

# Evaluation of Chronic Constriction Injury Induced Neuropathic Pain Using Chrysin in Rats

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## ABSTRACT

**Introduction:** The exhaustive literature review suggested the vast pharmacological profile of chrysin but this drug has not been scientifically reported in the healing of nerve pain till date. The nerve pain is linked with various diseases such as cancer, inflammation, diabetic and mental problems. Neuropathic pain has been viably prompted in rodents by chronic constriction injury (CCI) of sciatic nerve. **Objectives:** The natural phytoconstituent chrysin will be investigated for neuropathic pain using well established models. **Materials and Methods:** The neuroprotective action of chrysin on neuropathic pain and tissue biomarkers was measured using standardized experimental tests reported in references books. **Results:** The delivery of chrysin (50 and 100 mg/kg; *p.o.*) for 15 continuous days showed critical neuropathic pain inhibitory action with respect to control and measurably identical to standard medication in all experimental models via dose dependent manner. The level of TBARS was declined by chrysin for the 15 days while levels of reduced glutathione and total protein were expanded with respect to control and measurably identical to standard medication. Chrysin is one of huge herbal medication to rummage free radicals in the natural structure and it also control the free radical related lipid peroxidation process in the pathogenesis of neuropathic pain. **Conclusion:** It can be concluded that, extraordinary expansive assessments are required to develop this medicinal activity by using distinctive animal model of neuropathic pain. Additionally, growing the healing impact of chrysin and various flavonoids may give exceptional assistance to finish the further examination in clinical research plan. **Key words:** Biomarker, Chrysin, Flavonoid, Gabapentin, Neuropathy, Pain.

## INTRODUCTION

Neuropathic pain is a chronic maladaptive neurodegenerative disorder. Neuropathic pain refers to pain can arise after nerve injury, it is causing harm changes occur in injured neurons and then sensation pain and descending path in the central nervous system.<sup>1</sup>

The study of disease transmission of neuropathic pain uncovered that 1 and 2 % neuropathic pain patients of total population are available in the United Kingdom<sup>2</sup> and in United States of America, respectively.<sup>3</sup> The overall diabetic neuropathic pain was noted 2.8 % in 2000 and second most instances of neuropathic pain because of herpes zoster contamination which is seen in 24 % at 2004.<sup>4</sup> The more seasoned age over 70 are increasingly inclined to build up the neuralgia when contrasted with beneath

60 years of age, the thing that matters is watched just about 3 % of postherpetic neuralgia patients.<sup>5</sup> The sexual orientation inconstancy i.e., females are better than male species for the improvement of neuropathic pain because of the hormonal fluctuation.

Various synthetic medications are utilized for the healing of neuropathic pain like anticonvulsant, antidepressants and narcotics,<sup>6-9</sup> but the uses of these drugs has been accounted for to deliver the harmful reactions because of constant use in neuropathic patients.<sup>10</sup> Right now different herbal remedies such as curcumin, sodium ferulate, safranal, geniposidic acid, berberine, huperzine A, limonoids and nutrient E are assume a promising job in the enhancement of neurodegeneration and treatment of neuropathic pain.<sup>11</sup>

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Chrysin is a dietary phytochemical and found in many plants such as propolis, *Passiflora caerulea*, nectar and scientifically reported in various pharmacological activities such as anticancer,<sup>12</sup> anti-inflammatory,<sup>13</sup> neuroprotective,<sup>14</sup> antidiabetic,<sup>15</sup> antiarthritic,<sup>16</sup> antiasthmatic,<sup>17</sup> antidepressant<sup>18</sup> and antiviral.<sup>19</sup> Despite vast pharmacological profile of chrysin but this drug has not been scientifically reported in the healing of nerve pain till date. The nerve pain is linked with various abovementioned diseases. Thus, the natural phytoconstituent chrysin will be investigated for neuropathic pain using well established models.

## MATERIALS AND METHODS

### Drugs and Chemicals

Chrysin was procured from American company named "Sigma-Aldrich" and situated in St. Louis, United States. The analytical grade chemicals used in current investigations were purchased from SD Fine Chemicals Mumbai, India.

### Animals

Male Sprague Dawley rat of 200-250 g were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India. The present protocol was sanctioned by Institutional Animal Ethics Committee (IAEC No.: ATRC/13/19; Dated: 31/08/19).

### Induction of peripheral neuropathic pain

Neuropathic pain was generated in rats by standard protocol reported in scientific literature such as CCI of sciatic nerve.<sup>20</sup>

### Behavioral evaluation

Evaluation of behavioral parameters was carried out at different time span *i.e.*, 0, 3, 6, 9, 12 and 15<sup>th</sup> day between 09.00 am to 03.00 pm. The order of behavioural studies was carried out from low intense stimuli to high intense stimuli (allodynia followed by hyperalgesia) in paw as well as in tail employing different well standardized animal based tests like Von Frey hair filament test,<sup>21</sup> Hargreaves test,<sup>22</sup> pin prick test,<sup>23</sup> D'Aemour and Smith test,<sup>24</sup> tail pinch test<sup>25</sup> and acetone drop test.<sup>26</sup>

### Biochemical analysis

The animals of each group were sacrificed after 16<sup>th</sup> day of behavioural observation by cervical dislocation and complete right sciatic nerves were separated from the animal body immediately. The isolated part of nerve was mixed properly to prepare homogenized mixture (10 % w/v) with phosphate buffer (pH 7.4) and centrifuged at 3500 rpm for 10 min. The supernatant

was used for the determination of TBARS,<sup>27</sup> GSH<sup>28</sup> and total proteins levels.<sup>29</sup>

### Experimental protocol

In the present investigations, the animals (24 animals) were divided into four groups, each group comprising of six male SD rats. Group I (CCI + Control): Control group received vehicle (2.5 ml, *p.o.*) for 15 consecutive days; Group II (CCI + Gabapentin): Standard group received gabapentin (10 mg/kg, *p.o.*) for 15 consecutive days. Group III (CCI + Chrysin): Test group received Chrysin (50 mg/kg, *p.o.*) for 15 consecutive days and Group IV (CCI + Chrysin): Test group received Chrysin (100 mg/kg, *p.o.*) for 15 consecutive days.

### Statistical analysis

Data were expressed as mean $\pm$ SD,  $n=6$  rat per group. \* $P<0.05$  vs Control; <sup>a</sup> $P<0.05$  vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test using sigma stat software version 3.5.

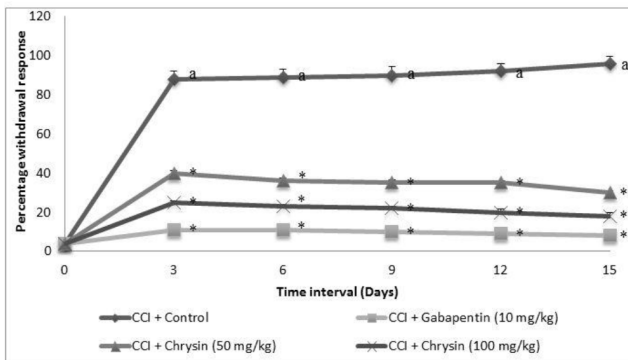
## RESULTS

### Effect of chrysin against neuropathic pain using Von Frey hair filament test

The higher value of percentage paw withdrawal response indicated neuropathic pain whereas minimum value of percentage paw withdrawal response indicated neuroprotective potential. The decreased percentage paw withdrawal response in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.o.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic pain in rats via decline in percentage paw withdrawal response and protected all animals from CCI induced neuropathic pain (Figure 1).

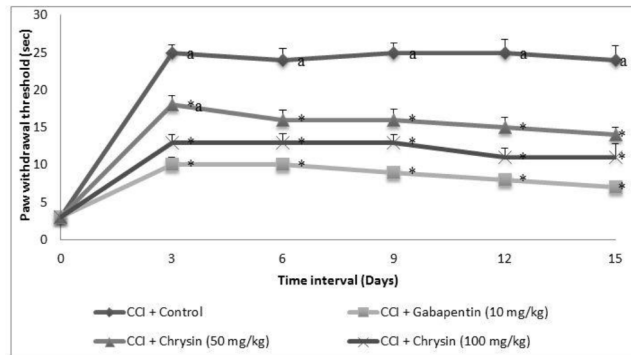
### Effect of chrysin against neuropathic pain using Hargreaves test

The lower value of right hind paw withdrawal threshold indicated neuropathic pain whereas higher value of right hind paw withdrawal threshold indicated neuroprotective potential. The increased value of the right hind paw withdrawal threshold in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.o.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic



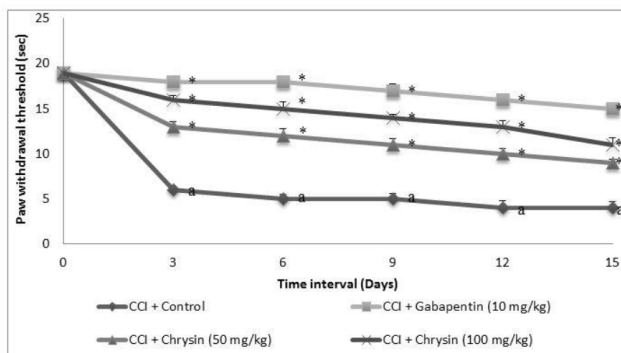
**Figure 1: Investigation of neuroprotective action of chrysin using Von Frey hair filament test.**

n=6; The data is expressed as Mean ± S.D.; \*P<0.05 vs Control; <sup>†</sup>P<0.05 vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.



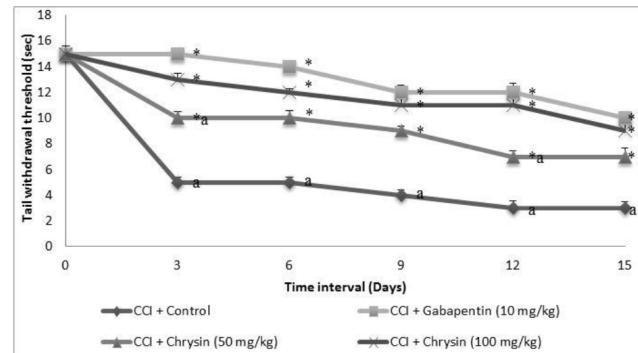
**Figure 3: Investigation of neuroprotective action of chrysin using pin prick test.**

n=6; The data is expressed as Mean ± S.D.; \*P<0.05 vs Control; <sup>†</sup>P<0.05 vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.



**Figure 2: Investigation of neuroprotective action of chrysin using Hargreaves test.**

n=6; The data is expressed as Mean ± S.D.; \*P<0.05 vs Control; <sup>†</sup>P<0.05 vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.



**Figure 4: Investigation of neuroprotective action of chrysin using D'Aemour and Smith test.**

n=6; The data is expressed as Mean ± S.D.; \*P<0.05 vs Control; <sup>†</sup>P<0.05 vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

pain in rats via increased value of the right hind paw withdrawal threshold and protected all animals from CCI induced neuropathic pain (Figure 2).

**Effect of chrysin against neuropathic pain using pin prick test**

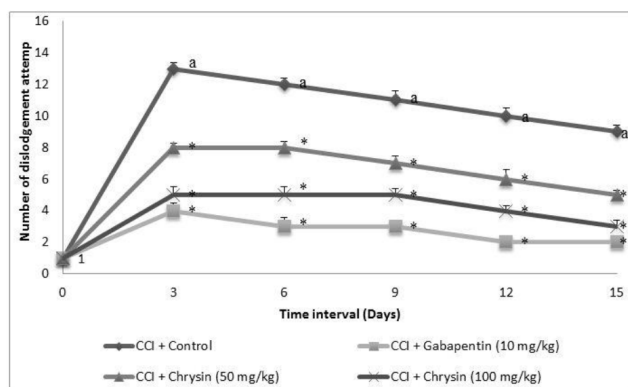
The higher value of the right hind paw withdrawal threshold (sec) indicated neuropathic pain whereas lower value of the right hind paw withdrawal threshold (sec) indicated neuroprotective potential. The decreased value of right hind paw withdrawal threshold (sec) in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.a.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic pain in rats via decline in right hind paw withdrawal threshold (sec) and protected all animals from CCI induced neuropathic pain (Figure 3).

**Effect of chrysin against neuropathic pain using D'Aemour and Smith test**

The lower value of the tail withdrawal threshold indicated neuropathic pain whereas higher value of the tail withdrawal threshold indicated neuroprotective potential. The increased value of tail withdrawal threshold in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.a.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic pain in rats via increase in tail withdrawal threshold and protected all animals from CCI induced neuropathic pain (Figure 4).

**Effect of chrysin against neuropathic pain using tail pinch test**

The higher value of the number of dislodgements attempt indicated neuropathic pain whereas lower value



**Figure 5: Investigation of neuroprotective action of chrysin using tail pinch test.**

$n=6$ ; The data is expressed as Mean  $\pm$  S.D.; \* $P<0.05$  vs Control;  $^{\#}P<0.05$  vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

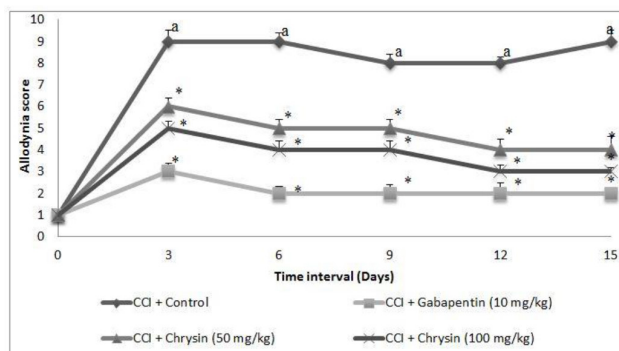
of the number of dislodgements attempt indicated neuroprotective potential. The decreased value of the number of dislodgements attempt in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.a.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic pain in rats via decrease in the number of dislodgements attempt and protected all animals from CCI induced neuropathic pain (Figure 5).

#### Effect of chrysin against neuropathic pain using acetone drop test

The higher value of the allodynia score indicated neuropathic pain whereas lower value of the allodynia score indicated neuroprotective potential. The decreased value of the allodynia score in the rats was noted after the treatment of chrysin (50 or 100 mg/kg, *p.a.*) in relation to control. The chrysin exhibited significant neuroprotective potential at all doses in relation to control in the dose dependent way and similar to gabapentin on the basis of statistical analysis during whole experimentation. The standard drug almost completely abolished neuropathic pain in rats via decrease in the allodynia score and protected all animals from CCI induced neuropathic pain (Figure 6).

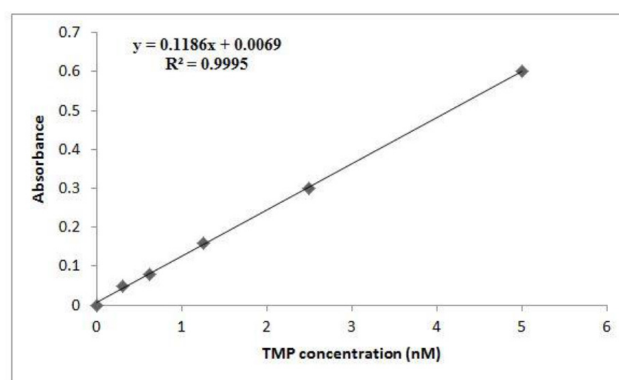
#### In vivo effects of chrysin on CCI induced tissue biomarker disturbances

The calibration curve of TMP ( $y = 0.1186x + 0.0069$ ;  $R^2 = 0.9995$ ; Figure 7), GSH ( $y = 0.0194x + 0.029$ ;  $R^2 = 0.9972$ ; Figure 8) and BSA ( $y = 0.1274x + 0.0084$ ;  $R^2 = 0.9980$ ; Figure 9) was prepared for the simultaneous estimation of TBARS, GSH and total

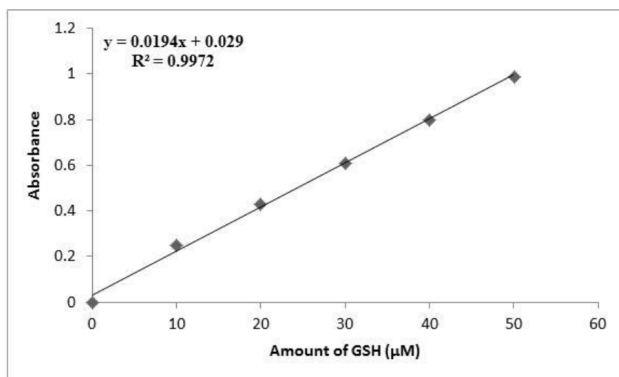


**Figure 6: Investigation of neuroprotective action of chrysin using acetone drop test.**

$n=6$ ; The data is expressed as Mean  $\pm$  S.D.; \* $P<0.05$  vs Control;  $^{\#}P<0.05$  vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.



**Figure 7: Calibration curve of TMP vs absorbance.**



**Figure 8: Calibration curve of GSH vs absorbance.**

proteins respectively. The CCI induced neuropathic pain produced various tissue biomarker disturbances such as higher level of TBARS, lower levels of GSH and total proteins. The neuropathic pain of animals was treated with oral administration of chrysin (50 or 100 mg/kg) via decline in TBARS level, raise in GSH and total proteins levels in relation to control and similar to gabapentin on the basis of statistical analysis (Table 1).

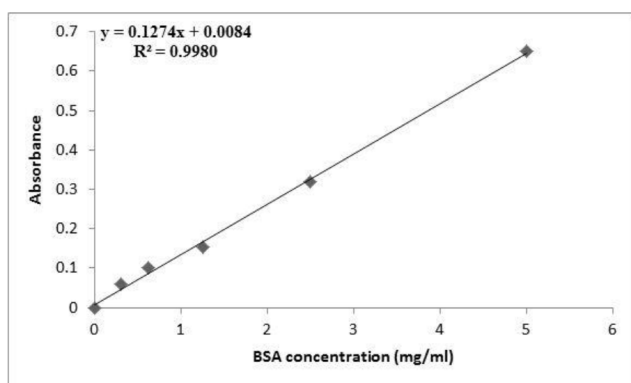


Figure 9: Calibration curve of BSA vs absorbance.

Table 1: *In vitro* effect of chrysin on CCI induced oxidative stress marker disturbances.

Groups	TBARS (nM / mg of protein)	GSH ( $\mu$ M / mg of protein)	Total proteins (mg / ml)
CCI + Control	13.45 $\pm$ 0.60 <sup>a</sup>	28.44 $\pm$ 2.05 <sup>a</sup>	57.64 $\pm$ 3.11 <sup>a</sup>
CCI + Gabapentin (10 mg/kg)	5.49 $\pm$ 0.48 <sup>*</sup>	65.17 $\pm$ 4.58 <sup>*</sup>	115.40 $\pm$ 3.56 <sup>*</sup>
CCI + Chrysin (50 mg/kg)	7.80 $\pm$ 0.98 <sup>*</sup>	50.56 $\pm$ 3.69 <sup>*</sup>	92.45 $\pm$ 5.79 <sup>*</sup>
CCI + Chrysin (100 mg/kg)	6.25 $\pm$ 0.79 <sup>*</sup>	58.98 $\pm$ 4.68 <sup>*</sup>	105.14 $\pm$ 4.49 <sup>*</sup>

n=6; The data is expressed as Mean  $\pm$  S.D.; \*P<0.05 vs Control; <sup>a</sup>P<0.05 vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

## DISCUSSION

The CCI model is a generally utilized animal model of nerve injury which can deliver allodynia/hyperalgesia and is appeared to share numerous pathophysiological states of neuropathic pain in people. CCI model of neuropathic pain is solid and effectively reproducible.<sup>20</sup> The current research finding recommended that CCI of sciatic nerve in rat has conveyed a basic augmentation in warm allodynia, warm hyperalgesia, mechanical hyperalgesia and mechanical allodynia in fringe and central site with alteration of various biochemical parameters i.e., increase in TBARS, decrease in reduced glutathione and proteins levels. The oral administration of chrysin (50 and 100 mg/kg; *p.o.*) fundamentally reduce the neuropathic pain induced via CCI of sciatic nerve and normalizes the social and biochemical changes. Flavonoids are plant specific metabolites that contain enormous gatherings of polyphenolic compounds, which present benefits to human wellbeing on account of their natural properties. Chrysin, which has the universal 15-carbon flavone spine, is one of the most

significant bioactive constituents of various organic products, vegetables and even mushrooms. Chrysin has a typical compound structure, comprising of two intertwined rings, A and C and a phenyl ring, B, appended to the second situation of the C ring. It shares the normal flavone structure, with an extra hydroxyl bunch at the fifth and seventh places of the A ring. Chrysin is generated from the amino acid phenylalanine.<sup>12-19</sup> The various pharmacological activities of chrysin have been reported scientifically such as anticancer – act via inhibition of tumour cell formation,<sup>12</sup> anti-inflammatory – act via attenuating cisplatin induced expression of COX-2,<sup>13</sup> neuroprotective – act via decreased ACR-induced cytotoxicity in a time and dose-dependent manner,<sup>14</sup> antidiabetic – act via prevented the progression of diabetic nephropathy and protected the kidney from damage,<sup>15</sup> antiarthritic – act via attenuation of testicular inflammation, oxidative stress, and apoptosis,<sup>16</sup> antiasthmatic – act via reduces allergic airway inflammation by degranulation of certain types of cells,<sup>17</sup> antidepressant – act via culminated in the upregulation of BDNF and NGF levels<sup>18</sup> and antiviral – act via strong inhibitory effect on EV71 replication.<sup>19</sup>

## CONCLUSION

Finally, it may be suggested that chrysin is utilized in the healing of nerve pain by means of increment in reduced glutathione, total protein level and decrease of TBARS level. Thus, chrysin is a more exceptional natural candidate in the administration of neuropathic pain.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

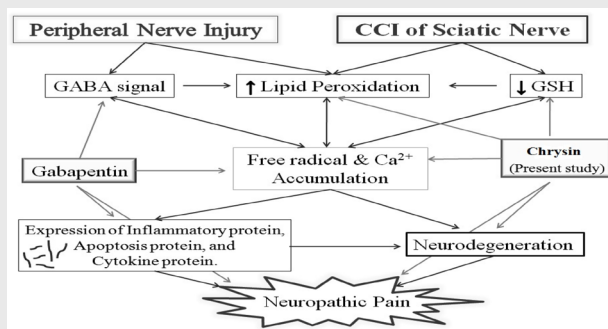
## ABBREVIATIONS

**ANOVA:** Analysis of variance; **BSA:** Bovine serum albumin; **CCI:** Chronic constriction injury; **GSH:** Reduced glutathione; **kg:** Kilogram; **mg:** Milligram; **p.o.:** Per oral; **SD:** Standard deviation; **Sec:** Second; **TBARS:** Thiobarbituric acid reactive substances; **TMP:** 1,1',3,3'-Tetramethoxy propane; **WHO:** World health organization.

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## PICTORIAL ABSTRACT



## SUMMARY

The present research work is summarized as chrysin can be useful in the treatment of neuropathic pain disorders. Finally, it can be concluded that, extraordinary expansive assessments are required to develop this medicinal activity by using distinctive animal model of neuropathic pain. Additionally, growing the healing impact of chrysin and various flavonoids may give exceptional assistance to finish the further examination in clinical research plan.

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