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EVALUATION OF CHRONIC CONSTRICTION INJURY INDUCED NEUROPATHIC PAIN USING FLAVONOID NARINGENIN IN RATS

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Abstract

The overview of writing available online suggested that naringenin was logically used in the treatment of different issue, for example, hepatotoxicity, hypertension, myocardial infraction, diabetes mellitus, obesity, epilepsy, parkinson, alzheimer, malignant growth, anxiety, rheumatoid joint pain, dengue and hypersensitive rhinitis. The current examination has been planned to explore the impact of naringenin in CCI of sciatic nerve prompted neuropathic pain in rats as standard procedures mentioned in scientific reports. The neuropathic pain was induced in rats using well established model i.e. chronic constriction injury model. The effect of naringenin on neuropathic pain was measured using standardized procedures such as Von Frey hair filament test, Hargreaves test, Pin prick test, D'Aemour and Smith test, Tail pinch test and Acetone drop test. The effect of naringenin on tissue biomarker changes induced during neuropathic pain such as thiobarbituric acid reactive substances, reduced glutathione and total protein content was studied using standardized procedures. The oral administration of naringenin (40 and 80 mg/kg) for 15 consecutive days exhibited significant neuropathic pain inhibitory activity with respect to control and statistically equivalent to standard drug. The test drug naringenin prevent the neuropathic pain in dose dependent manner. The level of TBARS biochemical marker was declined by oral administration of naringenin for the 15 days whereas other the level of other biochemical markers such as reduced glutathione and total protein was increased with respect to control and statistically equivalent to standard drug. The outcome of present research work suggests that the remedial effect of naringenin and different flavonoids may give extraordinary help to complete the further investigation in clinical arrangement.

Key word: Chronic constriction injury**,** Flavonoid, Naringenin, Neuropathic pain, Sciatic nerve.

Introduction

Neuropathic pain and/or nociceptive pain is a chronic neurodegenerative disorder which is induced due to lesion, disease of somatosensory nervous system or various disease conditions such as inflammation, cancer, diabetes and autoimmune impairment (Jensen *et al.,* 2011). The synthetic drugs used in the treatment of neuropathic pain includes anticonvulsants –gabapentin, pregabalin, carbamazepine, lamotrigine; serotonin norepinephrine reuptake inhibitors – duloxetine, venlafaxine; tricyclic antidepressant – amitriptyline, nortriptyline, lofepramine, duloxetine, venlafaxine and opioid – morphine, oxycodone, propoxyphene recommended as second line treatment; cannabinoids use as third line treatment; methadone, lamotrigine, lacosamide, tapentadol and botulinum toxin used as fourth line treatment (Attal *et al.,* 2010; Moulin *et al.,* 2014). These synthetic drugs have been associated with various side effects such as tingling, confusion, headache, burning and blurred vision (Medvedeva *et al.,* 2008; Mehta *et al.,* 2014). Therefore, the natural product scientists are working in various plant based phytoconstituents used in the treatment of neuropathic pain.

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Naringenin is a natural flavanone type of flavonoid. The "DE VRY" was firstly described the naringenin is present in the flowers of grape fruit and name of the naringenin is originate from the Sanskrit term "NARANGI" means "orange" (Rangaswami *et al.,* 1939, Sinclair *et al.,* 1972). Naringenin is a flavanone glycoside that is derived from the flavanone glycoside naringin. It is one of the main active components of Chinese herbal medicines, such as *Drynaria fortunei*, *Citrus aurantium* and *Citrus medica* (Zhang *et al.,* 2014).

The survey of literature revealed that naringenin was scientifically reported in the treatment of various disorders such as hepatotoxicity (Pari and Amudha, 2011), hypertension (Ikemura *et al.,* 2012), myocardial infraction (Bharti *et al.,* 2014), diabetes mellitus (Jung *et al.,* 2004,

Jung *et al.,* 2006), obesity (Alam *et al.,* 2013), epilepsy (Golechha *et al.,* 2011), parkinson (Kim *et al.,* 2009), alzheimer (Wang *et al.,* 2012), cancer (Manthey *et al.,* 2001), anxiety (Fernandez *et al.,* 2009), rheumatoid arthritis (Kawaguchi *et al.,* 2011), dengue (Zandi *et al.,* 2011) and allergic rhinitis (Oh *et al.,* 2014).

Despite the vast pharmacological profile of naringenin but this drug has not been scientifically reported in the treatment of neuropathic pain till date. The neuropathic pain is one of the important parameter involved in the various diseases such as cancer (Giacalone *et al.,* 2019), diabetic mellitus (Thakur *et al.,* 2020), epilepsy (Morano *et al.,* 2019), Adewusi *et al.,* 2018), alzheimer (Xu *et al.,* 2019) and hypertension (Wang *et al.,* 2017). Thus, the natural phytoconstituent naringenin will be investigated for neuropathic pain using well established models.

Materials and methods

Drugs and chemicals

Naringenin was purchased from Sigma-Aldrich, St. Louis, Missouri, United States. The various chemicals, reagents and solvents used in present investigations were obtained from S.D. fine chemicals Ltd., Mumbai, India; CDH Pvt. Ltd., New Delhi, India; Sisco Research Laboratories Pvt. Ltd. Mumbai, India and E Merck Limited, Mumbai, India. All the reagents used in the present study were of analytical grade.

Animals

Male Sprague Dawley rat weighing 200-250 g were employed in the present study. Animals were purchased from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India. Rats were maintained with standard laboratory diet (Markfed cotton seed processing plant, Gidderbaha, Mukatsar, Punjab India) and water *ad libtium*. Further animals were exposed to natural light and dark cycle. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC No.: ATRC/14/19; Dated: 31/08/19) and care of the animals were taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Environmental and Forest, Government of India (Reg. no.: 1407/a/11/CPCSEA).

Induction of peripheral neuropathic pain

Neuropathic pain was induced in male SD rats by standard protocol reported in scientific literature such as chronic constriction injury (CCI) of sciatic nerve as described by Bennett and Xie (1988).

Behavioral evaluation

Behavioural parameters were assessed on different time intervals *i.e.,* 0, 3, 6, 9, 12 and 15th day. In each day, behavioural observation was performed between 09.00 am to 03.00 pm. The order of behavioural observation was performed from low intense stimuli to high intense stimuli (allodynia followed by hyperalgesia) in paw as well as in tail using various experimental models such as Von Frey hair filament test (Chaplan *et al.,* 1994), Hargreaves test (Hargreaves *et al.,* 1988), pin prick test (Erichsen and Blackburn-Munro, 2002), D'Aemour and Smith test (D'Amour and Smith, 1941), tail pinch test (Takagi *et al.,* 1966) and acetone drop test (Flatters and Bennett, 2004).

Biochemical analysis

All the groups of animals were sacrificed after 15th day of behavioural observation by cervical dislocation and complete right sciatic nerves were isolated immediately. All part of nerves was used for the biochemical estimations. The sciatic nerve was homogenated (10 % w/v) with phosphate buffer (pH 7.4) and centrifuged at 3500 rpm for 10 min. The supernatant was used for the estimation of tissue thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al.,* 1979), reduced glutathione (GSH) (Ellman, 1959) and total protein levels (Lowry's *et al.,* 1951).

Experimental protocol

The experimental protocol of present studies was consists of four groups and each comprising 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 + naringenin): Test group received naringenin (40 mg/kg, p.o.) for 15 consecutive days and Group IV (CCI + naringenin): Test group received naringenin (80 mg/kg, p.o.) for 15 consecutive days.

Statistical analysis

All the results were expressed as mean ± SD. Data obtained from behavioral tests and tissue biomarker *i.e.,* TBARS, GSH and protein levels were statistically analyzed using two-way analysis of variance (ANOVA) followed by Student-Newman-test by sigma stat software version 3.5. A probability value of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

Results and discussion

Von Frey hair filament test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin

(10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI induced neuropathic pain rats using Von Frey hair filament test. The results of neuroprotective activity parameters such as percentage paw withdrawal response have been presented in Fig. 1. CCI of sciatic nerve resulted in a significant development of peripheral mechanical allodynia as indicated by increase in the percentage paw withdrawal response and decline in the percentage paw withdrawal response indicates the neuroprotective response. The both tested doses of naringenin significantly decline the percentage paw withdrawal response in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

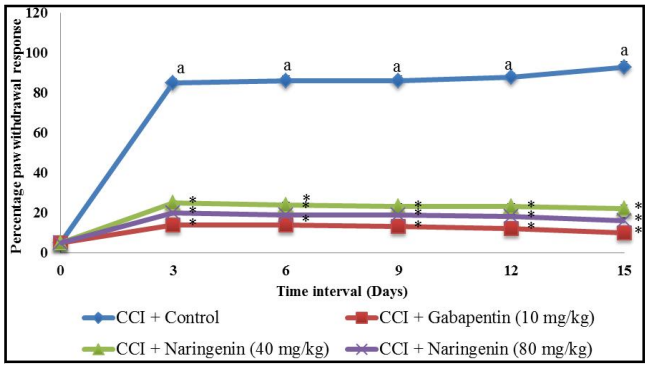

Fig. 1: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using Von Frey hair filament test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman- Keul's test.

Hargreaves test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin (10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI induced neuropathic pain rats using Hargreaves test. The results of neuroprotective activity parameters such as right hind paw withdrawal threshold (sec) have been presented in Fig. 2. CCI of sciatic nerve resulted in a significant development of thermal hyperalgesia as indicated by decrease in right hind paw withdrawal threshold and increase in the right hind paw withdrawal threshold indicates the neuroprotective response. The both tested doses of naringenin significantly increases the right hind paw withdrawal threshold in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

Pin prick test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin

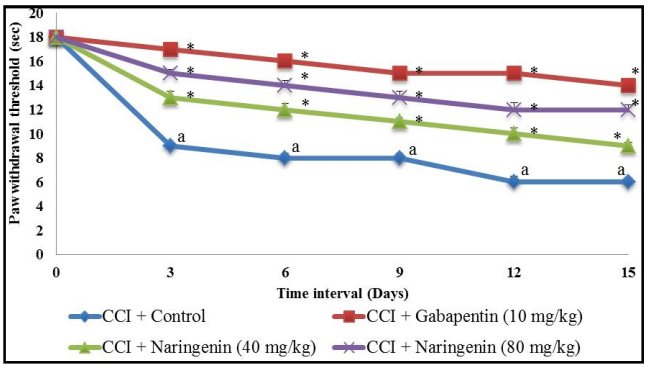

Fig. 2: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using Hargreaves test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

(10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI induced neuropathic pain rats using pin prick test. The results of neuroprotective activity parameters such as right hind paw withdrawal threshold (sec) have been presented in Fig. 3. CCI of sciatic nerve resulted in a significant development of peripheral mechanical hyperalgesia as indicated by increase in the right hind paw withdrawal threshold (sec) and decline in the right hind paw withdrawal threshold (sec) indicates the neuroprotective response. The both tested doses of naringenin significantly decreases right hind paw withdrawal threshold (sec) in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

D'Aemour and Smith test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin (10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI

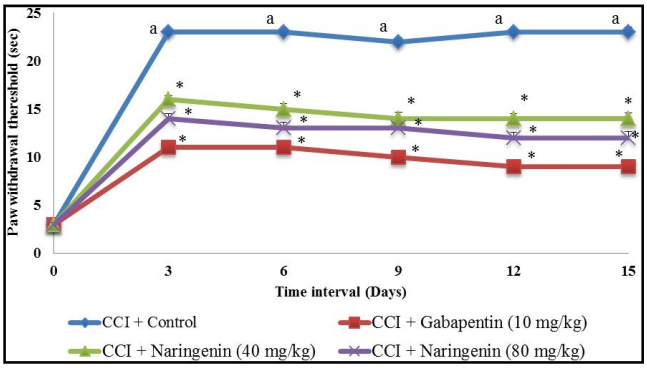

Fig. 3: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using pin prick test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

induced neuropathic pain rats using D'Aemour and Smith test. The results of neuroprotective activity parameters such as threshold of tail withdrawal (sec) have been presented in Fig. 4. CCI of sciatic nerve resulted in a significant development of thermal hyperalgesia as indicated by decrease in tail withdrawal threshold and increase in tail withdrawal threshold (sec) indicates the neuroprotective response. The both tested doses of naringenin significantly raises the tail withdrawal threshold (sec) in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

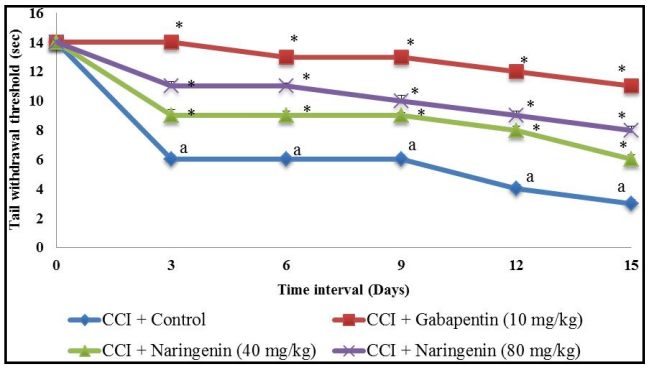

Fig. 4: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using D'Aemour and Smith test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

Tail pinch test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin (10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI induced neuropathic pain rats using tail pinch test. The results of neuroprotective activity parameters such as the number of dislodgment attempts have been presented in Fig. 5. CCI of sciatic nerve resulted in a significant development of central mechanical hyperalgesia as indicated by increase in the number of dislodgements attempt and decline in the number of dislodgements attempt indicates the neuroprotective response. The both tested doses of naringenin significantly decline the number of dislodgements attempt in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

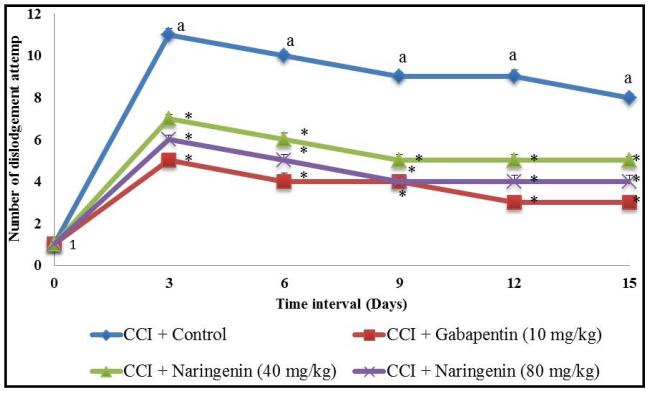

Fig. 5: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using tail pinch test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

Acetone drop test

The naringenin (40 or 80 mg/kg, *p.o.*), gabapentin (10 mg/kg, *p.o.*) and the control (vehicle, *p.o.*) were subjected to evaluate the neuroprotective activity in CCI induced neuropathic pain rats using acetone drop test. The results of neuroprotective activity parameters such as the number of allodynia score have been presented in Fig. 6. CCI of sciatic nerve resulted in a significant development of cold chemical sensitivity as indicated by increase in the number of allodynia score and decline in the number of allodynia score indicates the neuroprotective response. The both tested doses of naringenin significantly decline the number of allodynia score in the dose dependent way as compared to control and statistically equivalent compared to gabapentin during the entire period of experiment.

Potential of naringenin on CCI induced tissue biomarker changes

The CCI induced tissue biomarker changes were studies via estimation of biomarker such as TBARS, GSH and total proteins with the help of calibration curve of TMP Fig. 7, GSH Fig. 8 and BSA Fig. 9 respectively. CCI of sciatic nerve resulted in a significant increase in TBARS; decrease in reduced glutathione and total protein content represents the state of neuropathic pain whereas lower level of TBARS, higher level of GSH and total proteins represents the state of neuroprotective. The oral

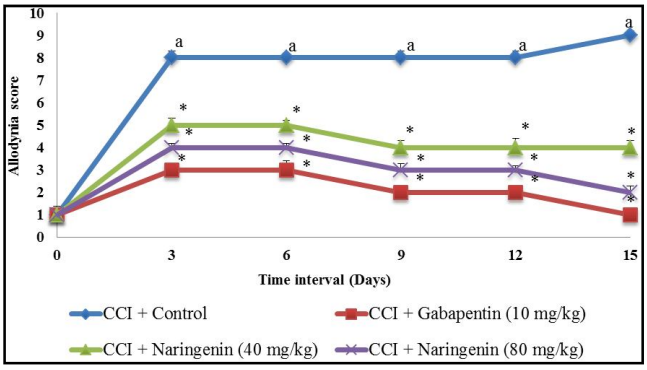

Fig. 6: Evaluation of neuroprotective potential of naringenin in CCI induced neuropathic pain using acetone drop test. n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs Control; [a]*P<0.05* vs gabapentin; one way ANOVA followed by Student-Newman-Keul's test.

administration of naringenin (40 or 80 mg/kg) attenuate CCI induced changes of tissue biomarkers in a dose dependent manner with respect to control and statistically equivalent to gabapentin.

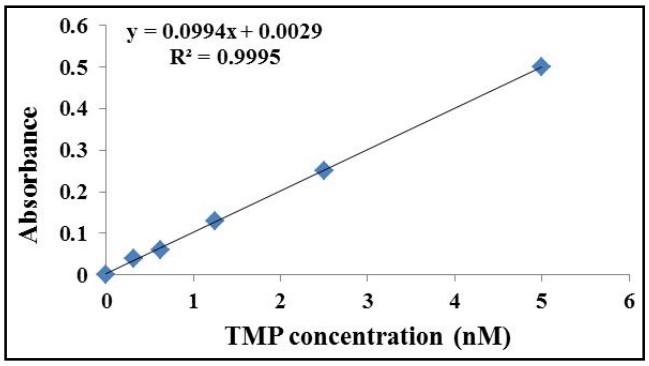

Fig. 7: Standard plots of TMP vs absorbance.

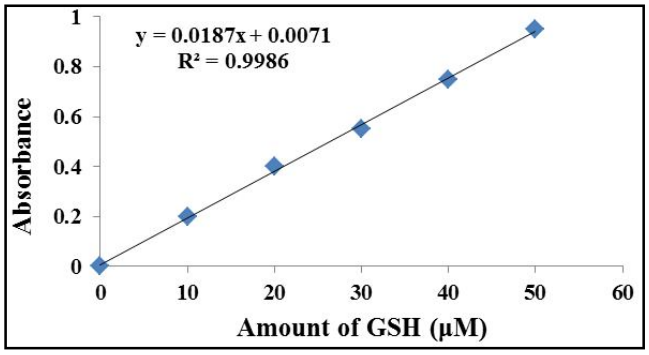

Fig. 8: Standard plots of GSH vs absorbance.

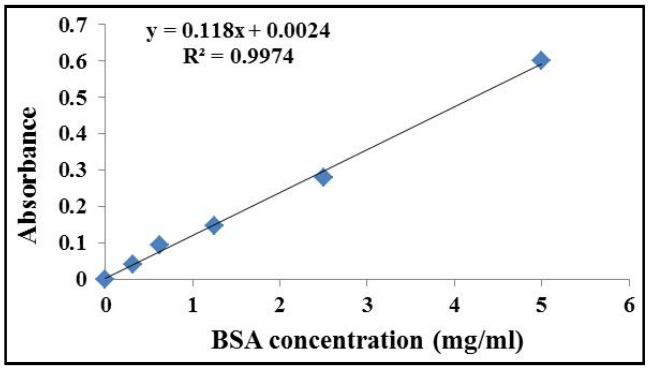

Fig. 9: Standard plots of BSA vs absorbance.

Table 1: Effect of naringenin on CCI induced oxidative stress marker changes.

Groups	TBARS (nM/mg of protein)	GSH (µM/mg of protein)	Total proteins (mg/ml)
CCI + Control	11.89 ± 0.45[a]	25.69 ± 1.50[a]	53.88 ± 3.45[a]
CCI + Gabapentin(10 mg/kg)	4.60 ± 0.60*	68.49 ± 3.25*	110.54 ± 2.88*
CCI + Naringenin(40 mg/kg)	5.25 ± 0.78*	53.44 ± 2.48*	90.47 ± 4.58*
CCI + Naringenin(80 mg/kg)	4.90 ± 0.99*	62.98 ± 2.81*	99.49 ± 4.69*

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

The CCI of sciatic nerve model is regularly utilized for introduce the mononeuritic neuropathic pain in rodent (Bennett and Xie, 1988). The production of free radicals, cytokines and adjustment of ionic developments are essential occasions in the peripheral nerve injury prompted neuropathic pain. The commonly aggregation of free radical, tumor necrosis factor and calcium in sciatic nerve is takes place in CCI instigated neuropathic pain (Hassani *et al.,* 2015). In addition, inexhaustible aggregation of free radicals is likewise liable for the induction of lipid peroxidation in the sensory system (Patel *et al.,* 2016). These progressions are responsible to deliver the ischemic condition in the peripheral sensory system and it experiences the upgrade of neurodegeneration at peripheral site (Nagamatsu *et al.,* 1996). Along these lines, peripheral nerve injury likewise modifies the GABAnergic signaling procedures in mind prompts produce the focal neuropathic pain (Arai *et al.,* 2013).

Naringenin or its glycoside naringin is used in the treatment of various disorders such as anxiety by its antinociceptive action to activate Gi/o protein, opening of voltage-gated, calcium-gated potassium channels and inhibition of calcium influx (Fernandez *et al.,* 2009); cancer by inhibition of development of mammary tumors induced by 7,12-dimethylbenz[a]anthracene (So *et al.,* 1996); hypertension by activate the production of 8-hydroxy-2'-deoxyguanosine demonstrated which provide strong antioxidant activity (Ikemura *et al.,* 2012); dengue by its inhibitory action against different stages of DENV-2 infection and replication cycle (Zandi *et al.,* 2011) and diabetes mellitus by lowered the activity of hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (Jung *et al.,* 2004). The neuropathic pain is one of the important parameter in the above mentioned disorders. Therefore, neuropathic pain in rats was treated by naringenin.

Finally, it can be concluded that naringenin is used in the treatment of neuropathic pain via increase in reduced glutathione, total protein level and reduction of TBARS level. Along these lines, naringenin is a more up to date natural applicant in the management of neuropathic pain.

Conclusion

Therefore, it might be concluded that, naringenin can be helpful in the management of neuropathic pain manifestations. Finally, it can be concluded that, increasingly broad examinations are required to build up mechanism of action by utilizing different animal model of neuropathic pain. Also, expanding the mechanism of action

of naringenin and different flavonoids may give extraordinary help to complete the further investigation in clinical arrangement.

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Declaration of interest

The authors report no declaration of interest.

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