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RESEARCH ARTICLE

Antiarthritic Effect of Polar Extract of *Curcuma longa* on Monosodium Iodoacetate Induced Osteoarthritis in Rats

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ARTICLE HISTORY

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Abstract: ***Background*:** *Curcuma longa* Linn, "the golden spice" is a common spice used in Southern Asia and Middle East countries. It has a history of ethnopharmacological use for its various functional activities like antiseptic, anti-inflammatory, antioxidant, antimicrobial, anticancer and so on.

***Objective*:** To investigate the effects of polar extract of *C. longa* (PCL) against monosodium iodoacetate (MIA) induced osteoarthritis in rat and to compare with curcuminoids, which are contemporarily believed to be the only active phytochemicals of *C. longa* for relieving pain in osteoarthritis.

***Method*:** Degenerative osteoarthritis in rats was induced by intra-articular injection of monosodium iodoacetate (MIA) in right knee. PCL or curcuminoids or tramadol was administered orally on the 5th day post MIA injection to rats. Weight bearing capacity and percentage inhibition of nociception of PCL treated groups were determined and compared with curcuminoids and tramadol (reference drug). In addition, gene expression of type II collagen and matrix metalloproteinases (MMP) in joint cartilage was measured by Reverse transcription polymerase chain reaction.

***Results*:** PCL significantly decreased the difference in weight distribution between left and right limb in a dose dependent manner. Anti-arthritic activity of PCL is evident from gene expression analysis, significantly up regulating type II collagen gene (*COL2A1*) and down regulating *MMP-3* and *MMP-7*.

***Conclusion*:** Polysaccharide extract of *Curcuma longa* showed beneficial effects on joints by exhibiting equilibrium between catabolism and anabolism of joint cartilage.

Keywords: *MMP-3*, *MMP-7*, osteoarthritis, pain, polar extract of *Curcuma longa,* turmacin, type II collagen, weight bearing capacity.

1. INTRODUCTION

Turmeric, scientifically known as *Curcuma longa,* belongs to the ginger family Zingeberaceae. It is extensively used as culinary spice, food preservative, colorant, cosmetic and medicine. Marco Polo, a Venetian merchant traveller portrayed this spice as "marvelling at a vegetable that exhibits qualities so similar to that of saffron". The "Golden spice" is also known as "Indian saffron" because of its saffron like colorant property. As rightly quoted by Hippocrates "Let thy food be thy medicine", turmeric despite of its spicing use, has a very long history of therapeutic use dating nearly 4000 years [1]. *C. longa* has a long spectrum of bioprotective functions like anti-oxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-

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diabetic, anti-fertility, anti-bacterial and anti-fungal activities [2]. Folklore claims *C. longa* could be used for the treatment of inflammatory conditions such as bruises, bursitis, arthritis, back pain *etc*. [3-5].

Even though traditional Indian and Chinese medicine advocates the use of decoction of this spice (polar extract) [3], many researchers pivot only on curcuminoids (non-polar extract) especially curcumin which is contemplated as an only active constituents. Turmeric is sold as a food spice in the form of crude powder which is constituted of 70 % w/w polar fraction and just around 3% w/w of non-polar fraction (curcuminoids) [6, 7]. Wittingly or unwittingly this astonishing spice is extensively used in the countries of southern Asia like India, China, Okinawa, Malaysia *etc*., in their routine life. The studies proved that regular turmeric consumption by people of these countries hold prolonged life time *via* its anti-ageing, cognition enhancing, anti-carcinogenic and anti-inflammatory properties [8-11]. Even previous reports enumerate the usefulness of polar (aqueous) extract as antitumor, antidiabetic, antimicrobial, hepatoprotective, antifertility, antidepressant, antioxidant, anti-inflammatory and anti-bacterial and immunomodulatory [12-19]. Thus, we hypothesized that it is not only curcuminoids but constituent other than curcuminoids *i.e.*, polar extract may also contribute to therapeutic activities equally or more efficiently than the former. We initiated our investigation with osteoarthritis because of its impact on the population by making them physically disable.

Osteoarthritis (OA) is a chronic joint disease affecting 10-15% of population worldwide aged above 60 [20]. OA expected to be the fourth leading cause of disability by 2020 [21]. It is characterized by structural degradation of articular cartilage extracellular matrix, synovial inflammation and subchondrial bone sclerosis. The mechanism behind the cartilage degradation is due to stimulation of collagenolytic matrix metalloproteinases (MMP) by upregulation of inflammatory cytokine like tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) levels. Although nonsteroidal anti-inflammatory drugs are used as symptomatic treatments for OA [22], these drugs have not proven positively against the natural progression of OA in humans.

To the best of our knowledge no studies have been conducted to evaluate the anti-arthritic effect of curcuminoids free polar extract of *Curcuma longa* (PCL). Hence, the present study is conducted to manifest the activity of PCL and to compare its activity with curcuminoids against MIA induced OA in rats that can reveal which of the turmeric extracts could be useful for the treatment of OA.

2. MATERIALS AND METHODS

2.1. Plant Material

Dried rhizomes of turmeric were collected from different parts of Tamil Nadu, India and authenticated at National Institute of Science Communication and Information Resources, New Delhi, India. A voucher specimen (No. 653) was deposited in Department of Pharmacognosy, Natural Remedies R&D centre, Karnataka, India.

2.2. Extraction of Polar Extract of *Curcuma longa* (PCL)

PCL (Turmacin^{TM}) was prepared as described earlier [23]. In brief, coarsely powdered *C. longa* rhizomes were subjected to steam distillation to remove the turmeric oil. The powder was then refluxed with water, concentrated and spray dried to obtain an oil-free extract. PCL was prepared by blending the aqueous extract and the turmeric oil at a ratio of 99:1 (w/w) followed by sieving. Yield of this extract is 10.5 % w/w. The total polysaccharides termed as turmerosaccharides were analysed using HPLC (Shimadzu - LC 2010CHT), equipped with Lichrosphere column C18. PCL (100 mg) was hydrolysed with 20 % trifluoroacetic acid (25 mL) and 10 µL sample was injected. Analysis was carried out at a flow rate of 1 ml/min with ammonium acetate (0.07 %) and acetonitrile as mobile phase in gradient proportion. The elution was monitored using UV detection wavelength at 307 nm with a run time of 50 min.

2.3. Extraction of Curcuminoids

Extraction of curcuminoids was described earlier [24]. Concisely, coarse ground rhizomes of *C. longa* were refluxed twice or more with ethyl acetate for 3 hours. The concentrated extract obtained was stirred thrice with petroleum ether and the insoluble matter was crystallized using isopropyl alcohol. Percentage yield of yellow powder of cur-

cuminoids is 2.5 % w/w (> 95% w/w determined by HPLC). HPLC analysis was performed with 5 µL of 0.025 % curcuminoids in 50 % methanol using 1mM potassium phosphate buffer and acetonitrile as mobile phase at a flow rate of 2 ml/min. observed at 420 nm for 10 min.

2.4. Animals and Groupings

Male Wistar rats of 6 - 8 weeks old weighing 195-225g were used for the experiment. Animals were maintained at ambient temperature (22 ± 3°C) and relative humidity 30-70 % on regular light-dark cycle (12 h light: 12 h dark) with free access to food and water. The study was approved by Institutional Animal Ethics Committee (IAEC) of Natural Remedies Private Limited (Approval no.: IAEC/PCL/05/06.15) and the animal experiments were conducted in accordance with current ethical regulations for CPCSEA, India.

After 5 days of acclimatization, animals were divided into nine groups of six rats:

Group I - Normal control (0.5% Carboxy Methyl Cellulose: 10 ml/kg *p.o.*)

Group II - Negative control (0.3 mg MIA: 25 µl/rat *i.ar.*)

Group III, IV, V - Treatment (PCL: 22.5, 45, 90 mg/kg bw *p.o.* respectively)

Group VI, VII, VIII - Treatment (Curcuminoids: 22.5, 45, 90 mg/kg bw *p.o.* respectively)

Group IX - Reference control (Tramadol 10 mg/kg *p.o.*)

2.5. Induction of Osteoarthritis

On the 0^{th} day, the rats were anesthetized by subcutaneous injection of ketamine and xylazine at 50 mg/kg and 10 mg/kg bw respectively. Osteoarthritis was induced in group II - IX by intra-articular administration of 25µl saline containing 0.3 mg monosodium iodoacetate (MIA) into the right knee of rats. For normal control group, an equivalent volume of saline was injected intra-articularly.

2.6. Treatment

On the 5^{th} day post MIA injection, after induction of osteoarthritis, single oral dose of PCL or curcuminoids or tramadol was administered to the respective groups at the mentioned dose levels. Tramadol was taken as a reference control.

The doses for PCL, curcuminoids and tramadol were selected based on previous studies [25].

2.7. Assessment of Nociception Percentage

The ratio of hind limb weight distribution was used to assess the progression of osteoarthritis [26]. Weight distribution between the sensitized right (osteoarthritic) and contralateral left (control) hind limb was measured using a static weight bearing incapacitance tester (Model: BIO-SWB 6.0-TOUCH) on day 0, 1, 3 and 5 (0h, 1h, 3h, 6h and 24 h post-test and reference item administration) (as shown in Fig. **1**). Animal was placed in an angled plexiglass chamber such that each hind paw is positioned on to separate sensor plates. The rats were acclimatized to the apparatus and readings were taken. The downward force exerted on the plate by each hind limb was recorded for five consecutive 5-sec period and averaged to obtain the mean score. The change in hind paw weight distribution was determined by calculating the difference between weight exerted by left and right limb.

Percentage inhibition of nociception was calculated based on the difference in hind limb weight bearing capacity of treatment group in comparison with MIA group [27].

$$\text{Percentage inhibition of nociception} = \frac{MIA\ group - Treatment\ group}{MIA\ group} \times 100$$

2.8. Analysis of Gene Expression

On the 6^{th} day (*i.e* 24^{th} h) of post MIA injection, the animals were sacrificed and the cartilage tissue of right knee were analysed for expression of anabolic type II collagen gene (*COL2A1*), and catabolic genes matrix metalloproteinase 3 (*MMP-3*) and matrix metalloproteinase 7 (*MMP-7*) using real-time PCR (CFX96-Bio Rad).

Total RNA was extracted from tissue homogenate using RNA isolation reagent (TRIzol method) and the isolated RNA was assessed for its quality and quantity using Nano Drop. Total RNA (200ng) was used for cDNA synthesis. The RNA and hexa primer were used for the first strand cDNA synthesis by Reverse transcriptase using kit

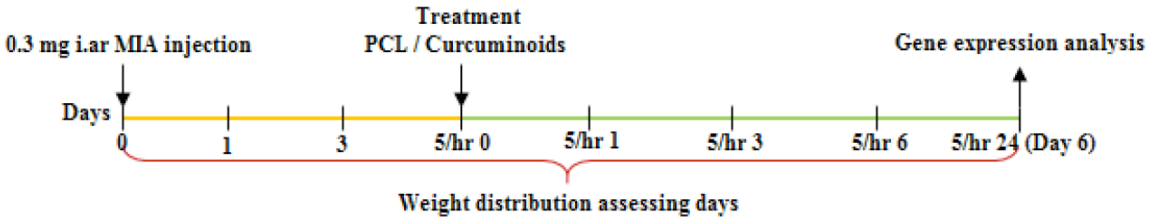

Fig. (1). Treatment and assessment duration.

Table 1. Primer sequence for RT-PCR analysis.

Gene	Forward Primer	Reverse Primer
GAPDH	5'-TGGCCTCCAAGGAGTAAGAAAC-3'	5'-CAGCAACTGAGGGCCTCTCT-3'
COL2A1	5'-GCAACAGCAGGT TCACGTACA-3'	5'-TCGGTACTCGATGATGGTCTT G-3'
MMP-3	5'-GAGTGTGGATTCTGCCATTGAG-3'	5'-TTATGTCAGCCTCTCCTTCAGAGA-3'
MMP-7	5'-ACTCTAGGCCATGCCTTTGC-3'	5-'CCATCCGTCCAGTACTCATCCT-3'

GADPH: Glyceraldehyde 3-phosphate dehydrogenase; MMP: Matrix metalloproteinase

method (Thermo scientific). The PCR amplification was carried out in a reaction volume of 20µl containing 2 µl of cDNA and 10 µl of SYBR Green Supermix (Bio Rad, USA). The Real Time PCR reaction was carried out on amplification of cDNA for 35 cycles of denaturation (95°C for 30sec), Annealing (56°C-66°C (Gradient) for 30sec) and extension (72°C for 45sec) using primers given in Table **1**. The RNA expression levels were normalized to that of housekeeping (GAPDH) gene expression and the results were analyzed.

2.9. Statistical Analysis

The data are expressed as mean ± standard error mean (SEM). Significance of result was analysed using statistical software IBM SPSS version 21. Bonferroni/Dunnet test was used as one-way ANOVA post-hoc test based on the test of homogeneity of variances. $P<0.05$ is considered as statistically significant.

3. RESULTS

3.1. HPLC Analysis

Chromatographic peaks of compounds were identified by comparing the retention time (RT) of individual standards (data not shown) with that of the extract. Chromatographic profile of PCL after acid hydrolysis showed the presence of galactouronic acid, glucose, galactose, mannose, arabinose, xylose, rhamnose confirming the presence of polysaccharides/turmerosaccharides (Fig. **2**). Turmerosaccharides content of PCL was determined as 12.6 % w/w by HPLC by the method of Gomis *et al*, 2001 [28]. HPLC profile of curcuminoids showed the presence of bismethoxy curcumin (4.602 %), demethoxy curcumin (20.638 %) and curcumin (74.760 %) compared to standards.

3.2. Nociception Inhibition

Intra-articular administration of MIA into right knee resulted in significant difference in distribution of its own bodyweight among its hind limbs in comparison to normal control. The maximum change in weight distribution was observed on the 5^{th} day of post MIA injection compared to day 0, 1 and 3. As demonstrated in Fig. (**3**), single oral dose treatment of PCL at 90 mg/kg on the 5^{th} day of post MIA injection reduced the weight bearing asymmetry.

The values are expressed as mean ± SEM; n=6. * $P < 0.05$ significantly different from normal control. # $P < 0.05$ significantly different from monosodium iodoacetate control group.

The percentage inhibition of nociception on single dose treatment with PCL/curcuminoids was calculated by comparing the weight bearing asymmetry with MIA group. Single oral dose administration of PCL produced dose-dependent reduction in nociception as in Table **2**. Curcuminoids (90 mg/kg bw) at 3^{rd} h showed only 38.67%

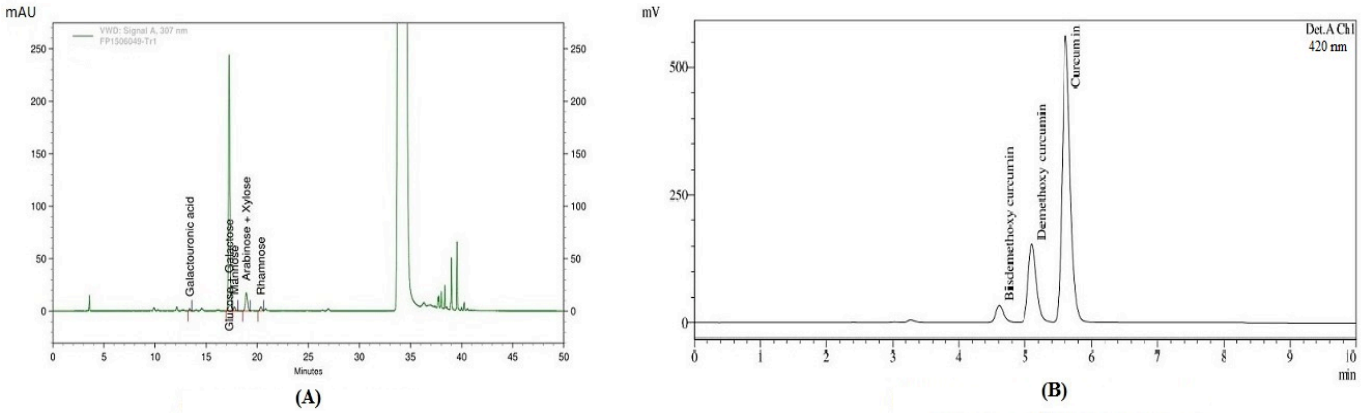

Fig. (2). HPLC Chromatogram of (**A**) PCL (**B**) Curcuminoids.

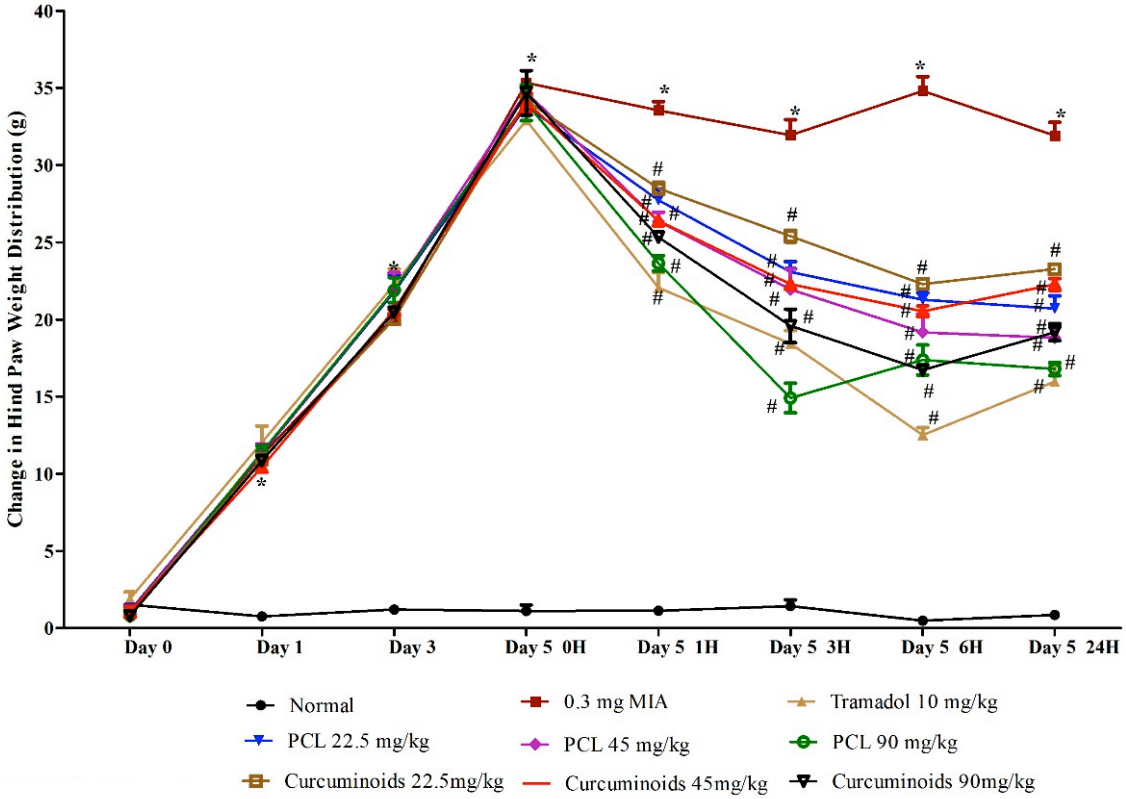

Fig. (3). Effect of PCL/curcuminoids on MIA induced change in hind paw weight distribution.

Table 2. Percentage inhibition of nociception after single dose treatment.

Day	% Inhibition						
	PCL (mg/kg)			Curcuminoids (mg/kg)			Tramadol (mg/kg)
	22.5	45	90	22.5	45	90	10
5 (0 HR)	4.27	1.69	3.67	3.28	3.56	1.38	6.84
5 (1 HR)	17.34	21.43	29.56	15.09	21.35	24.49	34.27
5 (3 HR)	27.74	31.28	53.29	20.48	30.21	38.67	42.27
5 (6 HR)	38.89	44.94	50.08	35.94	41.00	51.95	64.05
5 (24 HR)	35.13	41.06	47.39	27.08	30.19	39.91	49.95

PCL: Polar extract of Curcuma longa

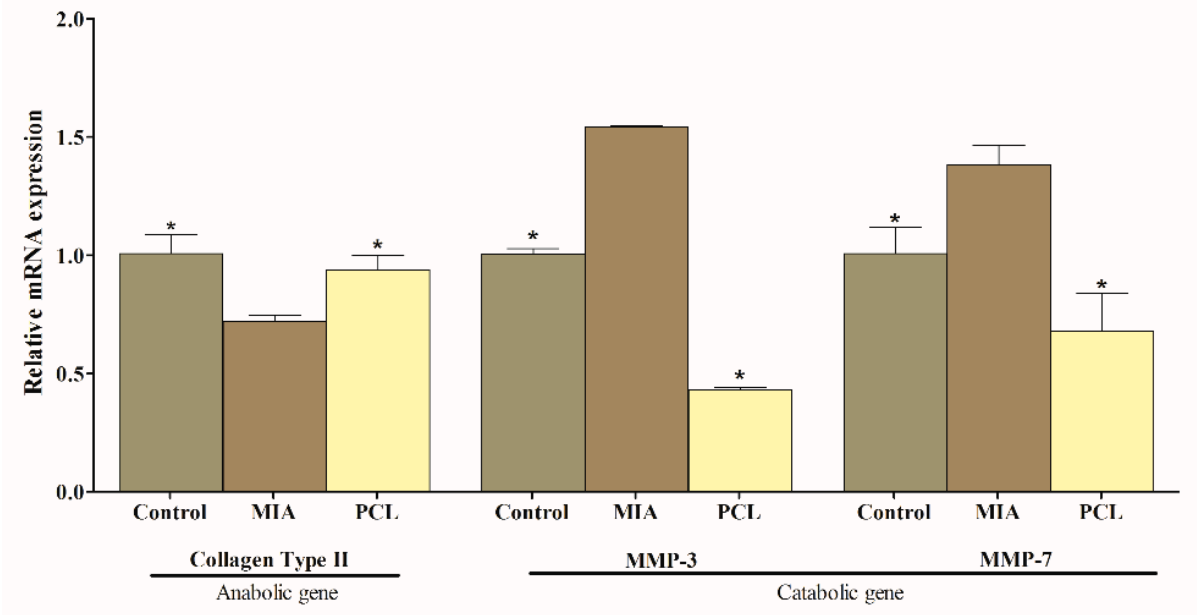

Fig. (4). Effect of PCL on gene expression in MIA induced rat cartilage tissue.

inhibition of nociception. Whereas PCL (90 mg/kg bw) showed maximum anti-nociceptive effect, 53.29% inhibition at 3rd h which is effective than curcuminoids. Tramadol (10 mg/kg) showed 42.27% inhibition of nociception. The activity of PCL in inhibiting the nociception was sustained up to 24 h.

3.3. Analysis of Gene Expression

In MIA treated group, expression of type II collagen significantly downregulated compared to normal control group. PCL (90 mg/kg) treated group demonstrated significant upregulation of type II collagen as compared to MIA treated group (Fig. **4**).

MMP-3 and *MMP-7* expression were increased significantly in MIA group compared to normal control. Whereas the expression of *MMP-3* and *MMP-7* gene were reduced significantly in PCL treatment in comparison to negative control group (Fig. **4**).

The values are expressed as mean ± SEM; n=3. * $P < 0.05$ significantly different from monosodium iodoacetate control group.

4. DISCUSSION

Turmeric, an Indian golden spice has a long history of use in cuisine of many Asian and European countries as well as medicine in many countries. The constituents of *C. longa* are the colouring agent called curcuminoids, volatile oil and the polar compounds such as polysaccharides and proteins [1]. The spice has contributed enormously to the field of traditional system of medicine like Ayurveda, Siddha, Unani and Chinese medicine (TCM). It is used in treating various conditions like cellular malfunctioning: cancer [9, 29]; inflammatory conditions [11, 30, 31]; skin disease: acne, psoriasis [32]; neurological disorder: Alzheimer's disease [8]; cell injury [33]. Though decoction of turmeric has claimed for its traditional use in treating ailments [34], many researches have focused only on curcuminoids considering them as the only known actives and not polar constituents. Our previous study revealed the anti-inflammatory activity of polar extract of *Curcuma longa* both *in vitro* and *in vivo* [23, 24]. In connection with these reports the study was designed to explore the anti-arthritic and analgesic activity of polar extract of *C. longa* in MIA induced osteoarthritic rats.

The model of MIA-induced osteoarthritis in rats has been validated and extensively used in the study of osteoarthritic pain involving inflammatory cytokines and a variety of chemical mediators [35]. MIA is a glycolysis inhibitor affecting glucose metabolism that extract energy. MIA when injected intra-articularly, inhibits the activity of glyceraldehyde-3-dehydrogenase (GADPH) consequently deranges the glycolysis pathway thereby leads to the death of chondrocytes, the sole cells of cartilage, followed by alterations in bone and induction of synovial inflammation and pain. This

model pathologically mimics human osteoarthritis and has been recommended as a suitable model for the study of drugs anti-arthritic activity [36].

In the present study, we determined the effect of PCL on osteoarthritic pain after single oral dose treatment against MIA induced OA rats. Change in weight distribution between OA induced and contralateral hind limb is a measure of pain [26]. The weight distribution was measured using a sensored incapacitance tester. The maximum change in weight distribution, the indication of peak osteoarthritic pain was observed on the 5th day in animals injected with MIA and the pain induced was observed to be maintained stably for 21 days (data not published). Thus the administration of PCL, curcuminoids and tramadol were carried out on the 5th day. Single oral administration of PCL and curcuminoids at three doses (22.5, 45 and 90 mg/kg) and tramadol at 10 mg/kg demonstrated significant decrease in weight distribution between the left and right hind limb in dose dependent manner in comparison to negative control group. PCL at all three dose levels demonstrated immediate onset of action at 1st h with high activity (up to ~30 % inhibition) than curcuminoids (up to ~25% inhibition). Moreover, PCL 90 mg/kg interestingly showed its highest anti-nociceptive activity within 3 h among all the investigated groups. Whereas curcuminoids at 90 mg/kg showed their peak therapeutic activity only at 6th h revealing PCL at 90 mg/kg could serve better in quick relief of osteoarthritic pain. As evident from Table **2**, PCL sustained its potential stably up to 24 h unlike curcuminoids treated group. Curcuminoids (90 mg/kg) dropped out its antinociceptive activity at 24th h *i.e.* decrease up to 12% from its peak activity (at 6th h). Whereas PCL (90 mg/kg) at 24th h showed only 6% decrease from its peak therapeutic response (at 3rd h) demonstrating that PCL holds long duration of action. Effective analgesic activity of PCL along with quick onset of action and long duration of action. Also the percent inhibition of nociception of PCL at 90 mg/kg was comparable to Tramadol. PCL showed peak response at 3rd h while Tramadol response was maximal at 6th h.

OA occurs in condition when injured chondrocytes, the specialized cells of cartilage, triggers the release of pro-inflammatory cytokines like IL-1β, IL-6 and TNFα, thereby up regulate the expression of matrix metalloproteinases (MMPs) leading to the destruction of cartilage. MMPs are degrading enzymes that degrades proteoglycans and collagen, the structural components of extracellular matrix (ECM) in which chondrocytes are embedded. Type II collagen is the major type of collagen found 85 - 90% in cartilage forming fibrillar network to give tensile strength to it. MMPs like *MMP-1, -2, -3, -7, -8, -9, -13* are majorly found in OA condition out of which *MMP-3* and *7* are over expressed [37-41]. *MMP-3* and *MMP-7* cause degradation of type II collagen by enhancing the activity of collagenase and also promote aggrecan cleavage leading to disease progression in OA [42-45]. Since PCL (90 mg/kg) showed comparable activity over curcuminoids in increasing the pain threshold, it was further subjected to RT-PCR analysis. MIA on intra-articular injection increased the expression of catabolic genes, *MMP-3* and *MMP-7*. However, PCL (90 mg/kg) treatment suppressed their expression significantly. Also, MIA upon articular injection into knee significantly reduced the expression of constructive gene *COL2A1* that encodes for type II collagen. PCL at 90 mg/kg has significantly increased the expression of *COL2A1* over MIA confirming the protection of cartilage. There exist many researches that worked on curcuminoids in gene regulation upon treatment of osteoarthritis. Curcuminoids, though significantly down regulated *MMP-3* and *COL2A1* gene [46], did not had effect on regulating *MMP-7* [47] Whereas PCL has significantly down regulated both the inflammatory genes *MMP-3* and *-7*. Also PCL strikingly upregulated the expression of *COL2A1* gene which is up to the normal control group confirming the potential of PCL in cartilage protection. The study revealed that unlike curcuminoids, PCL maintained the equilibrium between anabolic and catabolic genes involved in cartilage protection.

PCL has also been clinically proved to reduce the pain in OA patients [48]. But the mechanism is unknown. Polar extract of *C. longa* demonstrated anti-inflammatory activity *in vitro* in lipopolysaccharide (LPS) and concanavalin A (Con A) stimulated splenocytes by inhibiting PGE_2 and IL-12 [23] and the activity was confirmed *in vivo* on various inflammatory models in rats - carrageenan induced paw edema, Xylene induced ear edema and Cotton Pellet-induced Granuloma [49]. In addition to the gene regulating activity, PCL also inhibited inflammatory mediators and cytokines. The study unveiled that PCL can act effectively on

either condition, during the initial progression of OA by inhibiting the release of pro-inflammatory cytokines and inflammatory mediators like IL-12, IL-6, PGE2, NO [23] and even at the later stage of the disease by regulating and maintaining the equilibrium between gene expression as demonstrated in the current study. Thus the preclinical research studies indicate obvious chondroprotective function of PCL *via* promoting homeostasis between catabolic and anabolic process in the cartilage. Overall PCL appears to have promising clinical outcome in OA patients. However, further clinical trials are warranted to understand the clinically meaningful structure-modifying effects *via* imaging techniques.

CONCLUSION

Single oral dose administration of PCL exhibited anti-arthritic activity in MIA induced osteoarthritic model in rats. Anti-arthritic activity of PCL could be attributed by maintaining the equilibrium between catabolic and anabolic gene involved in cartilage protection. The study also reveals that it is not only curcuminoids that is active ingredient of "golden spice" but also the turmerosaccharides which showed comparable activity to the former. The study signifies that PCL can serve as an effective alternative treatment for osteoarthritis in human.

ABBREVIATIONS USED

MIA = Monosodium iodoacetate

MMP = Matrix metalloproteinase

OA = Osteoarthritis

PCL = Polar extract of *Curcuma longa*

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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