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ANTI-HYPERALGESIC EFFECT OF NARINGIN IN CHRONIC CONSTRICTION INJURY

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Abstract
Chronic neuropathic pain, caused due to damage to the central as well as peripheral nervous system is a debilitating and prevalent condition affecting 10-18% population. Which had a symptoms like pain, dysaesthesia, paraesthesia, allodynia, hyperalgesia. Chronic constriction induced neuropathic pain is a rat model-basedexperimentadopting chronic constriction injury model (Bennett and xie,1988). In this study an attempt was made to test the sensitivity of the rat to various clinically validated drugs likenaringin(20,40,80mg/kg) as well as gabapentin (100mg/kg),wister rats of both sexes were used and randallselittoanalgesymeter is used as an experimental toolforpre and post-surgery, thiopental sodium is used aaanesthesia. Naringin suspensions were prepared just 10 min before the administration.6 rats were taken as subjects for each group. By this study the results suggested that naringin 20,40,80mg has shown significant decrease in anti hyperalgesic effects and narangin 80mg has exhibited its maxiumum effects compared with the gabapentin.

Key-Words: Anti-hyperalgesic effects, Randall SelittoAnalgesymeter, Allodynia, Hyperalgesia.

INTRODUCTION

Chronic pain which is related to peripheral neuropathy is clinically important and isassociatedwith actual or potential tissue damage, or described in terms of such damage.Neuropathic pain is often characterized by pathological symptoms such as hyperalgesia and allodynia for spontaneous pain[1,2]. Opioid analgesics, anticonvulsive agents such as gabapentin, and topical anesthetics have been administered, for the treatment of neuropathic pain, and nonpharmacological treatments like acupuncture and electrical stimulation. New treatments and drugs are necessary as both the pharmacological and non- pharmacological treatments were not completely effective in ameliorating neuropathic pain[3].

Animal models of peripheral nerve injury are useful to elucidate the mechanisms underlying neuropathic pain and to improve current clinical treatments. Chronic constriction injury (CCI), an animal model of neuropathic pain proposed by Bennett and Xie, produces pronounced behavioral responses and it can reproduce signs noticed in humans, and also help to understand neural mechanisms in neuropathic pain disorders[4,5]. The Bennett and Xie model is widely used and accepted model for studies of mononeuropathy which is characterized by mechanical and cold allodynia that are produced by chronic constriction of the sciatic nerve[6,7].In this study, the efficacy of Naringin in Chronic Constriction Injury (CCI) model of neuropathic pain in rat model was studied.

MATERIALS AND METHOD

MATERIAL

Naringin (5,7-Dihydroxyflavone) was sourced from Sigma Chemical Co. (St. Louis. USA). Thiopentone sodium was purchased from National Chemicals (Vadodara, INDIA). Gabapentin was gifted by Hetero drugs, Hyderabad. All other chemicals and reagents used were of analytical grade.

ANIMALS

The wistar rats of either sex were used for the in-vitro glucose uptake studies. All the animals were fed with standard rat pellets and water ad libitum and maintained under standard laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government. KVSR Siddhartha College of Pharmaceutical Sciences, Regd No: 993/a/06/CPCSEA).

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Chronic Constriction Injury (CCI) model:

The Chronic Constriction Injury (CCI) model is the first model of post traumatic painful peripheral neuropathy. It produces a partial denervation of the sciatic nerve that affects myelinated afferent axons much more severely than unmyelinated. CCI model produces a partial denervation that allows for the analysis of pain behaviors evoked by stimulation of thenerve's target - the hind paw.

Surgical Procedure:

Rats were anesthetized with thiopental sodium 30mg/kg by intraperitoneal route

In this model of CCI , an incision of 1.5 cm was made 0.5 cm below the pelvis. Sciatic nerve was exposed and isolated after separation of the biceps femoris and the gluteous superficialis and four loose ligatures (5-0 chromic catgut) with 1-mm spacing were placed around it. Following surgery, Povidone-lodine was applied daily for 3 days and animals were left for one week for the assessment of mechanical hyperalgesia.

The **Randall selittoanalgesymeter**apparatus is used to assess the hyperalgesia in animal models of neuropathic pain.Baseline Paw withdrawal Threshold (PWD) was measured on left paw using Randall selitto one day before surgery. This is considered as basal threshold.Seven days after surgery, on 8th day, pre-dose PWT was recorded.PWT was again determined 0.5, 1, 2 and 3hr after the drug administration. Experimenter was blinded to treatment groups.Data was expressed as withdrawal threshold in gramsand percentage reversal ofhyperalgesia.

Percent reversal of Hyperalgesia =(post dose PWT- pre dose PWT) X 100
(Basal - pre dose PWT)

1.1. Experimental Design for CCI Induced Neuropathic Pain:

Group 1(n=6)	CCI surgical animals treated with sodium CMC.
Group 2(n=6)	CCI surgical animals treated with Naringin 20mg/kg. p. o.
Group 3(n=6)	CCI surgical animals treated with Naringin 40mg/kg, p. o.
Group 4(n=6)	CCI surgical animals treated with Naringin 80mg/kg, p. o.
Group 5(n=6)	CCI surgical animals treated with Gabapentin 100mg/kg,i.p

Drug suspension preparation and administration:

To prepare the suspension for oral route, Naringin was triturated with an addition of two drops of Tween-80. Drug was administered to the animals within 10 minutes of the preparation by oral route. Gabapentin and tramadol wereDissolved in distilled water and administered to animals.

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RESULTS:

Table 1: Paw withdrawal threshold (PWTs) of disease control animals treated with vehicle

Naïve threshold	Predose threshold	Post dose 0.5hr	Post dose 1hr	Post dose 2hr	Post dose 3hr
150	35	40	50	35	40
140	40	45	40	40	45
130	45	35	45	40	45
140	55	55	40	50	40
150	40	45	35	35	35
150	50	40	55	35	50
Mean-143.33	44.17	43.33	44.17	40.83	42.50
S.D-8.16	7.36	6.83	7.36	5.85	5.24

Table 2: Paw withdrawal threshold (PWTs) of animals treated with naringin 20mg/kg.p.o

Naïve threshold	Predose threshold	Post dose 0.5hr	Post dose 1hr	Post dose 2hr	Post dose 3hr
150	45	95	100	75	60
140	50	100	105	75	55
130	50	90	95	80	55
140	60	90	100	80	60
150	40	95	100	85	65
150	55	100	95	85	60
Mean-143.33	50.00	95.00	99.17	80.00	59.17
S.D-8.16	7.07	4.47	3.76	4.47	3.76

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Table 3: Paw withdrawal threshold (PWTs) of animals treated with naringin 40mg/kg,p.o.

Naïve threshold	Predose threshold	Post dose 0.5hr	Post dose 1hr	Post dose 2hr	Post dose 3hr
150	60	90	100	90	65
150	45	105	100	75	60
130	60	100	120	100	80
140	45	95	95	90	80
150	40	100	115	85	65
150	60	85	125	85	70
Mean-145.00	51.67	95.83	109.17	87.50	70.00
S.D-8.37	9.31	7.36	12.42	8.22	8.37

Table4: Paw withdrawal threshold (PWTs) of animals treated with naringin 80mg/kg,p.o.

Naïve threshold	Predose threshold	Post dose 0.5hr	Post dose 1hr	Post dose 2hr	Post dose 3hr
140	60	95	115	95	75
150	60	100	115	95	75
150	60	95	120	100	85
150	55	100	120	105	80
150	55	120	120	95	90
145	45	110	130	100	85
Mean-147.50	55.83	103.33	120.00	98.33	81.67
S.D-4.18	5.85	9.83	5.48	4.08	6.06

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Table5: Paw withdrawal thresholds (PWTs) of animals treated with gabapentin 100mg/kg,i.p

Naïve threshold	Predose threshold	Post dose 0.5hr	Post dose 1hr	Post dose 2hr	Post dose 3hr
130	50	120	130	105	70
150	55	110	130	95	75
140	60	120	130	95	70
140	60	100	120	95	65
150	55	120	115	100	70
150	55	110	130	100	80
Mean-143.33	55.83	113.33	125.83	98.33	71.67
S.D-8.16	3.76	8.16	6.65	4.08	5.16

Table6:% Reversal of Paw withdrawal thresholds (PWTs) of animals treated with naringin 20mg/kg,p.o.

Post dose- 0.5hr	Post dose- 1hr	Post dose- 2hr	Post dose- 3hr
47.62	52.38	28.57	14.29
55.56	61.11	27.78	5.56
50.00	56.25	37.50	6.25
37.50	50.00	25.00	0.00
50.00	54.55	40.91	22.73
47.37	42.11	31.58	5.26
Mean- 48.01	52.73	31.89	9.01
S.D- 5.93	6.42	6.14	8.13

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Table7:% Reversal of Paw withdrawal thresholds (PWTs) of animals treated with naringin 40mg/kg,p.o.

Post dose- 0.5hr	Post dose- 1hr	Post dose- 2hr	Post dose- 3hr
33.33	44.44	33.33	5.56
57.14	52.38	28.57	14.29
57.14	85.71	57.14	28.57
52.63	52.63	47.37	36.84
54.55	68.18	40.91	22.73
27.78	72.22	27.78	11.11
Mean- 47.10	62.60	39.18	19.85
S.D- 13.04	15.44	11.57	11.70

Table8:% Reversal of Paw withdrawal thresholds (PWTs) of animals treated with naringin 80mg/kg,p.o.

Post dose- 0.5hr	Post dose- 1hr	Post dose- 2hr	Post dose- 3hr
43.75	68.75	43.75	18.75
44.44	61.11	38.89	16.67
38.89	66.67	44.44	27.78
47.37	68.42	52.63	26.32
68.42	68.42	42.11	36.84
65.00	85.00	55.00	40.00
Mean- 51.31	69.73	46.14	27.73
S.D- 12.28	8.02	6.29	9.37

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Table9:% Reversal of Paw withdrawal thresholds (PWTs) of animals treated with gabapentin 100mg/kg,i.p

Post dose- 0.5hr	Post dose- 1hr	Post dose- 2hr	Post dose- 3hr
87.50	100.00	68.75	25.00
57.89	78.95	42.11	21.05
75.00	87.50	43.75	12.50
50.00	75.00	43.75	6.25
68.42	63.16	47.37	15.79
57.89	78.95	47.37	26.32
Mean- 66.12	80.59	48.85	17.82
S.D- 13.69	12.37	9.98	7.75

On acute treatment:

In all animals, cut off PWT was 160g. In normal control rats, PWT was not significantly altered In CCIcontrol rats declined from 150g (naive) to 70 g (pre-dose). In Naringin 20mg/kg treatment group, naive PWT, pre-dose 1/2 h, 1 h, 2 h and 3h post-dose PWT were found to be 143.33 ±8.16, 44.17±7.36, 43.33± 6.83, 44.17±7.36, 40.83±5.85 and 42.50 ±5.24 respectively.In Naringin (40mg/kg) treatment group, naive PWT, pre-dose 1/2 h, 1 h. 2 h and 3h post-dose PWT were found to be 145.0 ± 8.37, 51.67± 9.31, 45.83±7.36, 109.17 ±12.42, 87.50 ± 8.22 and 70.0± 8.37 respectively. In Naringin (80mg/kg) treatment group, naive PWT, pre-dose 1/2 h, 1 h, 2 h and 3h post-dose PWT were found to be 147.50 ± 4.18, 55.83± 5.85, 103.33 ± 9.83, 120.0± 5.48, 98.33 ± 4.08 and 81.67 ± 6.06 respectively. In Gabapentin (100mg/kg)treatment group, naive PWT, pre-dose 1/2 h, 1 h, 2 h and 3h post-dose PWT were found to be 143.33± 8.16, 55.83 ± 3.76, 113.33 ± 8.16, 125.83 ± 6.65, 98.33 ± 4.08 and 71.67 ± 5.16 respectively. Peak effectwas observed at 1 h post-dose for all treatment groups.

DISCUSSION

Our study results indicated that CCI surgery resulted in significant hyperalgesia in rat models. This result is strongly in accordance with previous findings [8]. Acute treatment of Naringin by oral route of administration at three different dose levels of 20mg/kg, 40mg and 80mg/kg have exhibited dose dependent and time dependent effect in reversing hyperalgesia on Paw Pressure Analgesymeter. Our results are also in consistent with the previous study in which neuroprotective action of Naringin by treating spinal cord injury (SCI) induces rats resulted in a significant increase ($P<0.05$) in lipid peroxidase, nitric oxide, tumor necrosis factor alpha, interleukin-16, and bax whereas expression of bcl-2 and caspase-3 were significantly ($P < 0.05$) reduced [9]. Similarly, reduction in pain by Naringin was due to inhibition of nitric oxide synthase which results in decreased amounts of NO which found to improve the recovery of neurological function in rats subjected to SCI [10]. In addition, previous reports disclosed that Naringin shows efficacy in animal models of inflammatory pain. The same suggest that the flavone Naringin possesses in vivo anti-inflammatory and anti-nociceptive potential, which are supported in silico by an interaction with COX-2 binding site [11]. On the contrary to our findings, one previous report implied that Naringinitself causes hyperalgesia by acting as ligand for BDZ receptor a subtype of GABA(A) receptor [12]. However, there are mixed results with regard to the efficacy of Naringin on hyperalgesia. Naringin and naringenin exert a potent anti-inflammatory effect and have been used successfully in the treatment of inflammatory diseases, such as Alzheimer's disease. In the previous study, we observed that repeated administration of naringenin was able to alleviate neuropathic pain in a

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dose-dependent manner. Furthermore, the anti-allodynia effect of naringenin (200 mg/kg)remained significant 7 days after discontinuation of the treatment. These data suggest that intrathecal treatment with naringenin exerts a strong antinociceptive effect. Once this effect is achieved, it may last over a long period of time. This characteristic indicates that naringenin may be suitable for the long-term treatment of chronic pain. Moreover, naringenin penetrates the blood brain barrier easily. Therefore, naringenin may be a useful drug in the treatment of neuropathic pain.

CONCLUSION

Hyperalgesia was significantly induced by CCI surgery in the rat model as paw withdrawal thresholds significantly decreased in all CCI surgery performed rats. Naringin 20mg, 40 mg and 80mg by oral route have significantly decreased the peak anti-hyperalgesia effect was observed at 1hour. Naringin 80 mg has exhibited maximum effect of 70% which is comparable with standard drug Gabapentin effect.

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