration of immobility in control group was70.33 ± 2.74sec, in diazepam treated group was 60.16±14.92, in *Tagara* treated group 70.67 ± 3.02 sec and in *Jatamansi* treated group 86.33 ± 6.98sec. Though an apparent moderate increase was observed in *Jatamansi* it was found to be statistically non-significant. Result has been represented in table 6.

Table- 6: Effect of test drug on the rat in behavioral despair test

Group	Dose	Immobility time (sec)
Control (normal water)	5ml/kg	70.033
Standard (diazepam)	2mg/kg	60.16±14.92
Tagara	1080mg/kg	70.66±3.02
Jatamamsi	1080mg/kg	86.33±6.97

Each value represented in Mean± SEM

Discussion

The present study was mainly undertaken to elucidate the CNS activity profile of the test formulations *Tagarachurna* and *Jatamansichurna* to provide pharmacological basis to their clinical use and to find out if any experimental basis exists for their therapeutic use as sedatives. Both the test drugs did not affect gross behaviour to significant extent. This clearly indicates that the test drug do not possess true sedative activity. To supplement the observations made in the gross behaviour tests the data obtained from actophotometer were analyzed. The results obtained in the present study showed a moderate non-significant increase in spontaneous motor activity in *Tagara* given group and a moderate non-significant decrease in *Jatamansi* treated group.The exact reason for this weak SMA stimulation and inhibition is not known. The reason may be the dose suitability to the rats- though they were equivalent to therapeutic dose or the complex nature of the *churna* from chemical point of view- being composed of more than one chemical component. The results obtained indicate that both the drugs, at the dose level studied do not have significant observable effects which can be linked to expression of sedative or hypnotic activity. This inference is further supported by the fact that both the drugs did not prolong pentobarbitone sleeping time – a primary test for prediction of presence of hypnotic or hypnotic potentiation effect in a test drug.

Dopamine's role in control of movement is well known. Mesolimbic and nigrostriatal dopamine systems has predominant role in this. Increased locomotor activity is indicative of enhanced dopaminergic activity especially, in rodents irrespective of it being horizontal or vertical. Even the stereotypy involving movement is also considered to involve dopaminergic system. However, locomotor activity is multi factorial and is influenced by other factors and neurotransmitters. In this regard it is pertinent to mention the involvement of glutamate receptor especially its variant N-methyl-D-aspartate (NMDA) receptor subtypes. Antagonist of this receptor types are reported to produce hyperactivity implicating its role in modulating the motor activity[12]. It is interesting to mention here that valerian extracts (extracts of *Tagara*) have reported to interact with GluR (Glutamate receptor) especially the NMDA subtype[13]. Activation of this receptor is reported to heighten the sensitivity to other neurotransmitters. It can be suggested that the weak stimulation observed in the present study may involve modification of this receptor. Similarly modification of one or both of the above neurotransmitter may also be involved in the activity observed with *Jatamansi*. In an earlier study by Prabhu*et al.*[14] alcoholic extract of *Jatamansi* root was found to increase the level of GABA on acute administration and increase the levels of most of the central biogenic amines and inhibitory neurotransmitters on chronic administration. Thus the weak depressant effect observed with *Jatamansi* may be due to modulation of inhibitory release or activity of inhibitory neurotransmitters. Since the drug also modulates turnover of many neurotransmitters the effect might not have reached significant level.

The result obtained from the zero maze test clearly show that both the test drugs have significant anti-anxiety activity. Both of them significantly prolonged the stay of the test animals in open tunnel- the primary index predictive of anti-anxiety activity. Further the open head dips were also higher in these groups.*Tagara* was found to produce slightly better effect in comparison to *Jatamansi*. Thus the results obtained in our study provide evidence for the presence of anti-anxiety activity in the test drugs. Clinically most active anxiolytic drugs are the positive modulators of the $GABA_A$ receptor systems. However, the benzodiazepine compounds represented by diazepam produce non-selective modulation on various sub-groups of these receptors including a1, a2, a3 or a5 subunit-containing receptors with comparable efficacy. This non-selective action is presumed to be responsible for their broad-range side effects, including muscle relaxant, sedative, ethanol-potentiating and amnesic effects[15]. Based on experimental evidence it has been hypothesized that the anti-anxiety activity of this class is predominantly mediated through α-2 or α-3 subunits of this receptors[16,17,18].At present there is good demand for drugs with specific anti-anxiety activity without the attendant side effects like sedation, muscle relaxation etc. The activity profile obtained in the present study clearly indicates that both the test drugs produce anti-anxiety activity without producing any remarkable sedative or hypnotic activity. Thus they have the potential to be used as specific anxiolytics without any potent sedative activity. Many neuronal circuitries are reported to be modulated by benzodiazepine when producing their neuropharmacological activity.As discussed above *Jatamansi* influences formation and turnover of many neurotransmitters including GABA. It is possible that the observed effect may due to modulation of this receptor activity. Most of the sedative- anxiolytic drugs possess both sedative and anxiolytic activity. Some of the sedative-anxiolytics also cause memory impairment especially for retrograde memory hence it would be desirable to have drug with predominant anti-anxiety activity.The test drug may have the potential for the development of this kind of herb based formulations.

Zolpidem, a preferential modulator of a1- GABAA receptors with predominant anti-anxiety activity over sedation and muscle relaxation effects, was found to modulate the neuronal circuits especially of hippocampal septal neurons. Some of the studies have shown that multi component drugs sometimes act preferably at specific nodes to produce the desirable effect without producing many side effects unlike pure component which act at many nodes leading to expression of many side effects[19]. The test *churnas* can be considered as examples of multi-component formulations they may have specific network modulating activity resulting in the observed anti-anxiety activity. This angle of investigation is worth investigating further. Based on the present data it can be suggested that both the test *churnas* have specific anti-anxiety activity which may possibly due to modulation of a1- $GABA_A$ sub types. It is also possible that like Zolpidem the test *churnas* may also have specific modulating activity on neuronal circuits and pathways in the hippocampal and septal regions.

In addition to the assessment of the test drug for sedative-hypnotic and anti-anxiety activities they were also subjected to additional testing in rota rod test, d-amphetamine and behavioural 'despair' tests. Rota rod test was carried out to assess whether the test drugs affect muscle tone and balance on this instrument which may be either due to central muscle relaxation or sedative activity. The results obtained indicated that *Jatamansi* did not modify the performance of the rats on the rota rod indicating that they do not possess remarkable sedative or central muscle relaxant activity. However, in *Tagara* administered group the performance of the rats was affected to certain extent, though not as remarkable as that of diazepam. This indicates the specific nature of the observed anti-anxiety activity in *Jatamansi* and anti-anxiety activity with weak muscle relaxation in *Tagara*.

The data related to the effect of test drugs on d-amphetamine induced stereotypy shows that both the drugs suppressed rearing and sniffing stereotype behavior elicited by d-amphetamine injection. This may be considered as an index of presence of weak to moderate anti-psychotic activity in the test drugs.The exact mechanism of action is not known. However, it is a well known fact that d-amphetamine stereotypy is due to stimulation of Dopamine- (D2) receptors, especially in the mesolimbic regions. It is possible that the test drugs may be modulating this effect of amphetamine on the dopamine receptors.

The test drugs were assessed for the presence of anti-depressant activity by employing behavioral 'despair' test. Both the test drugs did not affect the duration of mice immobility which is considered as the index of depression. This indicates that they do not possess significant anti-depressant activity.

As mentioned earlier the study was undertaken with a view to determine the CNS activity profile of the test formulations and to make attempt to find out experimental evidence for their therapeutic use as sedatives. Both the drug failed to exhibit the normal profile of a sedative-hypnotic. Thus it can be suggested that they do not have true sedative-hypnotic activity. However, they possess anti-anxiety activity which might have acted as a calmness inducing factor. It may potentiate the sedative effect of other drugs when used in combination.

Conclusion

It can be suggested that the test drugs *Tagara* and *Jatamansi* have complex CNS activity profile- which is not easy to categorize under general CNS activity profile. Both possess significant anti-anxiety activity without significant sedative-hypnotic activity. This increases their utility for the treatment of anxiety neurosis. The anti-anxiety activity of *Jatamansi* is more specific, the only other effect of note in it is the observation of the anti-psychotic activity in the d-amphetamine stereotypy test. *Tagara* in addition to anti-anxiety and anti-psychotic activities seems to possess weak central muscle relaxation effect. To confirm the presence of anti-anxiety activity in the test drugs, unequivocally, it is necessary to test them in additional models of experimental anxiety.

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