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RESEARCH ARTICLE

Cartilage protective effect of *Sida cordifolia* L. and *Piper longum* L. is through modulation of MMPs and TIMP

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Abstract

Context: *Sida cordifolia* L. (SC) and *Piper longum* L. (PL) has been used traditionally for the treatment of osteoarthritis (OA). However the underlying mechanism of their beneficial action is not known.

Objective: To investigate *in vitro* free radical scavenging potential and *in vivo* anti-osteoarthritic activity of SC and PL.

Materials and Methods: SC and PL were studied for free radical scavenging potential along with phenolic content. Anti-osteoarthritic effect of both plants was assessed using collagenase induced OA (CIOA) rat model and further molecular study of isolated synovium was carried out. Statistical analysis was carried out by one-way analysis of variance followed by Fisher's Least Significant Difference test.

Results: SC and PL showed free radical scavenging potential, which could be attributed to their phenolic content, which is 10.360±1.165 and 0.633±0.018 mg/gm dry mass, respectively. OA was induced in rats by intra-articular injection of collagenase II. Rats were divided into 5 groups: Healthy control, osteoarthritic control (CIOA), indomethacin, *S. cordifolia* (SC) and *P. longum* (PL). Treatment was administered for 20 days. SC and PL have up-regulated superoxide dismutase (p=0.0442, 0.0538), glutathione peroxidase (p=0.9214, 0.9127), catalase (p=0.8329, 0.7598), paraoxonase-1 (p=0.7883, 0.9901), TIMP1 (p=0.0934, 0.0300) and down-regulated MMP3 (p=0.0929, 0.0361) and MMP9 (p=0.4231, 0.1759) in knee synovium compared to CIOA, respectively.

Discussion and conclusion: SC and PL clearly attenuated OA-augmented expression of MMP and restored OA-reduced expression of TIMP in the synovium. This finding provides the first evidence that *S. cordifolia* and *P. longum* effectively operates through inhibition of cartilage matrix degradation in OA.

INTRODUCTION

Osteoarthritis (OA) affects about 3.6% of the global population (Manen et al., 2012). In India the prevalence of knee OA is estimated to be 5.8% (Chopra et al., 2001). Current therapeutic strategy is to use NSAIDs for joint pain relief. However use of NSAIDs is strictly limited due to severe side effects including adverse effects on cartilage (Huh et al., 2008). Unfortunately, there is no medicine or therapy that can target cartilage protection. OA patients are

therefore increasingly restoring to CAM that primarily thought to be chondroprotective, either in the form of nutraceuticals or in the form of herbals. CAM is however criticized for lack of evidences in favor of their claims and/or their mechanism of action.

Etiology of OA is not clear, although oxidative stress and inflammation are considered as the principal factors responsible for excessive degeneration. Oxidative stress is the action of free radicals and reactive oxygen species (ROS), which is generally an outcome of stress but become deleterious when not being eliminated effectively by the endogenous systems and leads to various diseases (Anu et al., 2013; Hazra et al., 2008). Oxidative stress is found to be elevated in cartilage and synovial tissues in human and in animal models of OA, which contributes to disease progression by increasing production of inflammatory mediators and matrix degrading enzymes (Moore et al., 2013). Inherent mechanism adopted by many cells to protect themselves against free radical damage is through anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) etc. (Altindag et al., 2007; Hazra et al., 2008). Previous studies on OA have shown that matrix metalloproteinases (MMPs) and their tissue inhibitors play an important function in cartilage matrix turnover (Park et al., 2005). Tissue inhibitors of metalloproteinases (TIMPs) bind to MMPs on a 1:1 basis by forming high affinity complexes. However, in OA affected joint tissues, up-regulation of MMPs is reported higher compared to TIMPs, which contribute to the accelerated degradation of extracellular matrix. Any imbalance in the ratio of TIMPs to MMPs results in the continued destruction of proteoglycan and collagen of cartilage in OA (Park et al., 2005).

In our earlier work, we have studied anti-osteoarthritic potential of six herbs, which are commonly used by Ayurvedic physicians for the treatment of OA viz. *Sida cordifolia* L., *Piper longum* L., *Zingiber officinale* Rosc., *Ricinus communis* L., *Vitex negundo* L. and *Tribulus terrestris* L., using collagenase type II induced OA (CIOA) rat model (Nirmal et al., 2013), in which *Sida cordifolia* (Bala) and *Piper longum* (Pippali) have shown appreciable potential on studied parameters. The present communication is an effort to evaluate mechanistic action of Bala and Pippali in OA. We have reported action of these herbs on synovium by studying modulation of key genes affecting oxidative stress and cartilage degeneration.

Materials and Methods

Processing of herbs

Roots of *S. cordifolia* and inflorescence of *P. longum* were obtained from Green Pharmacy (Pune, Maharashtra, India) in dried form. The drugs were then identified and authenticated in house by *Ayurvedic* experts using API parameters.

Chemicals

Chemicals used in this study include, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Sodium nitroprusside (SNP) purchased from Sigma, USA. Thiobarbaturic acid (TBA), N-1-Napthyl ethylene diamine dihydrochloride (NED) purchased from Loba Chemie, India. Ascorbic acid, Potassium ferricyanide purchased from Merck, India. Gallic acid (SRL, India), Ferric chloride ($FeCl_3.6H_2O$) (Qualigens, India) and all other chemicals used were of analytical grade.

Determination of total phenolic content

The total phenolic content of aqueous extracts of *S. cordifolia* and *P. longum* was determined as described in Narkhede *et al*. 2013 (Narkhede et al., 2013). Total phenolics were determined as milligrams of gallic acid equivalents (GAE) per gram of sample by computing it with standard calibration curve.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of aqueous extracts of *S. cordifolia* and *P. longum* were determined by comparing it with ascorbic acid as a standard, as described in Mukharjee et al (Mukherjee et al., 2011). The percentage of DPPH-scavenging activity was determined according to the formula:

$$\% \text{ inhibition} = \text{Absorbance}_{control} - \text{Absorbance}_{sample}/\text{Absorbance}_{control} \times 100$$

Determination of reducing power potential

Ferric reducing potential was performed as described in Narkhede et al (Narkhede et al., 2012)**.** Ascorbic acid was used as a standard for comparison at concentration range from 10-100 µg/ml.

Nitric oxide (NO) scavenging assay

The aqueous extracts of *S. cordifolia* and *P. longum* was used for estimation of NO scavenging assay as described in Pawar et al (Pawar et al., 2011). NO radical inhibition was estimated by using griess reagent. The NO radical scavenging activity was calculated according to the equation:
% Inhibition = $[(A_{Cont} - A_{test}) / A_{Cont}] \times 100$
Where, A_{Cont}: Absorbance of control reaction, A_{test}: Absorbance of test reaction

Anti-lipid peroxidation
Decomposition of lipid membrane in the body leads to formation of Malondialdehyde (MDA) along with other aldehydes and enols as the end product. These react with TBA to form colored complexes. Hence these are called as the TBA reactive substances (TBARS). The complex of TBA-MDA is selectively detected at 532 nm as given in Kulkarni et al., (Kulkarni et al., 2012). Inhibition of lipid peroxidation by aqueous extracts of *S. cordifolia* and *P. longum* was calculated using following formula:
% Inhibition = $[A_{induced} - A_{test} / A_{induced} - A_{normal}] \times 100$
Where, $A_{induced}$: Absorbance of $FeCl_3$, A_{normal}: Absorbance of control reaction,
A_{test}: Absorbance of test reaction.

Quantitative real-time reverse transcription-polymerase chain reaction (qPCR) analysis
Rat model was carried out with prior institutional animal ethics committee approval (BVDUMC/CPCSEA/1962/2011-12). Briefly, osteoarthritis was induced by giving intra-articular injection of collagenase type II (Sigma, USA) on days, 1st and 4th of the experiment. Treatment was given once daily from day 14th to 34th. Powders of herbs were administered orally at the dose of 270 mg/kg *p.o.* in the form of suspension prepared in water. Standard drug indomethacin (purchased from local pharmacy, manufactured by Jagsonpal pharmaceuticals Ltd, India) was given at the dose of 3 mg/kg b. wt. Group I (HC) served as a control (Normal saline 50µl intra-articularly to right knee), Group II (CIOA) was osteoarthritic control (50 unit of collagenase type II intra-articularly to right knee), Group III (INDO) was positive control, given standard drug indomethacin; Group IV (SC) served with test drug *S. cordifolia* and Group V (PL) served with test drug *P. longum*. Animals were killed at the end of the experiment and their synovial tissue was removed. The synovium were flash frozen immediately in liquid nitrogen and stored at -80^0C until further use.
For qPCR analysis, total RNA from isolated knee synovium tissue was extracted using TRIZOL reagent (Sigma Aldrich, USA) with PureLink RNA mini kit (Invitrogen CA, USA). Quality of the isolated RNA was determined using denaturing agarose gel electrophoresis followed by quantification by measuring absorbance at 260 nm. The first strand cDNA was synthesized from 1 µg of total RNA using the SuperScript first-strand synthesis system for quantitative real-time PCR (Invitrogen CA, USA). qPCR analysis was performed with the help of a StepOne realtime PCR system (Applied Biosystems, CA, USA) using TaqMan gene expression assays (Applied Biosystems, CA, USA). Taqman gene expression master mix was procured from Applied Biosystems (CA, USA). Cycling conditions were 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 s, 60°C for 1 min. The Taqman gene expression assays that were used in this study are SOD (Sod1; Rn00566938_m1), GPx (Gpx1; Rn00577994_g1), CAT (Cat; Rn00560930_m1), Paraoxonase-1 (Pon1) (Pon1; Rn01455909_m1), MMP-3 (Mmp3; Rn00591740_m1), MMP-9 (Mmp9 Rn00579162_m1), and TIMP-1 (Timp1; 1 Rn00587558_m1). The data was analyzed using Data Assist software version 3.0. The data is representative of synovium at least from three rats. The relative abundance of the RNA was calculated to the amount of β-actin (Actb; Rn00667869_m1) using StepOne software version 2.2.2 (Applied Biosystems, CA, USA), DataAssist version 3.0 (Applied Biosystems, CA, USA) and the ΔΔ Ct method (Schmittgen & Livak, 2008).

Statistical analysis
Statistical analysis was carried out by using GraphPad Prism software version 6.0 (GraphPad Software Inc., CA, USA). The data were expressed as mean ± SD or SEM, and statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test.

Results

Determination of total phenolic content
Total phenolic content in aqueous extracts of both *S. cordifolia* and *P. longum* was 10.360 ± 2.020 and 0.633 ± 0.030 mg equivalent GAE/g dry mass, respectively.

Determination of DPPH radical scavenging activity

Both plants extracts were found to be potent inhibitor of DPPH-free radicals. Higher activity (55.81 % at conc. 100 µg/ml) was observed in *S. cordifolia* extract followed by *P. longum* (51.55% at conc. 100 µg/ml) (Table 1). IC_{50} of *S. cordifolia*, *P. longum* and standard was 82.8 ± 3.7, 106.5 ± 1.7 and 6.02 ± 0.3 µg/ml, respectively.

Table (1): Anti-oxidant potential of *S. cordifolia* and *P. longum*. % scavenging activity of DPPH and reducing potential. All the values are expressed as Mean ± SD.

	% DPPH scavenging		Reducing power		
Conc. (µg/ml)	***S. cordifolia***	***P. longum***	**Ascorbic acid**	***S. cordifolia***	***P. longum***
10	28.318 ± 3.178	22.134 ± 2.673	0.190 ± 0.018	0.082 ± 0.011	0.061 ± 0.011
20	32.239 ± 4.383	23.115 ± 0.942	0.255 ± 0.022	0.091 ± 0.017	0.071 ± 0.006
30	34.502 ± 4.383	24.095 ± 1.148	0.345 ± 0.031	0.111 ± 0.005	0.073 ± 0.009
40	34.615 ± 1.741	24.623 ± 4.259	0.413 ± 0.012	0.126 ± 0.003	0.074 ± 0.007
50	41.365 ± 4.218	26.546 ± 3.620	0.516 ± 0.042	0.127 ± 0.016	0.079 ± 0.006
60	44.532 ± 5.861	30.128 ± 6.606	0.551 ± 0.031	0.152 ± 0.021	0.086 ± 0.002
70	44.645 ± 3.594	33.409 ± 7.388	0.646 ± 0.023	0.175 ± 0.012	0.089 ± 0.005
80	49.321 ± 3.831	39.743 ± 0.727	0.695 ± 0.024	0.189 ± 0.008	0.090 ± 0.008
90	51.961 ± 1.359	50.000 ± 2.167	0.873 ± 0.093	0.237 ± 0.087	0.103 ± 0.005
100	55.807 ± 0.942	51.546 ± 1.927	0.895 ± 0.084	0.284 ± 0.028	0.105 ± 0.013

Determination of reducing power potential

Both plants have shown ferric reducing potential. Aqueous extract of *S. cordifolia* showed maximum absorbance compared to *P. longum* extract at 100 µg/ml (0.284 ± 0.028 and 0.105 ± 0.013, respectively) (Table 1). Higher absorbance indicated higher reducing potential.

Nitric oxide (NO) scavenging assay

A trend observed in NO scavenging activities of *S. cordifolia* (IC_{50}: 1743.333 ± 62.512 µg/ml), *P. longum* (IC_{50}: 672.619 ± 49.886 µg/ml) extracts and standard ascorbic acid (IC_{50}: 140.265 ± 10.175 µg/ml) at different concentrations. Both the extracts have decreased the amount of nitrite generated from the decomposition of SNP *in vitro*. The scavenging of NO by plants increased in dose dependent manner. *P. longum* have shown maximum activity.

Anti-lipid peroxidation

Lipid peroxidation is one of the major effects of oxidative damage. Aqueous extract of *S. cordifolia* have shown significant anti-lipid peroxidation activity, which is maximum at a 1000 µg/ml. However, *P. longum* extract have not shown any inhibition of lipid peroxidation. IC_{50} of *S. cordifolia* and standard was 566.345 ± 3.768, 5.5 ± 0.13 µg/ml, respectively.

qPCR analysis

In this experiment, mRNA abundance was measured for specific genes and the data presented as fold increase or decrease with respective to the osteoarthritic control rats unless otherwise mentioned.

Effect of *S. cordifolia* and *P. longum* on expression of antioxidant genes in collagenase induced osteoarthritic rats

Osteoarthritic control rats showed significant down-regulation of SOD (p=0.0019) in the knee joint synovium by ~5.2-fold compared to control rats (Fig. 1A). On the contrary, osteoarthritic rats receiving *S. cordifolia* showed significant up-regulation in the synovial expression of SOD by ~3.3-fold (p=0.0442) (Fig. 1A). Rats receiving *P. longum* and indomethacin also showed up-regulation in SOD expression by ~3.2-fold (p=0.0538) and ~1.7-fold (p=0.4825), respectively (Fig. 1A). Down-regulation of synovial expression of GPx gene by ~2.6-fold (p=0.8206) was observed in osteoarthritic control rats, when compared to control (Fig. 1B). On the contrary, GPx expression was elevated by ~1.7-fold (p=0.9214), ~1.8-fold (p=0.9127) and ~19.4-fold (p=0.0167) in osteoarthritic rats receiving *S. cordifolia*, *P. longum* and indomethacin respectively (Fig. 1B). Compared to control rats, osteoarthritic

rats also down-regulated synovial expression of CAT by ~6.8-fold (p=0.6134) (Fig. 1C). Up-regulation in the CAT expression by ~3.4-fold (p=0.8329), ~4.5-fold (p=0.7598) and ~40.9-fold (p=0.0048) respectively was observed in osteoarthritic rats receiving *S. cordifolia*, *P. longum* and indomethacin (Fig. 1C). In addition, down-regulation in the synovial expression of PON-1 by ~4.9-fold (p=0.6065) was observed in osteoarthritic control rats compared to control rats (Fig. 1D). While osteoarthritic rats receiving *S. cordifolia*, *P. longum* and indomethacin have shown increased mRNA level of PON-1 by ~2.8-fold (p=0.7883), ~1.1-fold (p=0.9901) and ~12.1-fold (p=0.1205) respectively (Fig. 1D).

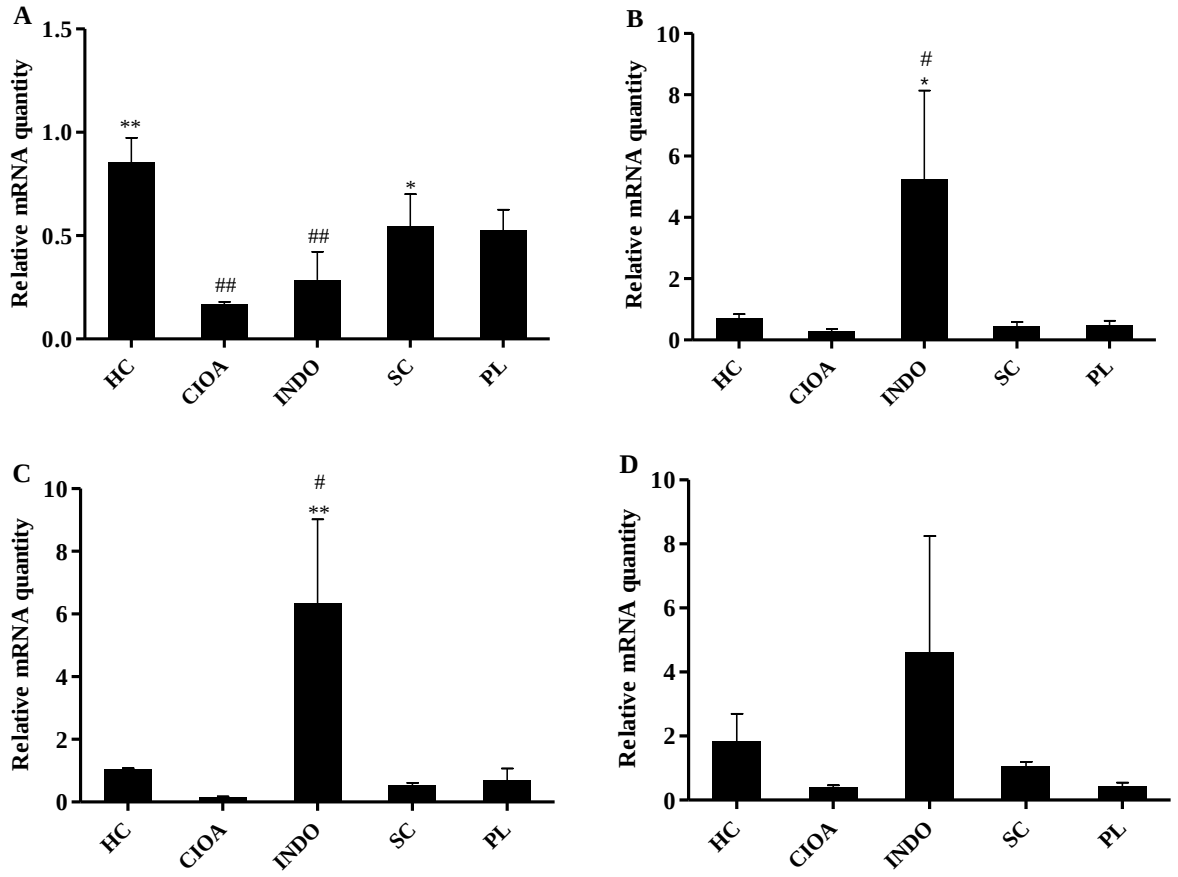

Figure (1): Effect of *S. cordifolia* (SC) and *P. longum* (PL) on the mRNA expression of A) SOD-1, B) GPx-1 C) CAT and D) PON-1 in the synovium of rats. All the values are expressed as Mean ± SEM (n=3). Comparisons were done between CIOA/HC and each individual treated group by Fisher's Least Significant Difference (LSD) test (*p<0.05, **p<0.01, #p<0.05, ##p<0.01, * =Compared to CIOA, # =Compared to HC).

Effect of *S. cordifolia* and *P. longum* on expression of MMPs and TIMP genes in collagenase induced osteoarthritic (CIOA) rats

In the knee synovium, osteoarthritic control rats showed up-regulation of MMP-3, MMP-9 and TIMP-1 by ~14.1-fold (p=0.0227), ~10.6-fold (p=0.1886) and ~1.2-fold (p=0.4845) when compared to control rats, respectively (Fig.2). Down-regulation of expression of MMP-3 by ~2.8-fold (p=0.0929) and ~3.6-fold (p=0.0623) was observed in the osteoarthritic rats receiving *S. cordifolia* and indomethacin, respectively (Fig.2A). *P. longum* treated group showed significant down-regulation in MMP-3 expression by ~6.1-fold (p=0.0361) (Fig.2A). In addition, both indomethacin and *S. cordifolia* treated osteoarthritic rats showed down-regulation in synovial expression of MMP-9 by ~9.9-fold (p=0.1918) and ~2.1-fold (p=0.4231), respectively (Fig.2B). Osteoarthritic rats receiving *P. longum* did not show down-regulation in the MMP-9 expression. Up-regulation of TIMP-1 was observed in rats treated with *S. cordifolia* and *P. longum* by ~1.5-fold (p=0.0934) and ~1.7-fold (p=0.03), respectively. On the contrary, indomethacin was unable to affect the level of synovial TIMP-1 in the osteoarthritic knee joints (p=0.4682) (Fig.2C).

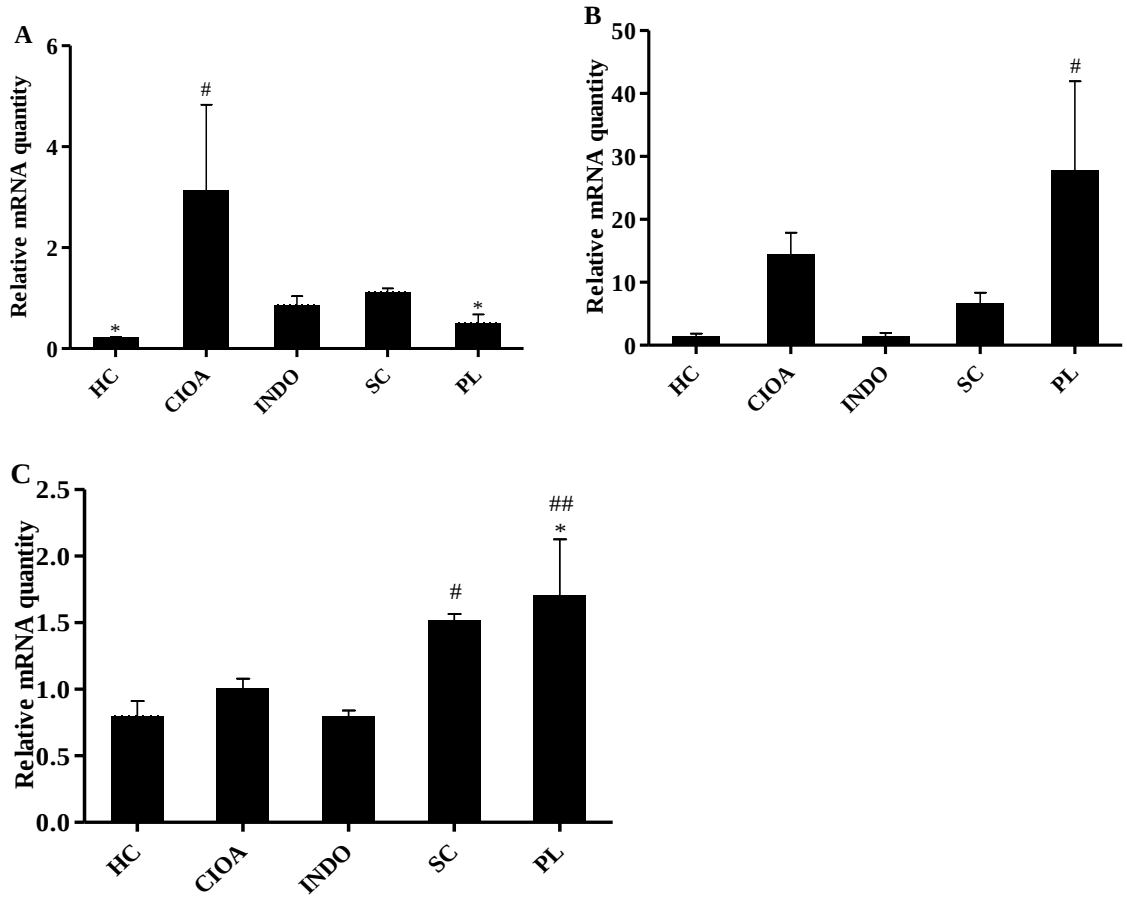

Figure (2): Effect of *S. cordifolia* (SC) and *P. longum* (PL) on the mRNA expression of A) MMP3, B) MMP9 and C) TIMP1 in the synovium of rats. All the values are expressed as Mean ± SEM (n=3). Comparisons were done between CIOA/HC and each individual treated group by Fisher's Least Significant Difference (LSD) test (*p<0.05, #p<0.05, ##p<0.01 *=Compared to CIOA, # =Compared to HC).

Discussion

Present study has evaluated the role of *S. cordifolia* and *P. longum* in treating osteoarthritis (OA). In testing therapeutic modalities for OA, it is valuable to study their role in synovitis. Our data has demonstrated anti-oxidant and anti-osteoarthritic role of oral administration of *S. cordifolia* and *P. longum* in rat model with respect to cartilage protection. In our previous studies, both plants have shown protective effects by controlling key components in OA pathogenesis; like knee and paw swelling, GAG release in serum and cartilage degradation as assessed by radiographs and histopathological investigation (Nirmal et al., 2013).

The antioxidant capacity of phenolic compounds possesses ability to scavenge radicals and thereby protecting cells against oxidative stress (Ndhlala et al., 2010). In OA, oxidative stress leads to structural and functional damage to chondrocytes as well as cartilage degradation via aggrecan and collagen (Aydogan et al, 2008; Watari et al., 2011). Our results showed presence of significant amount of phenolics in *S. cordifolia* and *P. longum*, which might have been responsible for their anti-oxidant activity. Both plants revealed scavenging potential against DPPH, possess strong reducing power and exhibited dose dependent efficacy along with the standard (Table 1). Release of (NO) and superoxide anion ($O2^-$) are the stress responses produced by chondrocytes (Henrotin et al., 2003). Suppression of released NO may be partially attributed to direct NO scavenging as both the plants scavenged NO in dose dependent manner. Interestingly we have got higher NO scavenging potential in *P. longum*, despite of less phenolic content, this could be attributed to different phytochemical compounds present in this extract. In OA, it is proposed that ROS contribute to matrix degradation through lipid peroxidation (Dawn et al., 2013). The percent

inhibition of peroxide formation by *S. cordifolia* was increased in a dose dependant manner, so it can prevent oxidative damage.

Interestingly, the action of these plant extracts is not confined only to scavenging free radicals. It could also be noted in this data that inherent anti-oxidant defense was also strengthened. Treatment with *S. cordifolia*, *P. longum* and indomethacin up-regulated SOD, CAT, GPx and PON-1 expression in synovium of osteoarthritic knee joint (Fig. 1), the major anti-oxidant enzymes against ROS (Anu et al., 2013), thus protecting tissue from deleterious effects of free radicals. The difference is not statistically significant due to high standard deviation. In OA, cartilage cumulative oxidative stress induces hypoxia-inducible factor 1-α in chondrocytes and cartilage leading to over expression of interleukin 1β, tumor necrosis factor alpha (Kulkarni et al., 2014), which accelerates cartilage degeneration (Alcaraz et al., 2010). SOD protects the tissue by catalyzing transformation of superoxide, CAT and GPx detoxifies H_2O_2 (Aydogan et al., 2008). PON1 is a high-density lipoprotein (HDL)-associated enzyme, which plays a protective role against lipid peroxidation of low-density lipoprotein (LDL), HDL; activity of PON1 decreases in OA (Aydin et al., 2012). Free radical scavengers and oxidative defense genes, including genes for superoxide dismutase (SOD) 2, SOD 3 and glutathione peroxidase 3 (GPx) have been identified as potential therapeutic agents for the protection of articular cartilage against progression of OA (Alcaraz et al., 2010). Thus, both the plants have increased anti-oxidant enzyme activities in response to increased oxidative stress.

ROS contributes to cartilage degradation through activating latent collagenase and by up-regulating the expression of genes coding matrix metalloproteinases (MMPs) (Henrotin et al., 2003). MMPs derived from chondrocytes, synovium and polymorphonuclear leukocytes have been proposed to play a major role in cartilage degradation in OA (Alam et al., 2011). *P. longum* have significantly (p=0.0361) downregulated levels of MMP3 in our observations (Fig. 2A). MMP-3 is the key enzyme involved in degradation of the non-fibrillar collagen and other components like proteoglycans of the ECM in cartilage (Sandya et al., 2009), its increased level leads to cell death and damaged cartilage (Huh et al., 2008). MMP mediated cartilage damage is initiated by synovial macrophage activation. These macrophages produce pro-MMP-9, which is activated via MMP-3 or MMP-13 pathway to active MMP-9 (Sellam & Berenbaum, 2010). *S. cordifolia* have downregulated both MMPs compared to osteoarthritic control rats (Fig. 2A, 2B). Increased levels of MMPs compared to tissue inhibitors of metalloproteinases (TIMPs) have been associated with the degradation of ECM of joint tissues in OA (Yoshihara et al., 2000). TIMP-1 has greater effectiveness in the inhibition of MMP-1, -3 and -9 (Lee et al., 2008). This explains the current observation wherein *P. longum* have downregulated MMP-3 while corresponding significant (p=0.03) upregulation in TIMP (Fig. 2); *S. cordifolia* administration showed upregulation of TIMP with corresponding reduction in MMP-3 and -9, an ideal situation to restore MMP-TIMP balance (Fig. 2). This activity of both extracts may be potentiated given to their ability to inhibit cyclooxygenase (COX) leading to the inhibition of prostaglandin (PGE) synthesis (Anilkumar & Chattopadhya, 2010; Bang et al., 2009), thereby preventing cartilage degeneration. Down-regulation of MMPs and Up-regulation of TIMPs prevents degradation of articular cartilage in OA. Therefore, inhibitors of MMP represent an attractive target in OA (Alcaraz et al., 2010). Down-regulation of both MMPs by *S. cordifolia* explains its GAG protective role (Nirmal et al., 2013). Indomethacin is also a COX inhibitor and prolong treatment with PGE2 blocking agent, decreases PGE2 production in synovial membrane of osteoarthritic rats (Alvarez-Soria et al., 2008). So, PGE2 may have reduced the observed expression of MMPs (Fig. 2A, 2B) as well as paw edema in indomethacin treated group (Nirmal et al., 2013). However, this action may not restore the balance in cartilage remodeling since indomethacin did not upregulate TIMP (Fig. 2C). Our result suggests that both the plants attenuated OA-augmented expression of MMPs; and restored the OA-reduced expression of TIMPs and anti-oxidants in the synovium.

Reducing synovial oxidative stress and inflammation could be a major target for cartilage protection in OA (Anu et al., 2013). Our study indicated that the oral administration of *S. cordifolia* and *P. longum* aqueous suspension may have therapeutic effects in osteoarthritis whereas cartilage protection was also achieved through controlling MMPs/TIMP and anti-oxidant levels in the synovium.

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Declaration of interest

The authors declare that they have no conflicts of interest.

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