Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Short Communication

COMPARATIVE ANALYSIS OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF *C. CASSIA* AND *C. ZEYLANICUM* IN RAW264.7, SW1353 AND PRIMARY CHONDROCYTES

PRERNA RAINA[1], CV. CHANDRASEKARAN[2], AMIT AGGARWAL[2], NARENDRA WAGH[3], RUCHIKA KAUL-GHANEKAR[1]*

[1]Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth University Medical College Campus, Dhankawadi, Pune 411043, India, [2]Natural Remedies Pvt. Ltd., Veersandra Industrial Area, 19 KM Hosur Road, Electronic City Post, Bangalore 560100, Karnataka, India, [3]Bharati Vidyapeeth University Medical College, Dhankawadi, Pune 411043, India
Email: ruchika.kaulghanekar@gmail.com

Received: 19 Aug 2015 Revised and Accepted: 05 Oct 2015

ABSTRACT

Objectives: The objective of this research was to compare the anti-inflammatory activity of aqueous and methanolic extracts of *C. cassia* (CC) and *C. zeylanicum* (CZ) in mouse macrophage (RAW264.7) and human chondrosarcoma (SW1353) cell lines as well as in human primary chondrocytes, to correlate their efficacy in management of osteoarthritis (OA) related pathophysiology.

Methods: RAW264.7, SW1353 and human primary chondrocytes were pre-treated with aqueous extracts of *C. cassia* (CC_W) and *C. zeylanicum* (CZ_W) and methanolic extracts of *C. cassia* (CC_M) and *C. zeylanicum* (CZ_M) at various concentrations (0.1-100 µg/ml) for 1 h, followed by stimulation with LPS and IL-1β, respectively. The effect of CC_M, CC_W, CZ_M and CZ_W on the production of nitric oxide (NO) was evaluated by Griess reaction. Evaluation of prostaglandin E_2 (PGE_2) and leukotriene (LTB_4) proteins was performed by EIA-Monoclonal based kits. The effect of these extracts on matrix metalloproteinase (MMPs-2, 9 and 13) levels was analyzed by SensoLyte® fluorimetric MMP assay kit.

Results: The methanolic extracts (CC_M, CZ_M) of both the varieties of cinnamon were found to be more effective than the aqueous extracts in terms of PGE_2, LTB4 and MMP inhibition. We found that in RAW 264.7, CC_M and CZ_M decreased NO and PGE_2 production by 45.4%±8.6; 65.6%±5.7 and 79.8%±1.2; 95.9%±0.3, respectively. Similarly, in SW1353 and chondrocytes, CC_M decreased PGE_2 production by 68.8%±6.4;36.1%±9.5, respectively whereas CZ_M reduced PGE_2 production by 70.2%±2.3; 52.3%±5.4, respectively. Moreover, in SW1353 and chondrocytes CC_M decreased LTB4 production by 85.47%±3.03; 99.6%±0.2, respectively whereas CZ_M reduced LTB4 production by 67.5%±5.6; 75.6%±1.2, respectively. In chondrocytes both CC_M and CZ_M significantly reduced the levels of MMP-2(55.7%±5.2; 73.1%±7.1), MMP-9 (57.5%±4.7; 74.5%±5.2) and MMP-13 (90.1%±2.6; 71.2%±12.5), respectively. However, on comparing the two species of cinnamon, *C. zeylanicum* was found to be more effective than *C. cassia* and thus could be considered for its potential therapeutic application in the management of inflammatory conditions associated with OA.

Conclusion: The present study would help in choosing better of the two species of cinnamon for their possible therapeutic application in the management of inflammatory condition associated with OA.

Keywords: *C. cassia*, *C. zeylanicum*, Inflammation, Osteoarthritis, Chondrocytes.

Cinnamon is widely used as a culinary spice and flavoring agent [1]. It has been extensively used in Indian traditional medicine for the management of various disease conditions [2]. Various studies have shown that Cinnamon has anti-inflammatory properties and decreased the expression of the inflammatory markers such as interleukin (IL)-1β, IL-6 and Tumor necrosis factor (TNF)-α [3]. Although there are many types of Cinnamon, only four varieties that are used for commercial purposes include *C. zeylanicum*, *C. cassia*, *C. saigon* and *C. korintje*. *C. cassia* (CC), is widely used as traditional Chinese medicine for treating blood circulation disturbances, gastritis and inflammatory diseases [4]. It has been shown to have various pharmacological properties, such as antiulcerogenic [5], anti-inflammatory [6], antipyretic [7], antimicrobial [8], antidiabetic [9] and antitumor activity [10]. Cinnamaldehyde, the active component of *cinnamon*, has been reported to down regulate the production of major inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, nuclear factor kappa (NF-κB) in RAW264.7 cells [11, 12]. *C. zeylanicum* (CZ), has been used traditionally for its anti-diabetic [13], anti-nociceptive [14], astringent [15] and diuretic activities [15]. Procyanidine polyphenols, a compound extracted from CZ has been reported to regulate inflammation and arthritis [16]. Gunawardena *et al.*, (2015) has recently demonstrated the anti-inflammatory activity of cinnamon (CZ and CC) extracts as well as its phytochemical compounds (E-cinnamaldehyde and o-methoxy cinnamaldehyde) *in vitro* [17]. Hong *et al.*, (2012) demonstrated that administration of water extract of cinnamon *in vivo* decreased the serum levels of TNF-α and IL-6. At *in vitro* level, it was shown to decrease the expression of TNF-α, inhibit LPS-induced degradation of IκBα as well as activate JNK, p38 and ERK1/2 [18].

Although several studies have reported anti-inflammatory activity of cinnamon bark from either CC or CZ, however, their efficacy in the management of osteoarthritis (OA) associated pathophysiology has not been compared. In the present work, we have for the first time compared the effect of two varieties of cinnamon on modulation of NO, PGE_2, LTB4 and MMP levels in human chondrocytic cell line (SW1353) and human primary chondrocytes. Such studies would help in selection of important medicinal plants that could be used for the prevention, cure and management of OA related pathogenesis. We found that compared to the aqueous extracts, the methanolic extract of *C. cassia* and *C. zeylanicum* significantly modulated NO, PGE_2, LTB4 and MMP levels in the tested cells. However, CZ proved to exhibit higher efficacy than CC and thus could be explored in the management of OA.

The materials used in the study included DMEM, L-15 media, Hams F12, FBS, penicillin and streptomycin, lipopolysaccharide (LPS), IL-1β, dexamethasone, 1400W dihydrochloride and (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA), L-glutamine was purchased from Himedia Corporation, Mumbai, India). MMP kit was purchased from Cisbio, PGE_2 and LTB4 kits were purchased from Cayman and tissue culture plasticware was purchased from BD Biosciences (San Diego, CA, USA).

The extracts of the barks of *C. cassia* and *C. zeylanicum* were procured from Natural Remedies, Pvt. Ltd. Bangalore. The plant materials were identified by National Institute of Science Communication and Information Resources (NISCAR), New Delhi

and Dr. P. Santhan, in-house taxonomist, Pharmacognosy department, R&D center, Natural Remedies Pvt. Ltd, Bangalore, India. The barks were sundried and stored. Voucher specimens (NRPL-569 and 570) were deposited in the herbarium of Natural Remedies, Pvt. Ltd. Bangalore.

For the preparation of CC_M and CZ_M, the coarsely powdered raw material (50 g) was extracted with methanol (~200 ml) under reflux at 70°C for 1h and the solvent was filtered. The remaining raw material was refluxed by adding 150 ml methanol for 1 h, repeated twice and again filtered. The liquid filtrate was combined and concentrated using rotavapor under vacuum to a thick paste at temperature NMT 60 °C and 10.0 g of crude extract was obtained. For the preparation of CC_W and CZ_W, the coarsely powdered raw material (50 g) was mixed with water and extracted at 85 to 90°C (3 times each with 200 ml water for 1 h each wash) and filtered each time. The combined liquid filtrates were concentrated using rota vapor under vacuum to a thick paste at temperature NMT 60°C and 15.0 g of crude water extract was obtained [19].

The cell lines RAW264.7 and SW1353 were purchased from American Type Culture Collection (ATCC, USA). The cell lines were maintained in DMEM and L-15 media containing 2 mM L-glutamine, respectively, (Himedia Corporation, Mumbai, India) supplemented with 10% FBS (Sigma, St. Louis, MO, USA), 20Units/ml penicillin and 20 µg/ml streptomycin (Gibco BRL, USA). Human cartilage sample was obtained from the patient undergoing knee replacement surgery after approval from institutional ethics committee (IEC) of Bharati Vidyapeeth Medical College (Ref: BVDU/MC/55) and informed consent from the patient. Chondrocytes were prepared by the enzymatic digestion of cartilage with 0.25% collagen and plated (1×10^6 cells/ml) in 35 mm primaria coated culture dishes. The cells were cultured in DMEM: Hams (1:1) F12 containing 2 mM L-glutamine, 10% FBS, 100Units/ml penicillin and 100 µg/ml streptomycin and incubated in 5% CO2 incubator at 37 °C.

For cell viability assay, RAW264.7, SW1353 and human primary chondrocytes were seeded at a density of 5×10^5cells/ml in 96-well plates. The cells were treated with different concentrations (0-100 µg/ml) of CC_M, CC_W, CZ_M and CZ_W for 24 h. Cell viability was determined by MTT assay as described previously [20, 21].

For evaluating nitric oxide (NO) release, RAW 264.7 cells were seeded at a density of 5×10^5cells/ml in 96 well plate and allowed to adhere for 24 h. The cells were pre-treated with different concentrations (0-100 µg/ml) of CC_M, CC_W, CZ_M and CZ_W for 1h, followed by stimulation with 1 µg/ml of LPS for 18 h. The amount of nitrite released was measured as described previously [21].

For PGE_2 and LTB4 assays, RAW 264.7 cells, SW1353 and human primary chondrocytes were seeded at a density of 5×10^5cells/ml in 96 well plate and allowed to adhere for 24 h. RAW 264.7 cells were pre-treated with CC_M, CC_W, CZ_M and CZ_W as described above. SW1353 and human chondrocytes were starved for 18 h in L-15 media containing 0.25% FBS and 1:1 DMEM/Hams F-12 respectively, prior to treatment with the test samples. The cells were pre-treated with different concentrations (0-100 µg/ml) of CC_M, CC_W, CZ_M and CZ_W followed by stimulation with 10 ng/ml of IL-1β for 18 h. PGE_2 concentration was determined in the cell supernatants by using PGE_2 EIA-Monoclonal based kits (Cayman Co., Ann Arbor, Mich., USA). LTB4 levels were determined in the supernatant by using LTB4 EIA-Monoclonal based kits, (Cayman Co., Ann Arbor, Mich., USA). For evaluating MMP levels, human chondrocytes were starved for 18 h and pre-treated with CC_M, CC_W, CZ_M and CZ_W as described above. MMPs (2, 9, and 13) were quantified in the supernatant by using commercial SensoLyte® 520 Generic MMP Activity Kit (Cysbio Anaspec Eurogentec group, USA).

For statistical analysis, all the experiments were performed in triplicates and the values have been presented as mean±SD. Differences among means were tested for statistical significance using one-way analysis of variance (ANOVA). For multiple comparisons,Tukeys test was used. The analyses were carried out using Graph-pad prism 5 software (San Diego, CA, USA). *$p<0.05$; **$p<0.01$; ***$p<0.001$ were considered to be statistically significant.

Raw264.7, SW1353 and human chondrocytes were treated with different concentrations of extracts (0-100 µg/ml) to test their effect on cell viability. CC_M and CC_W (table 1a); as well as CZ_M and CZ_W (table 1b) were found to be non-toxic to the cells, thereby suggesting them to be safe for use in further studies.

Table 1a: Effect of CC_M and CC_W on cell viability in RAW264.7, SW1353, human primary chondrocytes

	CC_M			**CC_W**		
Concentration of extracts (µg/ml)	**RAW264.7**	**SW1353**	**human primary chondrocytes**	**RAW264.7**	**SW1353**	**primary human chondrocytes**
0.1	101.1±1.4	100.2±0.1	100.4±0.3	101.4±2.3	102.7±3.4	101.8±2.4
1	100.1±0.8	100.1±0.04	100.1±0.1	101.8±2.5	103.6±3.6	104.3±0.9
10	100.2±0.8	100.0±0.2	100.8±0.4	100.7±0.7	105.8±3.1	109.7±3.3
100	100.5±0.5	102.8±2.5	104.0±1.1	100.8±0.8	104.3±1.1	112.0±1.7

Values have been represented as mean±SD of three independent experiments.

Table 1b: Effect of CZ_M and CZ_W on cell viability in RAW264.7, SW1353, human primary chondrocytes

	CZ_M			**CZ_W**		
Concentration of extracts (µg/ml)	**RAW264.7**	**SW1353**	**human primary chondrocytes**	**RAW264.7**	**SW1353**	**primary human chondrocytes**
0.1	100.1±0.1	100.0±0.01	100.4±0.4	102.1±1.4	101.06±1.4	100.05±0.04
1	100.7±0.9	102.1±1.3	102.0±0.5	104.6±0.9	101.04±0.1	101.6±0.6
10	101.8±1.9	101.8±1.9	110.2±2.2	106.8±2.1	105.06±2.0	108.02±0.7
100	105.4±4.3	104.3±0.5	118.6±0.8	109.9±0.7	105.6±0.7	115.5±1.1

Values have been represented as mean±SD of three independent experiments.

Raw264.7 cells were treated with different concentrations of CC_M, CC_W, CZ_M and CZ_W (0-100 µg/ml). A significant dose dependent decrease in nitrite production was observed with both the extracts as compared to LPS stimulated control cells. We found that at 100 µg/ml dose, CC_M exhibited 45.4 % ($p<0.001$) decrease in NO levels compared to CC_W (24.7 %; $p<0.001$) (table 2). At the same dose, CZ_M effectively reduced the NO levels by 65.6 % ($p<0.001$) compared to CZ_W (28.67 %; $p<0.001$) (table 2). The results showed that CC_M and CZ_M effectively reduced NO levels compared to their respective aqueous extracts.

Table 2: Effect of CC_W, CC_M, CZ_W and CZ_M on NO levels in LPS stimulated RAW264.7

Concentration of extracts (µg/ml)	CC_W	CC_M	CZ_W	CZ_M
		% decrease in NO levels		
0.1	5.2±4.5	5.7±4.9	7.4±3.8	14.8±8.4
1	11.5±5.4	9.5±3.8	14.9±9.2	19.7±4.5
10	12.7±9.1	23.1±6.9	18.4±7.1	48.3±7.6
100	24.7±6.1	45.4±8.6[a]	28.7±6.7[c]	65.6±5.7[b]

Values have been represented as mean±SD of three independent experiments. Tukey's multiple comparisons test: [a]p<0.05 compared to CC_W, [b]p<0.01 compared to CZ_W, [b]p<0.05 compared to "a", [c]p>0.05 compared to CC_W

We compared the effect of CC_M, CC_W, CZ_M and CZ_W on PGE_2 levels in RAW264.7, SW1353 and primary human chondrocytes. Since the extracts induced maximum inhibition in the nitrite levels in RAW264.7 cells at 100µg/ml dose, this dose was selected for our further experiments. It was observed that at 100 µg/ml dose, CC_M and CC_W reduced the PGE_2 production by 79.8 % (p<0.001) and 80.1 % (p<0.001), respectively in RAW264.7 cells. At the same dose, CZ_M reduced PGE_2 levels by 95.9 % (p<0.001), compared to CZ_w (11.2 %) (table 3). Both the extracts of CC seemed to be equally effective in reducing PGE_2 levels in RAW264.7 cells. In IL-1β stimulated SW1353 cells, at 100µg/ml dose, CC_M significantly reduced PGE_2 production by 68.8 % (p<0.001) compared to CC_w (22.36 %; p<0.001) whereas CZ_M was found to decrease PGE_2 production by 70.2 % (p<0.001) compared to CZ_w (59.93 %; p<0.001) (table 3). Interestingly, in human primary chondrocytes, the methanolic extracts of cinnamon reduced PGE_2 levels more effectively compared to the aqueous extracts. At 100µg/ml dose, CC_M reduced PGE_2 production by 36.1 % (p<0.01), compared to CC_W (6.7 %) whereas CZ_M decreased the PGE_2 production by 52.3 % (p<0.001), compared to CZ_W (16.2%) (table 3). The data showed that CC_M and CZ_M reduced PGE_2 levels significantly in chondrocytic cell line and primary chondrocytes.

Table 3: Effect of CC_W, CC_M, CZ_W and CZ_M on PGE_2 levels in RAW264.7, SW1353 cells and primary human chondrocytes

	% decrease in PGE_2 levels		
Concentration of extracts (100µg/ml)	RAW264.7	SW1353	primary human chondrocytes
CC_W	80.1±3.8	22.4±20.7	6.7±4.2
CC_M	79.8±1.2[a]	68.8±6.4[d]	36.1±9.5[g]
CZ_W	11.2±11.6[c]	59.9±4.8[f]	16.2±3.7[i]
CZ_M	95.9±0.3[b]	70.2±2.3[e]	52.3±5.4[h]

Values have been represented as mean±SD of three independent experiments. Tukey's multiple comparisons test: [a]p>0.05 compared to CC_W, [b]p<0.001 compared to CZ_W, [b]p<0.001 compared to "a", [c]p<0.001 compared to CC_W, [d]p<0.05 compared to CC_W, [e]p>0.05 compared to CZ_W, [e]p>0.05 compared to "d", [f]p<0.05 compared to CC_W, [g]p<0.05 compared to CC_W, [h]p<0.05 compared to CZ_W, [h]p>0.05 compared to "g", [i]p>0.05 compared to CC_W

CC_M, CC_W, CZ_M and CZ_W were further compared for their potential to modulate IL-1β induced LTB4 production in SW1353 and human chondrocytes. In SW1353, at 100µg/ml dose, CC_M reduced LTB4 levels by 85.5 % (p<0.001) compared to CC_W (61.6 %; p<0.001) (table 4). At the same dose CZ_M reduced LTB4 by 67.5 % (p<0.001) as compared to CZ_W (26.8 %; p<0.001). In human primary chondrocytes, at 100µg/ml dose, both CC_M and CC_W significantly reduced the LTB4 levels by 99.6 % (p<0.001) and 90.27 % (p<0.001), respectively. On the other hand, CZ_M reduced LTB4 levels by 75.6 % (p<0.001) compared to CZ_W (48.8 %; p<0.001) (table 4). Thus, CC_M and CZ_M showed more decrease in LTB4 production compared to the aqueous extracts.

Table 4: Effect of CC_W, CC_M, CZ_W and CZ_M on LTB4 levels in SW1353 cells and primary human chondrocytes

Concentration of extracts (100µg/ml)	% Decrease in LTB4 levels	
	SW1353	primary human chondrocytes
CC_W	61.6±4.6	90.3±0.1
CC_M	85.5±3.0[a]	99.6±0.2[d]
CZ_W	26.8±6.1[c]	48.8±0.9[f]
CZ_M	67.5±5.6[b]	75.6±1.2[e]

Values have been represented as mean±SD of three independent experiments. Tukey's multiple comparisons test: [a]p<0.05 compared to CC_W, [b]p<0.01 compared to CZ_W, [b]p>0.05 compared to "a", [c]p<0.01 compared to CC_W, [d]p<0.01 compared to CC_W, [e]p<0.001 compared to CZ_W, [e]p<0.001 compared to "d", [f]p<0.001 compared to CC_W

We compared the effect of CC_M, CC_W and CZ_M, CZ_W on IL-1β induced MMP levels in primary chondrocytes. Compared to control stimulated cells, at 100µg/ml dose, CC_M reduced MMP 2, 9 and 13 production by 55.7 % (p<0.001), 57.5 % (p<0.001) and 90.1 % (p<0.001), respectively. At the same dose, CC_W reduced MMP 2, 9 and 13 production by 16.1 %, 59.5 % (p<0.001) and 41.5 % (p<0.001), respectively (table 5). Similarly, at 100µg/ml dose, CZ_M significantly decreased MMP 2, 9 and 13 production by 73.1 % (p<0.001), 39 % (p<0.001) and 71.2 % (p<0.001), respectively, whereas CZ_W reduced MMP 2, 9 and 13 production by 15.6 %, 6.4 % and 40.1 % (p<0.01), respectively, compared to the control cells (table 5). Altogether, the data showed that methanolic extracts significantly reduced MMP levels compared to the aqueous extracts, however with few exceptions.

The present study compared the anti-inflammatory activity of aqueous and methanolic extracts of *C. cassia* and *C. zeylanicum* in RAW264.7, SW1353 and human primary chondrocytes. We found that in LPS activated RAW264.7 cells, CC_M, CZ_M attenuated NO release more significantly than CC_W, CZ_W. NO is a signalling molecule implicated in a broad spectrum of pathophysiological processes such as inflammation, apoptosis, regulation of enzyme activity and gene expression [22]. In an earlier study, it had been reported that the water extract of CC could not inhibit LPS-induced NO production in RAW 264.7 cells at 100 µg/ml concentration [25]. Interestingly, we found that at 100 µg/ml dose, CC_W significantly inhibited LPS-induced NO production in RAW 264.7 cells. The difference in these results could be attributed to the method of preparation of the extracts, source variation, time of collection of the material and so on

that may affect the presence of phytoactives in the extract, which contribute to their biological activity. Elevated levels of NO have been reported to play a critical role in the aggravation of chronic inflammatory conditions such as osteoarthritis [22-24]. Therefore, reducing NO production would be an important therapeutic target in the development of anti-inflammatory agents.

Table 5: Effect of CC_W, CC_M, CZ_W and CZ_M on MMP levels in primary human chondrocytes

Concentration of extracts (100µg/ml)	Primary human chondrocytes		
	% decrease in MMP levels		
	MMP-2	MMP-9	MMP-13
CC_W	16.1±17.0	59.5±4.2	41.5±7.8
CC_M	55.7±5.2[a]	57.5±4.7[d]	90.1±2.6[g]
CZ_W	15.6±22.1[c]	6.4±3.2[f]	40.1±5.7[i]
CZ_M	73.1±7.1[b]	74.5±5.2[e]	71.2±12.5[h]

Values have been represented as mean±SD of three independent experiments. Tukey's multiple comparisons test: [a]p>0.05 compared to CC_W, [b]p>0.05 compared to CZ_W, [b]p>0.05 compared to "a", [c]p>0.05 compared to CC_W, [d]p>0.05 compared to CC_W, [e]p<0.001 compared to CZ_W, [e]p>0.05 compared to "d", [f]p<0.001 compared to CC_W, [g]p<0.01 compared to CC_W, [h]p>0.05 compared to CZ_W, [h]p>0.05 compared to "g", [i]p>0.05 compared to CC_W

It was further observed that CC_M and CZ_M effectively decreased PGE_2 production in RAW264.7, SW1353 and human primary chondrocytes compared to the aqueous extracts. However, CZ_M was found to be more effective than CC in reducing PGE_2 production. PGE_2 is an important inflammatory mediator and is produced from arachidonic acid metabolites by the catalysis of COX-2. It is one of the major catabolic mediators involved in cartilage degradation and chondrocyte apoptosis [26]. The water extract of CC was earlier shown to decrease PGE_2 production by almost 34% at 100 µg/ml concentration in RAW 264.7 cells, [25] whereas our study showed almost 80% reduction in PGE_2 production at the same concentration of the extract. Moreover, we have analysed the effect of the extracts on PGE_2 production in SWI353 and primary chondrocytes as well. OA cartilage spontaneously releases more PGE_2 than the normal cartilage [27, 28]. Thus, blocking of PGE_2 production by cinnamon in OA could be a promising strategy in preventing cartilage degradation and chondrocyte apoptosis.

In SW1353, the methanolic extracts of CC and CZ reduced LTB4 levels more effectively than the aqueous extracts. In primary human chondrocytes, CC_W, CC_M induced an enhanced decrease in LTB4 levels that went below the basal values and hence needs careful evaluation. Since LTB4 is involved in a number of important cellular processes in the body [29] and its down regulation below the basal level may lead to severe complications [30-32].

However, CZ_M effectively reduced LTB4 levels than CC_W. Thus, CZ appears to be better option than CC in terms of LTB4 inhibition as it does not reduce LTB4 below the basal values. LTB4 plays a direct role in OA pathogenesis. Its increased synthesis has been found in the synovial tissue and synovial fluid of patients with OA. Thus, reducing LTB4 production in OA could help in modulating the pathophysiological conditions associated with this disease.

CC_M and CZ_M effectively decreased the levels of MMPs 2, 9 and 13 compared to CC_W, CZ_W. In human chondrocytes, MMPs are synthesized and secreted by chondrocytes in response to cytokines. The expression of gelatinases (MMP-2 and MMP-9) is either low or absent in most normal tissues, and markedly elevated during inflammation [33]. MMP-13 is secreted by chondrocytes in response to cytokines (IL-1β), causing digestion of type II collagen in cartilage [34]. It has also been reported to be associated with cartilage hypertrophy and calcification [35]. Thus, modulating the expression of MMPs 2, 9 and 13 by CC_M and CZ_M could prevent continued degradation of articular cartilage.

Compared to the aqueous extracts of cinnamon, the methanolic extracts significantly reduced the production of NO, PGE_2, LTB4 and MMPs. *C. cassia* has been reported to contain high amounts of coumarins, which may cause liver damage [36] whereas *C. zeylanicum* hardly contains any coumarin [36]. On comparing the two species of cinnamon, *C. zeylanicum* appears be a better modifier of the inflammatory cascade in OA related pathology. Thus, CZ_M could be proposed for its use in the modulation of major inflammatory mediators in OA, which would help in the regulation of chondrocyte survival, production of pro-inflammatory cytokines, prostaglandins, leukotrienes and production of ECM degrading enzymes such as MMPs.

In conclusion, these results suggested that compared to CC, CZ exhibited excellent anti-inflammatory activity through suppression of NO, PGE_2, LTB4 and MMP production. Due to the serious side-effects associated with the use of NSAIDs, the focus of drug industries has shifted towards towards evaluation of anti-inflammatory activity of medicinal plants that are rich in phytochemicals. The search for natural products that would regulate the inflammatory cascade associated with OA without affecting chondrocytes survival is of pivotal importance. This work is a small step towards comparing the natural products that would not only be effective in managing OA but would also be safe for chondrocyte health, which in turn would protect the degradation of cartilage.

The authors would like to acknowledge Department of Biotechnology (DBT), Government of India, for funding the project (BT/PR10467/PBD/17/561/2008).

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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