

Bioactive Turmerosaccharides from *Curcuma longa* Extract (NR-INF-02): Potential Ameliorating Effect on Osteoarthritis Pain

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ABSTRACT

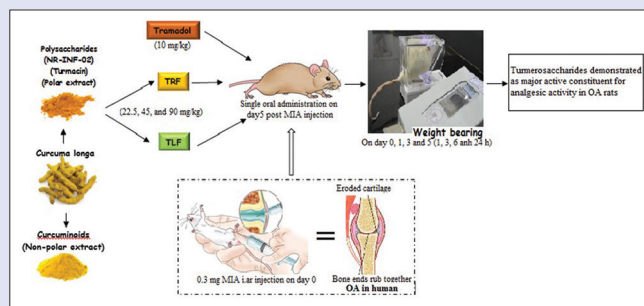
Background: *Curcuma longa* has long history of medicinal use in Ayurveda. A unique product NR-INF-02 was prepared from *C. longa* that was standardized to contain turmerosaccharides. **Objective:** The present study investigated the effect of turmerosaccharides rich fraction of NR-INF-02 on monosodium iodoacetate (MIA)-induced OA pain animal model that mimics human OA. Further, the analgesic effect of turmerosaccharides rich fraction was compared to turmerosaccharides less fraction of NR-INF-02. **Materials and Methods:** OA pain was chemically induced by intra-articular administration of single dose of 25 μ l of 0.9% saline containing 0.3 mg MIA into the right knee of male albino Wistar rat. Turmerosaccharides rich fraction and turmerosaccharides less fraction (at 22.5, 45 and 90 mg/kg rat body weight dose levels) were administered as a single dose orally on day 5 of post-MIA injection. OA pain was measured using hind limb weight-bearing ability at 1, 3, 6, and 24 h post-test substance administration on day 5. **Results:** Oral administration of turmerosaccharides rich fraction and turmerosaccharides less fraction (at 45 and 90 mg/kg) although significantly decreased the OA pain at all the intervals, the effect of turmerosaccharides rich fraction (57%) on OA pain was superior to turmerosaccharides less fraction (35%). **Conclusion:** Bioactive turmerosaccharides from *C. longa* extract contribute to the observed anti-arthritis effect in rats.

Key words: Analgesia effect, *Curcuma longa*, monosodium iodoacetate, NR-INF-02, osteoarthritis, turmerosaccharides

SUMMARY

- Osteoarthritic pain was induced by intra-articular injection of MIA into the right knee
- Single administration of TRF/TLF on day 5 resulted in dose-dependent significant reduction of OA pain

- TRF showed better analgesic activity than TLF
- TRF at 45 and 90 mg/kg has similar effects on OA pain as that of tramadol
- Turmerosaccharides identified as bioactive constituents of *C. longa* extract.



Abbreviations used: MIA: Monosodium iodoacetate; i.ar: Intra-articular; OA: Osteoarthritis; TRF: Turmerosaccharides rich fraction; TLF: Turmerosaccharides less fraction; PGE2: Prostaglandin E2; ROS: Reactive oxygen species.

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INTRODUCTION

Turmeric (*Curcuma longa*) belonging to *Zingiberaceae* family has a very long history of culinary, cosmetic, and medicinal use. In Ayurveda practices, turmeric is well-documented to have several medicinal properties for the treatment of various respiratory conditions (e.g., asthma, bronchial hyperactivity, and allergy) as well as for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough, sinusitis, sprains, inflammation, and swelling. While in traditional Chinese medicine, it is used to treat diseases associated with abdominal pain.^[1,2] Thus, there is substantial evidence in traditional system of medicine pertinent to the effectiveness of turmeric against inflammation and pain.

Ayurvedic herbal medicine preparation methods as mentioned in classical Ayurvedic books, namely, Bhaishajya kalpanā vijñānam and Śārngadhara Samhitā of ŚārngadharaĀcārya, include dosage forms such as svarasa (juice), kalka (bolus/paste), kvatha (decoction), hima (cold infusion), phaṇṭa (hot infusion), and curna (powder). The solvents generally used for the traditional herbal preparation include water,

ghee/oil, and milk. This clearly provides evidence that organic solvent extraction is not defined in Ayurveda for most of the herbal medicines and possibly the water/aqueous extraction procedures yield herbal medicines that are efficacious in treating diseases/disorders.^[3] In addition, few scientific reports on the pharmacological activities such as antioxidant, anti-tumor, anti-diabetic, immune modulatory and anti-depressant activity of aqueous extracts of *C. longa* are available.^[4-7]

In view of the above, a formulation was developed from *C. longa*

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using the decoction method with water as solvent and was termed as NR-INF-02 or Turmacin™. NR-INF-02 was standardized to contain turmerosaccharides (>10% w/w) and negligible amount of curcuminoids.^[8]

NR-INF-02 rich in turmerosaccharides was extensively studied in *in vitro* and *in vivo* safety and efficacy studies. NR-INF-02 demonstrated significant anti-inflammatory activity in acute (carrageenan and xylene) and chronic (cotton pellet granuloma) inflammatory *in vivo* models.^[9] In addition, NR-INF-02 was fractionated into turmerosaccharides rich fraction and turmerosaccharides less fraction. These fractions were further assessed for anti-inflammatory activity and safety. *In vitro* and *in vivo* studies indicated that anti-inflammatory activity of turmerosaccharides rich fraction of NR-INF-02 was superior to turmerosaccharides less fraction.^[8] Further, the LD₅₀ was found to be >5000 mg/kg rat body weight in acute oral toxicity – fixed dose procedure using the OECD test guideline No. 420.^[10] Thus, these studies clearly indicated that the possible anti-inflammatory activity of NR-INF-02 is primarily due to turmerosaccharides. However, the turmerosaccharides rich fraction was not investigated in *in vivo* monosodium iodoacetate (MIA)-induced osteoarthritic (OA) pain model.

Hence, the present study was conducted to investigate if turmerosaccharides fraction of NR-INF-02 contributes for relieving pain in an MIA-induced OA rat model, which mimics the pain and changes associated with human OA. In addition, the study also compared the analgesic effects of turmerosaccharides rich fraction and turmerosaccharides less fraction of NR-INF-02.

MATERIALS AND METHODS

Animals

Male albino Wistar rats bred at central animal facility, Research and Development Center, Natural Remedies Private Limited, were acclimatized for 5 days before experimentation. Rats weighing 240–250 g at the start of experimentation were kept under optimal temperature (25 ± 2°C) and 30%–70% relative humidity. Rats were provided access to rodent feed pellets (VRK Nutritional Solutions) and ultraviolet purified water *ad libitum*. All the animal procedures were approved by the Institutional Animal Ethics Committee of Natural Remedies, Bengaluru.

Preparation of test substances

Fractionation of NR-INF-02 into turmerosaccharides rich fraction and turmerosaccharides less fraction has been described in our earlier publication.^[8] In brief, NR-INF-02 was dissolved in water, and 5 volumes ethanol was added. The mixture was centrifuged for 20 min at 2000 rpm. The precipitate obtained after centrifugation was stirred with 5 volumes of ethanol at room temperature for 10 min and filtered. After filtration, the retentate obtained was dried under vacuum at <70°C to obtain turmerosaccharides rich fraction. While the supernatant obtained was concentrated under vacuum to obtain turmerosaccharides less fraction.

Experimental procedure

The animals were randomly assigned into nine groups as G1 = Normal/vehicle control, G2 = MIA control, G3 = Tramadol at 10 mg/kg as a reference control, G4–G6 = Turmerosaccharides rich fraction at 22.5, 45, and 90 mg/kg dose levels, respectively, and G7–G9 = Turmerosaccharides less fraction at 22.5, 45, and 90 mg/kg dose levels, respectively. Each group was assigned six rats that were housed two in number per cage. Individual rats were identified by cage card and markings.

OA was induced by intra-articular (i.ar) injection of MIA (Sigma-Aldrich, USA) solution into the knee joint. Anesthetized rats of all the groups except normal/vehicle control received single dose of 25 µl of 0.9% saline containing 0.3 mg of MIA into the i.ar space of the right knee through the infrapatellar ligament. While left knee was injected with 25 µl of 0.9% saline only. While normal/vehicle control rats received 25 µl of 0.9% saline into the right and left knee joints. MIA and saline were freshly prepared under sterile conditions and were injected into the knee using 27-gauge needle into the joint space.^[11]

Tramadol at one dose level, turmerosaccharides rich fraction and turmerosaccharides less fraction at three dose levels were administered to the respective groups as a single oral administration on day 5 post-MIA injection. Analgesic effects of the test substances were measured on day 5 at various time intervals (1, 3, 6, and 24 h).

Assessment of pain behavior

A hind limb static weight-bearing incapacitance tester (Bioseb, France) was used to assess the pain behavior. Pain associated with OA is characterized by hind limb weight-bearing distribution asymmetry. The difference in the distribution of weight on the left and the right hind limbs was measured as an objective measurement of pain.

All the groups were evaluated for hind limb weight bearing on day 0, 1, 3, and 5 (1, 3, 6, and 24 h post-test substance administration on day 5). While the analgesic effects of tramadol, turmerosaccharides rich fraction and turmerosaccharides less fraction was measured at 1, 3, 6, and 24 h post-test substance administration on day 5 following MIA injection. Animals were placed into a holder where the animal was comfortably maintained while their hind paws rest on two separate sensor plates. The animal was allowed to become accustomed to the apparatus. When stationary, the force exerted on the plate by each hind paw was recorded over a period and the same is expressed in grams. Four–five consecutive 5-s readings were taken and averaged to obtain the mean score. Results were expressed as difference between hind paw weight distribution, percentage weight born, and area under curve (AUC) from the time of test substance administration to 24 h post-test substance administration.

Statistical analysis

The difference between hind paw weight distribution values was expressed as mean ± standard error mean. Data were analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test for multiple group comparison. If error variance was found to be heterogenous, logarithmic transformation of raw data was performed and analyzed accordingly. Values of $P \leq 0.05$ were considered statistically significant. Data were processed using statistical software IBM SPSS version 21. AUC was calculated using GraphPad prism version 5, GraphPad software Inc., California, USA.

RESULTS

Effect of oral administration of turmerosaccharides rich fraction and turmerosaccharides less fraction on monosodium iodoacetate-induced osteoarthritic pain

Intra-articular injection of MIA (0.3 mg in 25 µl of 0.9% saline) into the right knee-induced OA pain. OA pain resulted in statistically significant reduction on weight bearing on the right hind limb as compared to normal control rats indicating increased pain response. Single oral administration of turmerosaccharides rich fraction or turmerosaccharides less fraction on day 5 resulted in dose-dependent significant reduction of OA pain response induced by MIA.

The difference in the weight distribution between left and right hind limbs (left-right) was significantly reduced in tramadol, turmerosaccharides rich fraction (45 and 90 mg/kg), and turmerosaccharides less fraction (45 and 90 mg/kg)-treated groups at all the time intervals (1, 3, 6 and 24 h) as compared to MIA-treated group. However, turmerosaccharides rich fraction showed superior activity on OA pain when compared to turmerosaccharides less fraction [Figure 1].

The total AUC for difference in weight distribution (left and right hind limbs) was calculated from 0 to 24 h post-test substance administration. MIA-treated group demonstrated a significant increase in AUC as compared to normal control group. While the AUC for tramadol, turmerosaccharides rich fraction, and turmerosaccharides less fraction-treated groups were significantly reduced as compared to MIA-treated group indicating reduction in OA pain over a period of 24 h [Figure 2].

Comparison of tramadol-treated group with turmerosaccharides rich fraction and turmerosaccharides less fraction-treated groups were

performed. There was no significant difference between tramadol treatment and turmerosaccharides rich fraction (90 mg/kg) treatment on OA pain at all intervals (1, 3, 6, and 24 h) evaluated on day 5. Whereas no significant difference between tramadol and turmerosaccharides rich fraction (45 mg/kg) treatment on OA pain was observed at all the intervals evaluated except for the first hour post treatment. Thus, turmerosaccharides rich fraction at 45 and 90 mg/kg has similar effects on OA pain as that of tramadol. Turmerosaccharides rich fraction was compared with turmerosaccharides less fraction-treated groups with their corresponding dose levels. Statistical analysis indicated that at all intervals turmerosaccharides rich fraction 90 mg/kg was significantly more effective on OA pain over turmerosaccharides less fraction 90 mg/kg [Table 1].

The effect of oral administration of test substance was also calculated as percentage inhibition of pain with respect to MIA control group. Tramadol demonstrated maximal pain reduction by 55%, 6 h postadministration. The turmerosaccharides rich fraction at 45 mg/kg dose level showed maximal pain reduction by 50%, 6 h postadministration. Correspondingly, turmerosaccharides less fraction at 45 mg/kg dose level showed maximal pain reduction by

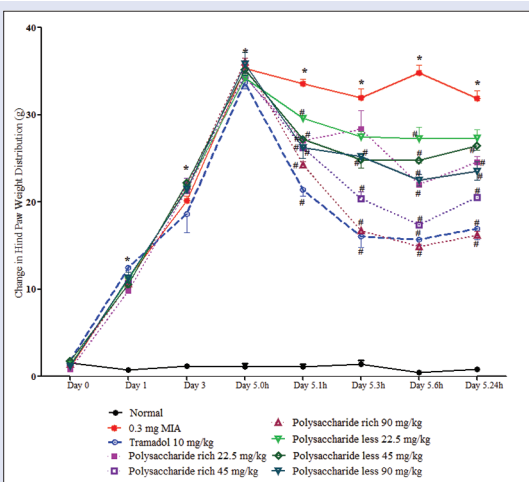


Figure 1: Effect of oral administration of turmerosaccharides rich fraction and turmerosaccharides less fraction at day 5 after monosodium iodoacetate (0.3 mg in 25 μ l) intra-articular injection. The effect on osteoarthritic pain evaluated at 1, 3, 6, and 24 h following oral administration of test substances. Data were expressed as difference in weight distribution on hind limbs (left-right). Each value is mean \pm standard error mean ($n = 6$) of four to five experiments. * $P < 0.05$ significantly different from normal control group. # $P < 0.05$ significantly different from monosodium iodoacetate control group

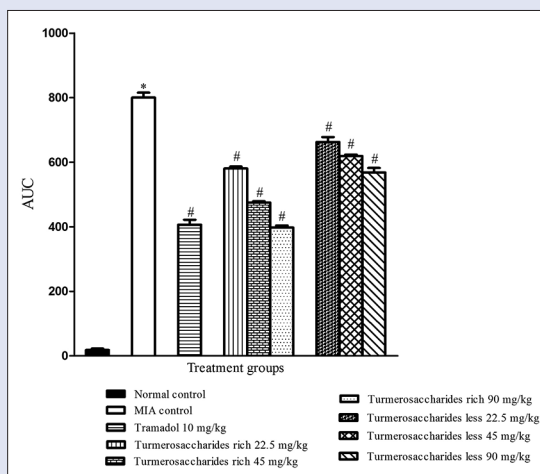


Figure 2: Total area under the curve for difference in weight distribution on hind limbs from 0–24 h on day 5. Results are expressed as mean \pm standard error mean ($n = 6$). * $P < 0.05$ significantly different from normal control group. # $P < 0.05$ significantly different from monosodium iodoacetate control group

Table 1: Multiple comparison of the effects of tramadol, turmerosaccharides rich fraction, and turmerosaccharides less fraction at day 5 after monosodium iodoacetate injection

Group	Change in hind paw weight distribution (g)				
	Day 5/0 h	Day 5/1 h	Day 5/3 h	Day 5/6 h	Day 5/24 h
Normal control 10 ml/kg 0.5% CMC p.o (25 μ l saline i.ar)	1.11 \pm 0.39	1.12 \pm 0.29	1.42 \pm 0.41	0.48 \pm 0.15	0.85 \pm 0.18
MIA 0.3 mg 10 ml/kg 0.5% CMC p.o (0.3 mg in 25 μ l saline i.ar)	35.33 \pm 0.31*	33.55 \pm 0.55*	31.93 \pm 1.02*	34.80 \pm 0.92*	31.91 \pm 0.86*
Tramadol 10 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	33.69 \pm 0.70	21.40 \pm 0.74	16.04 \pm 1.21	15.70 \pm 0.69	16.96 \pm 1.30
TRF 22.5 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	34.31 \pm 0.84	27.01 \pm 0.33 ^a	28.33 \pm 2.19 ^a	22.01 \pm 0.39 ^a	24.57 \pm 0.66 ^a
TRF 45 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	34.51 \pm 0.89	26.25 \pm 0.33 ^a	20.37 \pm 0.84	17.39 \pm 0.21	20.55 \pm 0.28
TRF 90 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	36.06 \pm 0.42	24.28 \pm 0.36	16.68 \pm 0.26	14.88 \pm 0.30	16.17 \pm 0.47
TLF 22.5 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	34.25 \pm 0.51	29.63 \pm 0.20 ^{a,b}	27.50 \pm 0.55 ^a	27.29 \pm 1.30 ^a	27.31 \pm 0.98 ^a
TLF 45 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	35.17 \pm 0.40	27.17 \pm 0.97 ^a	24.82 \pm 0.96 ^a	24.80 \pm 0.32 ^{a,c}	26.47 \pm 0.55 ^{a,c}
TLF 90 mg/kg p.o (0.3 mg MIA in 25 μ l saline i.ar)	35.81 \pm 1.31	26.27 \pm 1.25	25.18 \pm 0.27 ^{a,d}	22.51 \pm 0.64 ^{a,d}	23.55 \pm 1.06 ^d

Data were expressed as difference in weight distribution on hind limbs (left-right). Each value is mean \pm SEM ($n=6$). * $P < 0.05$ significantly different from normal control group; ^a $P < 0.05$ tramadol versus TRF and TLF; ^b $P < 0.05$ TRF (22.5 mg/kg) versus TLF (22.5 mg/kg); ^c $P < 0.05$ TRF (45 mg/kg) versus TLF (45 mg/kg); ^d $P < 0.05$ TRF (90 mg/kg) versus TLF (90 mg/kg). i.ar: Intra-articular; CMC: Carboxymethylcellulose; MIA: Monosodium iodoacetate; SEM: Standard error of mean; TRF: Turmerosaccharides rich fraction; TLF: Turmerosaccharides less fraction

Table 2: Effect of oral administration of turmerosaccharides rich fraction and turmerosaccharides less fraction on percentage inhibition of osteoarthritis pain at 1, 3, 6, and 24 h on day 5

Groups	Percentage reduction of pain severity (%)			
	Day 5/1 h	Day 5/3 h	Day 5/6 h	Day 5/24 h
Tramadol 10 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	36.21	49.77	54.89	46.84
TRF 22.5 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	19.48	11.25	36.74	23.00
TRF 45 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	21.75	36.18	50.02	35.60
TRF 90 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	27.63	47.75	57.24	49.33
TLF 22.5 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	11.69	13.86	21.58	14.42
TLF 45 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	19.01	22.26	28.74	17.05
TLF 90 mg/kg p.o (0.3 mg MIA in 25 µl saline i.ar)	21.69	21.13	35.33	26.18

Values in treated groups are expressed as percentage inhibition with respect to MIA control group. Inhibition (%): $([MIA\ control - treatment\ group] / MIA\ control) \times 100$. MIA: Monosodium iodoacetate; TRF: Turmerosaccharides rich fraction; TLF: Turmerosaccharides less fraction; i.ar: Intra-articular

29%, 6 h postadministration. While turmerosaccharides rich fraction at 90 mg/kg dose level showed maximal pain reduction by 57%, 6 h postadministration. Correspondingly, turmerosaccharides less fraction at 90 mg/kg dose level demonstrated maximal pain reduction by 35%, 6 h postadministration [Table 2].

DISCUSSION

OA is the most common form of joint disease affecting over one-half of people older than 65 years of age. OA primarily affects weight-bearing joints such as knee and hip. Currently, acetaminophen, nonsteroidal anti-inflammatory drug, opioids, and corticosteroids are used for treating OA pain however they are associated with adverse effects.^[12,13] For these reasons, new agents with ability to attenuate OA-associated pain and improve joint function are a welcome addition. In addition, the penchant toward natural products as a safe alternative to current pharmacological therapies^[14] also promulgated to develop NR-INF-02 for OA pain. NR-INF-02 is prepared from polar fraction of *C. longa* and is standardized to contain turmerosaccharides.

In recent times, research has been focused on understanding the role of phytoconstituents such as flavonoids, polyphenols in modulating OA pain.^[14] Hence, the present study has investigated whether turmerosaccharides fraction of NR-INF-02 contributes for relieving OA pain. In addition, the study also compared the effects of turmerosaccharides rich fraction and turmerosaccharides less fractions of NR-INF-02 on OA pain.

The effects of turmerosaccharides rich fraction and turmerosaccharides less fractions on OA pain were investigated in MIA-OA pain model. MIA is a metabolic inhibitor; injection of MIA into the joints inhibits glyceraldehydes-3-phosphate dehydrogenase activity in chondrocytes, resulting in disruption of glycolysis and eventual cell death. This model has been well established to test pharmacological agents for their ability to treat OA pain and mimics behavioral (pain), pathologic, and pharmacologic features associated with human OA.^[12] Hence, attenuation of MIA-OA pain by turmerosaccharides rich fraction directly connotes its effect on OA pain. In addition, it gives indication that turmerosaccharides may contribute to effect of NR-INF-02 on OA pain.

MIA injection into the right hind paw in the present study resulted in a significant decrease on weight bearing in the right hind paw. When administered at 0.3 mg per joint, the change in weight distribution was found to reach a maximum level on day 5 and maintained the level for 21 days (unpublished observations). Hence, the turmerosaccharides rich fraction and turmerosaccharides less fractions were administered on day 5 when the pain behavior reached maximal level and were assessed for analgesic effects. The results of the study indicated that turmerosaccharides rich fraction and turmerosaccharides less fractions administered orally produced significant analgesic activity. However, the analgesic effect produced by turmerosaccharides rich fraction was

superior to turmerosaccharides less fraction. Also, duration of action of turmerosaccharides rich fraction was higher than turmerosaccharides less fraction. We observed that 57% of analgesic activity was exhibited by turmerosaccharides rich fraction while turmerosaccharides less fraction produced 35% of analgesic activity. Thus, the current study findings indicate that turmerosaccharides are the major phytoactives that contribute to the analgesic activity of NR-INF-02. These observations are in agreement with the findings of Chandrasekaran *et al.* 2013^[8] and Illuri *et al.* 2015^[10] These two studies indicated that turmerosaccharides of NR-INF-02 contributed to the anti-inflammatory effects. The analgesic effect of turmerosaccharides rich fraction appears to be similar to the anti-nociceptive activity of synthetic drugs, tramadol that resulted in 55% of anti-nociceptive activity.^[15] Hence, the present study clearly indicates that turmerosaccharides are the phytoactives responsible for analgesic activity of NR-INF-02.

Induction of several factors such as NF- κ B, inflammatory mediators, eicosanoids and cytokines are responsible for OA pain.^[15,16] The inflammatory mediators released following MIA injection cause acute synovial inflammation. As the pain fibers are present in synovium, ligaments, bone, muscle, and meniscus of knee, acute synovial inflammation induced by MIA causes peripheral and central sensitization. The process of sensitization is thought to be the basis of OA pain.^[17] The turmerosaccharides of NR-INF-02 may exert its analgesic activity on the MIA-induced model of OA pain through its anti-inflammatory activity. The turmerosaccharides were shown to have anti-inflammatory activity as it inhibited PGE₂^[8] that contribute to structural damage of knee joint, and subsequently, OA pain. Hence, PGE₂ would have contributed to analgesic effect.

In addition, MIA also induces chondrocytes apoptosis primarily through ROS and ROS generation results in inflammation of articular cartilage.^[18] Thus, anti-oxidant ability or the oxidative defences will decrease chondrocyte damage. The turmerosaccharides from *C. longa* demonstrated marked radical scavenging activity in *in vitro* assays^[19] Hence, the anti-oxidant effects of turmerosaccharides would have resulted in scavenging ROS and thus resulted in chondrocyte protection and further analgesic activity. Thus, modulation of inflammatory mediators and chondrocyte protection would have contributed to the activity of turmerosaccharides rich fraction on OA pain.

Eventhough, turmerosaccharides less fraction showed significant analgesic effect, it is inferior to turmerosaccharides rich fraction. This indicates that turmerosaccharides less fractions might contain phytoactives responsible for analgesic effect. As indicated by Ramadas and Srinivas^[20] and Angel *et al.*^[21] water extract of *C. longa* contains glycoprotein, which might have contributed to the observed effect on OA pain. However, the phytoactives of turmerosaccharides less fraction are yet to be explored. Further, the mechanism of action of turmerosaccharides at molecular level are yet to be explored.

CONCLUSION

The current study demonstrated that turmerosaccharides rich fraction attenuated MIA-OA pain. Thus, the study findings suggest that turmerosaccharides remain the major phytochemical actives of *C. longa* (NR-INF-02) in decreasing the OA pain.

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Conflicts of interest

There are no conflicts of interest.

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