Effect of Chrysin on Mechanical Hyperalgesia in Chronic Constriction Injury-Induced Neuropathic Pain in Rat Model

Abstract

Objective: The present study aimed to assess the effect of Chrysin on mechanical hyperalgesia in chronic constriction injury (CCI)-induced neuropathic pain in Wistar rats. Materials and Methods: Neuropathic pain was induced by CCI to the sciatic nerve in rats. Oral treatment of chrysin was given at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg in neuropathic rats. Mechanical hyperalgesia (in terms of paw withdrawal threshold [PWT]) was measured using Randall–Selitto analgesy-meter, and percent PWT was determined. Statistical analysis was carried out using GraphPad Prism 5 tool. Results: In mechanical hyperalgesia test, treatment with chrysin 200 mg/kg, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 141 ± 8.94 g, 60 ± 7.91 g, 107 ± 9.08 g, 113 ± 5.70 g, 106.0 ± 7.42 g, and 97 ± 9.08 g, respectively. The peak effect was observed at 2 h posttreatment for 50 mg and 100 mg while the peak effect for 200 mg was reached at 1 h, and the same was maintained till 2 h posttreatment. Chrysin 200 mg dose has shown maximal percent reversal (74%) at 2 h posttreatment. The percent reversal PWT of 50 mg/kg, 100 mg/kg, and 200 mg/kg at 2 h were 68%, 67%, and 74%, respectively. Chrysin has exhibited dose-dependent efficacy in CCI-induced neuropathic pain. In mechanical allodynia test, In chrysin (200 mg/kg) treatment group, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 60.0 ± 0.0 g, 5.0 ± 1.10 g, 22.45 ± 6.62 g, 52.64 ± 18.29 g, 37.33 ± 17.56 g, and 29.83 ± 9.22 g, respectively. The percent reversal PWT of 50 mg/kg, 100 mg/kg, and 200 mg/kg at 2 h were 43%, 68%, and 87%, respectively. Conclusion: Chrysin attenuates neuropathic pain by ameliorating mechanical hyperalgesia and allodynia. Further studies are warranted to establish the mechanism.

Keywords: Analgesy-meter, chrysin, flavone, hyperalgesia, neuropathic pain, paw withdrawal threshold

Introduction

Neuropathic pain is now defined by the International Association for the Study of Pain as “pain caused by a lesion or disease of the somatosensory nervous system.” This replaces the older definition of “pain initiated or caused by a primary lesion, dysfunction, or transitory perturbation of the peripheral or central nervous system.”[1] Neuropathic pain affects 7%–10% of the general population.[2] Multiple causes of neuropathic pain have been described, and its incidence is likely to increase owing to the aging global population, increased incidence of diabetes mellitus, and improved survival from cancer after chemotherapy.[3]

Neuropathic pain can substantially impair the quality of life of the patient in both diabetic and nondiabetic patients.[4] Due to undesirable side effects of treatments and unknown mechanisms of pathological pain states,[4] the treatment of neuropathic pain is tough with conventional methods. Currently, pharmacotherapy of neuropathic pain is largely limited to mainly “off-label” use of drugs approved for other conditions, especially tricyclic anti-depressants, and anticonvulsants. Current first-line therapies used for neuropathic pain are calcium channel α2-delta ligands (pregabalin and gabapentin), tricyclic antidepressants (amitriptyline, nortriptyline, and desipramine), serotonin–norepinephrine reuptake inhibitors (duloxetine and venlafaxine), and second-line drugs used are opioids (tramadol and tapentadol). The use of pregabalin and gabapentin are limited as both drugs have a risk of increased suicidal thoughts or behaviors. In addition, both should be dose adjusted for patients with renal impairment. Similarly, amitriptyline has elicited pain relief only in a minority of the population. On the
other hand, duloxetine and venlafaxine are associated with increasing blood pressure and cardiac conduction abnormalities; hence, they should be used cautiously in patients with cardiac disease. Hence, there is a growing necessity for the discovery of new therapeutic interventions for neuropathic pain.[5]

Neuropathic pain is an important problem that needs to be addressed with flavonoids are the largest group of plant secondary metabolites with immense biological activities desired for human health. Chrysin is a hydroxylated flavone derivative mainly found in honey, propolis, and many plant species, for example, *Pelargonium crispum* (*P. J. Bergius*) *L. Her., Passiflora incarnata L.* Chrysin has been shown to be a very active flavonoid exerting a vast number of pharmacological properties such as anti-diabetic,[6] neuroprotective,[7] anticancer,[8] anti-inflammatory,[9] nephroprotective,[10] and anti-arthritic.[11] Considering neuroprotective potential of Chrysin, the study was planned to assess mechanical hyperalgesia in chronic constriction injury (CCI)-induced neuropathic pain in the rat model.

**Materials and Methods**

**Materials**

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

**Animals**

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 of KVSR Siddhartha College of Pharmaceutical Sciences, and studies were carried out as per the guidelines of CPCSEA (Regd No: 993/PO/Re/S/06/CPCSEA).

**Experimental design**

A total of 30 rats (weighs between 200 and 220 g) were subjected to CCI surgery. Animals were allowed for 7 days for the development of neuropathic pain which is characterized by hyperalgesia and allodynia. Five animals were excluded from the study, as they have not exhibited pain. There was no mortality of animals during the study period. Hence, 25 animals were randomly divided into five groups, with five animals each. Group 1, Control group (CCI-induced animals) treated with 1% sodium carboxymethyl cellulose (CMC); Group 2, CCI-induced animals treated with Chrysin (50 mg/kg, p.o.); Group 3, CCI-induced animals treated with Chrysin (100 mg/kg, p.o.); Group 4, CCI-induced animals treated with Chrysin (200 mg/kg, p.o.); Group 5, CCI-induced animals treated with gabapentin (100 mg/kg, i.p). The day on which CCI surgery was carried out is considered as day 0. Chrysin acute treatment was given as a single dose on day 8 after induction of neuropathic pain. Readouts of paw withdrawal threshold (PWT) were taken at 0.5 h, 1 h, 2 h, and 4 h following treatment of Chrysin. Chrysin was suspended in 1% sodium CMC with an addition of 0.1 ml Tween-80.

**Induction of neuropathic pain**

Rats were anesthetized with thiopental sodium 30 mg/kg by intraperitoneal route. In the CCI model, a 1.5-cm incision was made 0.5 cm below the pelvis. The biceps femoris and the gluteus superficialis were separated, and the sciatic nerve was exposed and isolated, and four loose ligatures (5-0 chronic catgut) with 1-mm spacing were placed around it. Following surgery, Povidone-iodine was applied daily for 3 days, and animals were left for 1 week for the assessment of mechanical hyperalgesia. Animals found to have developed neuropathic pain after surgery were included in the study. On the 8th day after surgery, animals were evaluated for the development of symptoms of mechanical hyperalgesia. Animals were considered to be neuropathic when the same exhibited mechanical hyperalgesia (i.e., paw withdrawal response was observed at a paw pressure of ≤70 g).

**Determination of mechanical hyperalgesia (Randall–Selitto paw pressure test)**

PWT, an index of mechanical hyperalgesia, was assessed by the previously described method.[12] The PWT was quantified using the Randall–Selitto paw pressure algosy-meter (model, 37215; UGO Basile, Italy). Increasing pressure at a linear rate of 10 g/s was applied to the center of the hind paw. The pressure at which animal withdraws its paw was recorded and expressed in mass units (g), with a cut-off of 150 g to avoid potential tissue injury. PWT was recorded for the left hind paw before and up to 4 h after treatment. Percent reversal of PWT was determined using the formula: \((\text{postdose threshold − predose threshold})/(\text{naive threshold − predose threshold})\) × 100.

**Determination of mechanical allodynia**

The rats were placed individually in plastic cages with a plastic mesh floor to determine the withdrawal threshold. The animals were left in the cage for 20–30 min to acclimatize to the test. The PWT in response to mechanical stimulation was measured using the up-and-down method[13] by applying calibrated von Frey filaments (Aesthesio®; Ugo basile, Italy) to the hind paw from underneath the cage through openings in the mesh floor. A series of von Frey filaments (0.4, 0.7, 0.16, 0.40, 0.60, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, 15, 26, and 60 g) were applied vertically to the plantar surface of the hind paw for 5 s while the hair was bent. Brisk withdrawal of paw or paw flinching was considered a positive response. The absence of a response...
in the animals at a pressure of 60 g was considered the cutoff value. The stimulation with one filament was repeated five times at 10–15 s intervals, when lack of a response, the next filament with greater bending force was applied. The lowest force required to elicit a paw withdrawal response was recorded as the PWT (g). The animals that exhibited paw withdrawal response or flinching response at <4 g were considered to have developed the allodynia. Percent reversal of PWT was determined using the formula: \((\text{postdose threshold} - \text{predose threshold})/\text{(naive threshold} - \text{predose threshold})\) \times 100.

Statistical analysis

The results were expressed as mean ± standard error of the mean. Differences in PWT in hyperalgesia were determined by two-way analysis of variance followed by the Bonferroni post hoc test. Differences at \(P < 0.001\) were considered statistically significant.

Results

Effect of chrysin on paw withdrawal thresholds in chronic constriction injury-induced neuropathic pain

In all animals, the cutoff PWT was 150 g. Figure 1 shows PWT was not significantly altered at any time point in control rats. In CCI surgery rats, predose PWT (on the 8th day before treatment) were found to be significantly \((P < 0.001)\) low when compared to Naive PWT. In chrysin (50 mg/kg) treated animals, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWTs were found to be 146 ± 4.18 g, 60 ± 7.91 g, 85 ± 8.66 g, 100 ± 6.12 g, 118 ± 5.7 g, and 95 ± 6.12 g, respectively. In the animals treated with Chrysin 100 mg/kg, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 144 ± 8.94 g, 65 ± 9.62 g, 95 ± 10 g, 105.0 ± 3.54 g, 118.0 ± 7.58 g, and 115 ± 14.58 g, respectively. In the animals treated with Chrysin 200 mg/kg, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 141 ± 8.94 g, 60 ± 7.91 g, 107 ± 9.08 g, 113 ± 5.70 g, 106.0 ± 7.42 g, and 97 ± 9.08 g, respectively. The peak effect was observed at 2 h posttreatment for 50 mg and 100 mg while the peak effect for 200 mg was reached at 1 h, and the same was maintained till 2 h posttreatment. Dose-dependent increase in the effect was evident at 0.5 and 1 h posttreatment [Figure 1].

Effect of chrysin on percent reversal paw withdrawal thresholds in chronic constriction injury -induced neuropathic pain

Figure 2 shows data of percent reversal of PWT. In chrysin (50 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose were found to be 28.97 ± 6.81, 46.53 ± 6.80, 67.88 ± 7.55, and 40.35 ± 9.38, respectively. In chrysin (100 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 37.24 ± 7.84, 50.55 ± 10.40, 67.52 ± 10.0, and 63.55 ± 15.27, respectively. In chrysin (200 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 59.51 ± 13.74, 64.49 ± 5.71, 74.45 ± 5.86, and 53.02 ± 8.24, respectively. The percent reversal of PWT was found to be significantly high at 1 h posttreatment of Chrysin. The percent reversal PWT of 50 mg/kg, 100 mg/kg, and 200 mg/kg at 2 h were 68%, 67%, and 74%, respectively. Chrysin 200 mg dose has shown maximal percent reversal (74%) at 2 h posttreatment [Figure 2].

Effect of chrysin on mechanical allodynia in chronic constriction injury-induced neuropathic pain

PWT of animals in allodynia test is presented in Figure 3. In all animals, cut off PWT was 60 g. In chrysin (50 mg/kg) treated animals, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 144 ± 8.94 g, 65 ± 9.62 g, 95 ± 10 g, 105.0 ± 3.54 g, 118.0 ± 7.58 g, and 115 ± 14.58 g, respectively. In the animals treated with Chrysin 100 mg/kg, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 141 ± 8.94 g, 60 ± 7.91 g, 107 ± 9.08 g, 113 ± 5.70 g, 106.0 ± 7.42 g, and 97 ± 9.08 g, respectively. The peak effect was observed at 2 h posttreatment for 50 mg and 100 mg while the peak effect for 200 mg was reached at 1 h, and the same was maintained till 2 h posttreatment. Dose-dependent increase in the effect was evident at 0.5 and 1 h posttreatment [Figure 1].

Figure 1: Effect of chrysin on mechanical hyperalgesia (in terms of paw withdrawal threshold) in chronic constriction injury-induced rats. All values are mean ± standard error of the mean (n = 5). \(P < 0.001\) compared to Naive threshold. \(*P < 0.001\) compared to the respective control group at each time point. Two-way ANOVA followed by the Bonferroni post hoc test to compare to each column to column

Figure 2: Effect of chrysin on percent reversal of paw withdrawal threshold in mechanical hyperalgesia study. All values are mean ± standard error of the mean (n = 5)
found to be 60.0 ± 0.0 g, 5.0 ± 1.10 g, 27.54 ± 6.455 g, 42.21 ± 12.45 g, 35.67 ± 5.68 g, and 31.23 ± 6.24 g, respectively. In chrysin (200 mg/kg) treatment group, naive PWT, predose PWT, 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 60.0 ± 0.0 g, 5.0 ± 1.10 g, 22.45 ± 6.62 g, 52.64 ± 18.29 g, 37.33 ± 17.56 g, and 29.83 ± 9.22 g, respectively.

Effect of chrysin on percent reversal paw withdrawal thresholds in chronic constriction injury-induced neuropathic pain

Figure 4 shows data of percent reversal of PWT in mechanical allodynia. In chrysin (50 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose were found to be 26.47 ± 3.20, 42.98 ± 3.60, 43.87 ± 4.10, and 33.55 ± 3.60, respectively. In chrysin (100 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 40.98 ± 5.20, 67.65 ± 7.50, 55.76 ± 6.20, and 47.69 ± 4.30, respectively. In chrysin (200 mg/kg) treatment group, percent reversal of PWT at 0.5 h, 1 h, 2 h, and 4 h postdose PWT were found to be 31.73 ± 5.40, 86.62 ± 9.50, 58.78 ± 4.30, and 45.15 ± 4.40, respectively.

Discussion

The present study results indicated that CCI surgery resulted in significant hyperalgesia in rat models. This result is strongly in accordance with previous findings. Acute treatment of Chrysin by oral route of administration at three different dose levels of 50 mg/kg, 100 mg/kg, and 200 mg/kg have exhibited substantial and moderate efficacy at 1 h posttreatment while moderate efficacy at 2 h post and at 4 h also increased efficacy treatment in reversing hyperalgesia on Paw Pressure Analgesy-meter. The observed effect was not on par with standard drug, gabapentin. The results are inconsistent with the previous study in which Chrysin offered neuroprotective action against spinal cord injury (SCI). Attenuation of pain by Chrysin is probably attributed to its property of inhibition of inducible nitric oxide synthase, which results in decreased amounts of NO. Thus, there was an improvement in the recovery of neurological function in rats subjected to SCI. In addition, previous reports disclosed that Chrysin shows efficacy in animal models of inflammatory pain. It is suggested that the flavone, Chrysin possesses in vivo anti-inflammatory and anti-nociceptive potential, which are supported in silico by an interaction with COX-2 binding site. Similar to Chrysin, other flavones are also reported to have anti-hyperalgesic effects in various animal models of pain. For instance, our recent research findings in our laboratory demonstrated that Biochanin A, isoflavone, has exhibited efficacy in reversing mechanical hyperalgesia in animal models of diabetic neuropathic pain. However, on the contrary to our findings, a previous report implied that Chrysin itself causes hyperalgesia by acting as a ligand for BDZ receptor α subtype of GABA_ receptor. Chrysin treatment shows the antinociceptive effect in the formalin test and also declined corticosterone and noradrenaline levels; therefore, Chrysin might cause the analgesic effect. However, there are mixed results with regard to the efficacy of Chrysin on hyperalgesia. To the best of our knowledge, there are no studies conducted to assess the effect of chrysin on mechanical allodynia. In our study, Chrysin 200 mg/kg exhibited better efficacy (87%) than Gabapentin (69%) in reversing allodynia at 1 h posttreatment. On the other hand, Gabapentin has shown more efficacy (80%) at 0.5 h than Chrysin (32%).

Conclusion

Chrysin attenuates neuropathic pain by ameliorating mechanical hyperalgesia and allodynia. Further studies are warranted to establish the mechanism.

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Conflicts of interest
There are no conflicts of interest.

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