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A STUDY ON ANALGESIC ACTIVITY OF VALERIANA WALLICHI (TAGARA) WITH COMPARISON TO STANDARD DRUG PIROXICAM IN MALE ALBINO RATS

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ABSTRACT
Background: Aim and objectives: the present study was carried out 1) To evaluate the analgesic activity of Valeriana wallichi in Male Albino rats. 2) To compare the analgesic activity of Valeriana wallichi with a standard drug Piroxicam in Male Albino rats. **Materials and methods:** Digital analgesiometer was used to evaluate analgesic activity of marketed preparation of Valeriana wallichi (Tagara from Himalaya Herbal products) by tail flick method. Male wistar albino rats weighing 150 - 250g are divided into 3 groups consisting of 6 rats in each group. First group of rats (control group) were treated with 0.2 ml normal saline orally. Second group were designated as standard group and were treated with 2mg/kg of Piroxicam orally. Third group were designated as test group and were treated with 120mg/kg of Valeriana wallichi orally. One way ANOVA was used for statistical calculation. **Results:** The test drug Piroxicam and the standard drug Valeriana wallichi showed significant analgesic activity and the analgesic activity of Piroxicam is higher when compared to V. wallachi. **Conclusion:** Valeriana wallichi showed significant analgesic activity.

KEYWORDS: Analgesic, Valeriana wallichi, Piroxicam, Tail flick method, Digital analgesiometer.

INTRODUCTION
Pain is an unpleasant sensation caused by noxious stimulation of the sensory nerve endings. It is a subjective feeling and an individual response to the cause. Pain is a cardinal symptom of inflammation and is valuable in the diagnosis of many disorders and conditions. It may be mild or severe, chronic or acute, lancinating, burning, dull or sharp, precisely or poorly localized, or referred. Experiencing pain is influenced by physical, mental, biochemical, psychological, physiological, social, cultural, and emotional factors[1],[2] Acute pain is of short duration but it gradually resolves as the injured tissues heal. Acute pain is distinct from chronic pain and is relatively more sharp and severe. Chronic pain continues or recurs over a prolonged period and can be caused by various diseases or abnormal conditions. The person with chronic pain does not usually show autonomic reactions to pain like increased pulse and rapid respiration because these cannot be sustained for long periods. Referred pain is felt at site different from that of an injured or diseased organ or body part. Analgesics are the drugs that relieve pain. The opioid analgesics act on the central nervous system and alter the patient's perception; they are more often used for severe pain. The non-opioid analgesics act primarily at the periphery, do not produce tolerance or dependence, and do not alter the patient's perception; they are used for mild to moderate pain[3],[4] According to the recent National Centre for Health Statistics report, analgesics constitute the most dangerous group of medications used legally. Opioids alone attribute to more than 15,000 fatalities annually with 343,000 emergency medicine (EM) department attendances in the United States alone due to drug overdose.[5] The effectiveness of an analgesic drug will vary depending on species, strain, sex, age and other parameters. As the analgesic drugs may cause some side effects like gastric lesions, tolerance and dependence, there is a need to explore the natural analgesic agents of plant origin to overcome these side-effects of analgesics like opioids.[6] Some of the herbal plants produce secondary metabolites like glycosides, flavonoids, alkaloids, terpenoids and steroids which have a wide range of pharmacological activities like analgesic, anti-inflammatory and anti-pyretic activities etc.[7] The perennial plant *Valeriana wallichi*, commonly called Tagara belonging to the family *Valerianaceae* was selected in the present research work on the basis of ethanobotanical information which reveals that the plant

is widely distributed throughout the world specifically in India, Nepal and China.[7] Valeriana spp. is now listed in the European and USA pharmacopeias. It is also sold as a diet supplement in the USA and is one of the highest selling natural medicines in Europe and the USA.[8] The rhizomes and roots of plant contain several chemical constituents like cyclopentapyrans, valtrate, iridoid ester glycoside-valerosidatum, acacetin-7-O rutinosides and valepotriates. The essential oil derived from plant root contains beta-bargamotene, calarene, ar-curcumene, valeranone, maalioxide and maalitol. The major acids of the plant are isovaleric acid and beta-methyl valeric acid [8]. The plant extracts are widely used in the ayurveda for the treatment of arthritis pains, pain in abdomen, paralysis, convulsions, hysteria, wounds, cough, asthma, skin diseases, fever and cases of animal bites. It is perceptible that the aforementioned chemical constituents have been reported to possess analgesic properties. Piroxicam is used to reduce pain, swelling, and joint stiffness from arthritis. It is known as a nonsteroidal anti-inflammatory drug (NSAID). It works by reducing the production of prostaglandins which are responsible for inflammation, fever and pain. It is a non-narcotic reliever of mild to moderate pain including menstrual cramps, injuries and any other musculo-skeletal conditions.[9] Piroxicam may cause adverse effects like abdominal pain, gastric ulcers, stomach and intestinal bleeding, black tarry stools, weakness and dizziness upon standing may be the only sign of the bleeding if it occurs.[10] So the present investigation was designed to compare the analgesic activities of Valeriana wallichi a natural plant extract with standard drug Piroxicam.

MATERIALS AND METHODS

Inbred pathogen free healthy albino rats of male sex weighing between 150-250g were used. Animals were maintained under standard conditions in the animal house of Dr. Pinnamenani siddhartha institute of medical sciences & Research foundation.

Preliminary procedure: The herbal preparation used for this study is taken from Himalaya Herbal Healthcare, in which extracts of this herbal capsules are available.

After weighing, the albino rats which showed 250gms on average are selected for the studies. The animals were divided into 3 groups, each group containing six animals. For identification three different colours were used to mark each group by darkening a portion of the tail, using ink, at approximately 3 cm from the tip of the tail. Control group of animals were marked with black ink, standard group with blue ink and test group of animals with red ink. Group-I is the control group in which the rats received 0.2 ml normal saline orally and group-II was the standard group in which the rats received the standard drug Piroxicam orally in a dose of 0.33mg/kg after dissolving in water whereas group-III was the test group where the rats received V.wallichi extract orally in a dose of 120mg/kg after dissolving in water.

The Tail Flick Method: Analgesic effect in rats was studied by tail flick model, using Digital analgesiometer.[11] The instrument was operated at 2.5amps current throughout the experiment. It contains an arrangement for holding the tail of the rat and also has a metallic rat carrier for holding the rat properly. Heat is generated in a wire connected between two terminals of the instrument. The tail of the rat was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. A current of 5 volts was applied and the time taken by the animals to withdraw (flick) the tail was taken as the reaction time. Prior to the experiment all rats normal reaction time for radiant heat stimulus on analgesiometer was noted for at least 5 times and average of all the 5 readings were taken as mean reaction time at 0 minutes. After recording the mean reaction time, control group were administered with normal saline, standard group with piroxicam and test group with V. wallichi. For the above mentioned drugs, reaction time was recorded after administration to their respective groups at 30 minutes, 60 minutes and 90 minutes. At least 3-5 reading were taken for each rat at a gap of 5 min to the normal behavior of the animal. All recordings were tabulated separately. A cutoff time of 20 seconds was used to avoid tail injury by heat. Any animal failing to withdraw its tail within cut off period is rejected from the study.

Statistical analysis: The results of the experimental data were analyzed statistically by One-way analysis of variance (ANOVA) at 95% confidence level and the sample means were analysed by Independent t-test at significance level of $p \leq 0.05$ using STATISTICA 6.0 (Stat-Ease Inc., Tulsa, 130 OK, USA). One-way analysis of variance (ANOVA) test was used to ascertain the significance of variations between the times of flicking in Tail flicking test.

RESULTS AND DISCUSSION

The results of mean reaction times of three different groups which contain 6 rats in each group group-I (control-normal saline), group-II (standard-piroxicam) and group-III (test-V.wallichi) were tabulated in Table-1, Table-2 and Table-3. The results showed that the mean reaction time at the initial period i.e. 0 min does not show significant ($p > 0.05$) analgesic activity in all the three groups whereas there was a highly significant analgesic activity in the standard and the test groups at mean reaction time 30, 60 and 90 min (Table-1) (Figure-1). The mean reaction time at 30 min was not significant between the two groups normal saline (control) and V.wallichi (test) but it was highly significant between control and piroxicam (standard) and also between standard and test as shown in Table-2. It was also found that there was a highly significant analgesic activity between the groups control vs standard, control vs test and test vs standard at 60 and 90 minutes of time period ($p < 0.01$). By comparing the mean reaction times at different time intervals (Table-3), it was found that, with the control group normal saline there was no significant

analgesic activity at different time intervals where as with the standard piroxicam the mean reaction times at 0-30 min and 60-90 min were highly significant than at 30-60 min which was significant ($p<0.05$). When mean reaction times were observed with V.wallichi (test) at different time intervals it was evaluated that, the mean reaction time at initial period of time 0-30 min was non-significant when compared to the other two time intervals 30-60 min and 60-90 min which were highly significant. So it was concluded from the results that, the standard drug piroxicam has more analgesic activity than the test drug V.wallichi.

Table 1: Comparison of Mean reaction time of normal saline, Piroxicam and *V.wallichi* at different intervals of time.

Time (Min)	Drug	Mean	SD	F-value	p-value & Inference
At 0	Normal saline	7.63	0.46	0.79	p=0.47 NS
	Piroxicam	7.47	0.21		
	V.wallichi	7.23	0.84		
At 30	Normal saline	7.5	0.55	43.53	p<0.01 HS
	Piroxicam	11.5	1.05		
	V.wallichi	8.14	0.71		
At 60	Normal saline	7.17	0.75	61.5	p<0.01 HS
	Piroxicam	13	1.27		
	V.wallichi	9.58	0.58		
At 90	Normal saline	7.5	0.55	154.63	p<0.01 HS
	Piroxicam	14.83	0.98		
	V.wallichi	12.84	0.64		

SD: Standard deviation, F-value: Fisher value, p: Probability value, NS: Non-significant, HS: Highly significant

Table 2: Comparison of mean reaction time between two different groups at 30-90 min of time interval period.

Time (Min)	Drug		p-value	Inference
At 30	Normal saline	Piroxicam	<0.01	HS
		V.wallichi	0.11	NS
	Piroxicam	V.wallichi	<0.01	HS
At 60	Normal saline	Piroxicam	<0.01	HS
		V.wallichi	<0.01	HS
	Piroxicam	V.wallichi	<0.01	HS
At 90	Normal saline	Piroxicam	<0.01	HS
		V.wallichi	<0.01	HS
	Piroxicam	V.wallichi	<0.01	HS

p: Probability value, NS: Non-significant, HS: Highly significant

Table 3: Pair wise comparison of mean reaction times of three different groups using paired t-test.

Drug	Comparison between time (min)	Paired t-value	p-value	Inference
Normal saline	At 0 to 30	0.44	0.67	NS
	At 30 to 60	0.87	0.41	NS
	At 60 to 90	0.87	0.41	NS
Piroxicam	At 0 to 30	9.22	<0.01	HS
	At 30 to 60	2.23	<0.05	S
	At 60 to 90	2.79	<0.01	HS
V.wallichi	At 0 to 30	2.03	0.07	NS
	At 30 to 60	3.85	<0.01	HS
	At 60 to 90	9.25	<0.01	HS

t-value: Tukey's value, p: Probability value, S: Significant, NS: Non-significant, HS: Highly significant

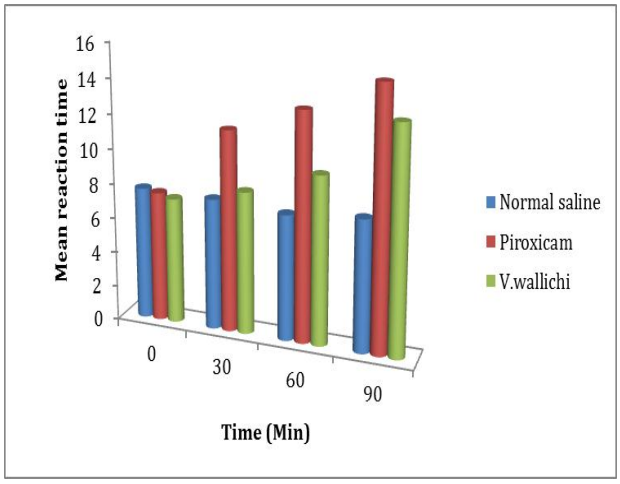

Figure 1: Comparison of Mean reaction time of normal saline, Piroxicam and V.wallichi at different intervals of time.

DISCUSSION

While the traditional use of herbal medicines in rural areas is more or less obsolete today, it is still used for the remedy of diseases in a large number of patients throughout the world. Many plant metabolites are being successfully used in the treatment of variety of diseases. Even today, many people of the world population rely upon the plant resources for their medication.[12] The herbal formulations are relatively free from adverse effects and chances of drug dependence with them are very less. Valeriana wallichi is an important medicinal plant widely used in Ayurveda because it has important pharmacological actions like anti-inflammatory, analgesic and antipyretic activities. Various methods for the experimental evaluation of analgesic activity have been described. They are all based upon the change which occurs in the response of the experimental subject to a painful stimulus after dosage with an active compound. Various animal species, as well as man, have been used as an experimental subject, and a variety of stimuli have been employed. Peripheral analgesic activity was evaluated by using writhing test in mice according to the method of Koster et al (1959).[13] However in the current investigation male albino rats were selected as experimental models and Tail and flick method was employed to evaluate the analgesic activity. In the present investigation the plant Valeriana wallichi was shown to have significant analgesic activity against pain induced by experimental Tail and flick method. The tail-flick response is believed to be a spinally mediated reflex and the μ receptor has generally been associated with pain relief and has been reported to be potent in regulating thermal pain.[14] Activation of μ_2 opioid subtype receptor produces spinal analgesia and commonly leads to the adverse effect of producing constipation.[15] The effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain. In this study, results indicated that Valeriana wallichi has analgesic effect against tail-flick test but its activity was less when compared to standard drug piroxicam. The findings of the present investigation were in accordance with the studies of Shrivastava and Sisodia (1970)[16], Sangeetha Pikhwal Sah *et al.* (2010)[17] and Sah *et al.* (2010)[18] which show that Valariana wallichii extract has effective analgesic activity as shown by tail flick method in addition to anti-inflammatory activity shown in carrageenan induced inflammation model in albino rats. Since Valariana wallichii contain various chemical constituents like alkaloids, glycosides, flavonoids, steroids etc, the observed analgesic activity may be attributed to any of these phytoconstituents.

CONCLUSION

In the current study, using Tail flick method in male Albino rats it was concluded that Valeriana wallichi has potent analgesic activity at a dose of 120mg/kg. The results were found to be highly significant with $p<0.05$ with standard and test drugs but the standard drug, piroxicam was more efficient than Valeriana wallichi in terms of its analgesic activity. However all these preliminary laboratory scale experiments give a clear idea that Valeriana wallichi possess analgesic activity but technology transfer to large scale level is needed for its final assessment of analgesic activity.

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