Research Article

Central and Peripheral Acting Analgesic Activity of Karunkali Ver (Acacia Catechu)

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ABSTRACT

Diabetic neuropathy, Equated to Vathakarshanam in Siddha is one of the complications of diabetes which cripple the patients. In India, the prevalence of diabetic neuropathy has been estimated as high as 62% based on subjective complaints, 55% by signs and 100% by nerve conduction studies. On an average, medical expenditures are thought to be 2.3 times higher in diabetics as compared with non diabetics. The present study was aimed at evaluation of the central and peripheral acting analgesic activity of total aqueous extract of root of Acacia catechu (karunkali ver – a Siddha Drug*)* in mice by Eddy's hot plate method and Writhing test. Karunkali (*Acacia catechu*) is an herb described in Gunapadam mooligai vaguppu. It is therapeutically quoted for Diabetes (Mathumegam*)* and Thimiru (Neuritis*).* The results of hot plate model indicated that the total aqueous extract of karunkali ver Kudineer (kvk) shows a significant increase ($p < 0.01$) in reaction time at a 3, 4 and 6 hours comparable to the reference drug Pentazocin but lesser ($p < 0.05$) at 2 hour. The tail immersion and hot plate test reveal that this has high analgesic activity. The bio chemical parameters never show any untoward changes during study period. Karunkaliver Kudineer showed maximum analgesic effect after 90min of administration. In conclusion, the Karunkaliver Kudineer was proved as a safe Siddha remedy for the treatment of algesia at the dose level of 4ml/kg body weight orally.

Keywords: Central and peripheral acting analgesic, karunkali ver (*Acacia catechu*), tail emersion method and writhing test

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INTRODUCTION

The manifold increase in Diabetes mellitus and its complications are ascending in the past two decades (1). Diabetic neuropathy is one of the complications which cripple the patients.

In India, the prevalence of diabetic neuropathy has been estimated as high as 62% based on subjective complaints, 55% by signs and 100% by nerve conduction studies (2). Socio-economic burden of the disease is worsening as it leads to foot ulcer. In urban India there are wide social and economic disparities challenging a better treatment (3). On an average, medical expenditures are thought to be 2.3 times higher in diabetics as compared with non diabetics. Many of these expenditures are headed to co morbidities associated with diabetic foot ulcer and lower extremity amputation (4).

Even though many herbals are time tested for their efficacy in diabetes, seldom are tested for diabetic neuropathy. Karunkali (*Acacia catechu*) is an herb described in Gunapadam mooligai vaguppu. It is therapeutically quoted for Diabetes (mathumegam*)* and Thimiru (5) (neuritis*).* The term "**thimiru**" mentioned in the literature is in analogy with neuritis a symptom in Diabetic neuropathy (6). The efficacy of the drug in diabetic neuropathy is not evidence based. The symptoms of peripheral neuropathy disease are analogy with Vatha karsanam which is given in the

book Yugi vaithiya sinthamani (7). Nociception is the neural processes of encoding and processing of noxious stimuli (8). It is the afferent activity produced in the peripheral and central nervous system by the stimuli that have potential to damage the tissues (9). To prove scientifically, the efficacy of the drug karunkali ver (Acacia root) in Diabetic neuropathy central and peripheral acting analgesic activity has been carried out.

Fig. 1: Root of Karunkali

Stock solution preparation

As recommended in SOP, 100 grams fine powder of dried root of karungali ver (*Acacia catechu*) is added in 200 ml of water. Then it was boiled continuously till the total volume is concise to 1/4th as a decoction. The filtered supernatant fluid was employed for the preclinical study.

Animals

Albino mice (22–28 g) either sex were obtained from the animal house of animal housing facility of department of pharmacology, Vels University, Chennai. Animals were maintained at standard laboratory conditions and fed with standard feeding pellets (Sai durga foods, Bangalore). Prior to treatment, the animals were fasted for 10 and 12 h respectively (10). However, water was made available ad libitum. (*ApprovalnumberXIII/VELS/PCOL/05/2000/CPCSEA/IAEC/08.08.2012*)

Experimental Methods

Acute toxicity safety Study

Acute oral toxicity test for the Karunkaliver Kudineer was carried out as per OECD Guidelines 425 (11). As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the

MATERIALS AND METHODS

Drugs and chemicals

Acetic acid, and CMC, all from Sigma-Aldrich Chemicals were the chemicals used. The standard drugs aspirin and Pentazocin was procured from the local market. All the other chemicals and drugs used were of analytical grade.

Fig. 2: Prepared Drug of Karunkali Ver Kudineer

entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behavior and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals.

However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal. General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.

Evaluation of analgesic activity by Eddy's Hotplate method

The hot-plate test method was employed to assess the analgesic activity. The temperature of the cylinder was set at 55±0.5^{0}C. The experimental mice were divided into four groups. Each mouse acted as its own control. Prior to treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0 and 10min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of the Karunkaliver Kudineer (1, 2, 4ml/kg, p.o.), Pentazocin (5mg/kg) and Saline (p.o.), was measured at 30, 60, 90 and 120 minutes after a latency period of 30 mins. The Percentage analgesic activity was calculated (12).

Anti nociceptive testing

The anti nociceptive property of Karunkaliver Kudineer was tested using the model of writhing response in mice. Swiss albino mice of either sexes weighing 20-30 g were used. The writhing syndrome was elicited by an intra peritoneal injection of 0.7% acetic acid at the dose of 0.1ml/10 g body weight. For the test group of animals Karunkaliver Kudineer at the dose level of 1, 2, 4ml/kg, per oral and for control group vehicle saline and Aspirin 100mg/kg was orally administered into the mice 30 min before acetic acid and the number of writhes was noted for 15 min beginning 5 min after acetic acid injection (13).

Statistical data

Data were presented as mean ± S.E.M. Statistical differences between control and treated groups were tested by one way ANOVA followed by dunnet's test.

RESULTS AND DISCUSSION

Karunkaliver Kudineer was found safe at all test doses (5, 10 and 20ml/kg p.0.). During 24h assessment time, test animals were found normal. Hence the therapeutic dose was fixed as 2 and 4ml/kg according to the safety guidelines. Acetic acid, which is used as an inducer for writhing syndrome, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings. It was found that Aspirin caused an effective or significant inhibition on the writhing response induced by acetic acid.

Table 1: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose ml/ kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	5	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	10	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	20	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmia 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality

Doses of 1, 2 and 4ml/kg of the Karunkaliver Kudineer were evaluated to verify the peripheral analgesic effect. But the results for the animal group treated with Karunkaliver Kudineer did not differ significantly from negative control. Hence, it is assumed that Karunkaliver Kudineer has no statistically significant peripheral analgesic effect. Therefore, despite of the mild effect observed for the doses of 4ml/kg for this test, it was not statistically significant to that of control. Similarly, the result of the analgesic activity evaluated using hot plate method revealed that the reaction time for mice was significantly increased in a dose dependent manner after 90minutes of oral administration. The Karunkaliver Kudineer at the both 2&4ml/kg doses remarkably protected the mice against thermally induced noxious stimuli, which was evidenced from the hot plate test. Hot plate test was assayed to characterize the central analgesic activity. The results showed that the pain relief was achieved in a dose dependent manner, at both test doses (1, 2 and 4 ml/kg).

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas local anesthetics and narcotics do. The acetic acid induced abdominal writhes were observed to be 40.44 ± 6.90 over the period of 10 min in the control. The number of abdominal writhes were not significantly ($p>0.05$) inhibited by Karunkaliver Kudineer. Standard drug, acetyl salicylic acid significantly inhibited writhes by about 73.04% over the control. The pain protective effect exerted by the Karunkaliver Kudineer on the mouse by hot plate method, suggest that the analgesic effect of the drug may be centrally mediated but not peripherally. Acetic acid-induced writhing is a well recommended protocol in evaluating medicinal agents for their analgesic property. The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclo-oxygenase, and prostaglandin biosynthesis.

This pain paradigm is widely used for the assessment of peripheral analgesic activity due to its sensitivity and response to the compounds at a dose which is not effective in other methods. The local peritoneal receptor could be the cause of abdominal writhings. Pain sensation in acetic acid induced writhing paradigm is elicited by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increases in peritoneal fluids. These prostaglandin and lipoxygenase products cause pain by increasing capillary permeability. The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. Thermal nociception models such as hot plate test used to evaluate central analgesic activity.

Table 2: Effect of Karunkaliver Kudineer on pain induced by hot plate method

	Dose	Reaction time in sec. before drug	% increase in reaction time after drug treatment 30 min	60 min	90 min	120 min
Control	Saline 2 ml/kg	3.2±0.05	8.1±0.02	12.6±0.5	15.48±0.6	15.30±0.5
Karunkaliver Kudineer	1 ml/kg	3.2±0.04	19.6±0.32**	25.42±1.18**	37.25±2.23**	35.38±1.12**
Karunkaliver Kudineer	2 ml/kg	3.1±0.05	27.2±0.30**	36.20±1.42**	55.39±2.81**	46.20±1.20**
Karunkaliver Kudineer	4 ml/kg	2.4±0.05	32.4±0.26**	39.56±1.33**	64.00±2.48**	53.18±1.20**
Pentazocin	5 mg/kg	3.0±0.04	54.1±1.33**	65.72±2.88**	69.10±2.45**	66.04±2.00*

Values expressed in mean ±SEM, Significant **P<0.01 (n=6)

Table 3: Effect of Karunkaliver Kudineer on writhing response in mice

Treatment	Dose (mg/kg)	Number of writhes	Inhibition (%)
Control	Saline 2 ml/kg	40.44±6.9	-----
Karunkaliver Kudineer	1 ml/kg	35.18±5.67	13.00
Karunkaliver Kudineer	2 ml/kg	33.24±4.00	17.80
Karunkaliver Kudineer	4 ml/kg	30.01±2.82	25.79
Acetyl salicylic acid	100 mg/kg	10.9±3.00**	73.04

Values are expressed as Mean±S.E.M. Drug and test compounds were given orally 30 min before 0.3% acetic acid injection. **P<0.01; significantly different from the control group (N=6)

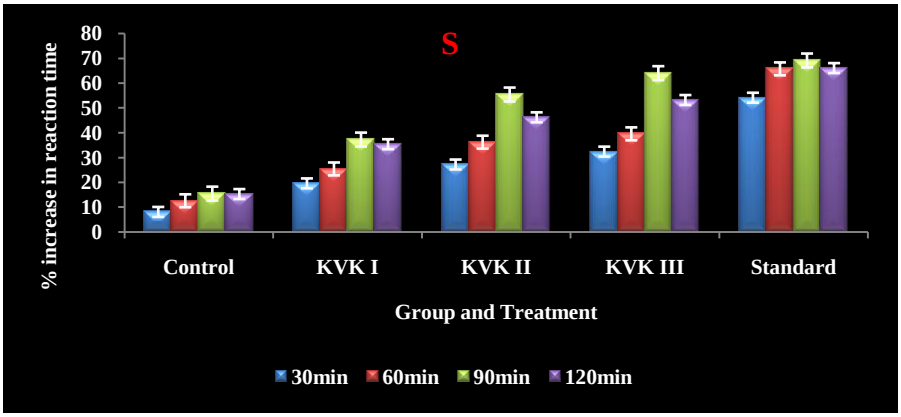

Fig 3: Reaction time

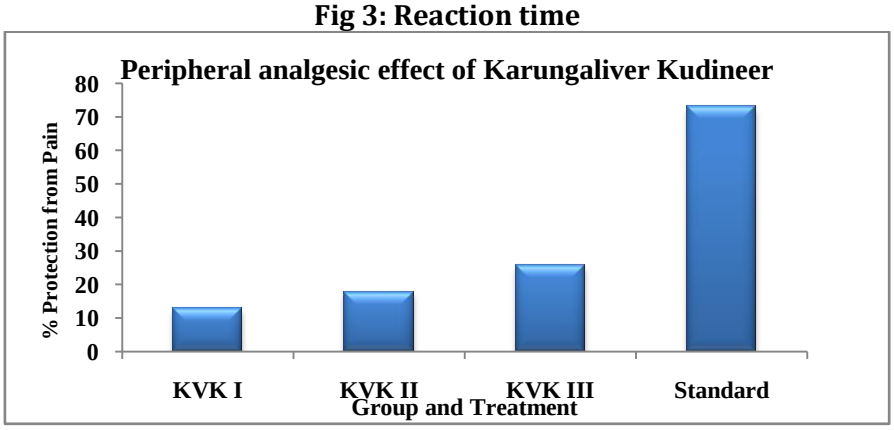

Fig 4: Peripheral analgesic effect of Karungaliver Kudineer

CONCLUSION

Karunkaliver Kudineer showed significant (P<0.01) analgesic effect in the hot plate test, implicating supra spinal analgesic pathways. In these pain paradigms Pentazocin, which is similar to the action of opioid agonists (e.g. morphine), raised the pain threshold level within 30 min of administration. Karunkaliver Kudineer showed maximum analgesic effect after 90 min of administration. In conclusion, the Karunkaliver Kudineer was proved as a safe Siddha remedy for the treatment of algesia at the dose level of 4ml/kg body weight orally.

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