

Full Length Research Paper

Zerumbone significantly improved immunoreactivity in the synovium compared to *Channa striatus* extract in monosodium iodoacetate (MIA)-induced knee osteoarthritis in rat

Al-saffar, F. J.¹, Ganabadi, S.^{1*}, Fakurazi, S.² and Yaakub, H.³

¹Department of Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra, 43400, Serdang, Selangor, Malaysia.

²Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra, 43400, Serdang, Selangor, Malaysia.

³Department of Animal Science, Faculty of Agriculture, Universiti Putra, 43400, Serdang, Selangor, Malaysia.

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The main aim of this study was to compare the immunoreactivity of some osteoarthritis related neuropeptides following oral administration of two natural remedies that is *Channa striatus* extract and zerumbone against monosodium iodoacetate induced knee osteoarthritis changes in the rat's synovial membrane. Assay of PGE₂ and PGF₂α in the serum were performed to evaluate their role during osteoarthritis events and post oral application of the treatment. Forty rats were divided equally into four groups. Rats in the first and second groups were received *channa* extract and zerumbone, respectively. Rats in the third group were treated with celecoxib, whereas the fourth group was treated with normal saline. Evaluation of immunoreactivity of the following neuropeptides: Protein gene product 9.5, calcitonin gene related peptide and neuropeptide Y in the synovial membranes was implemented with the aid of both histopathology and immunohistochemistry approaches. Results revealed lower pathology score in both first and second groups accompanied with markedly improved immunoreactivity in zerumbone treated groups compared to *channa* extract group. Significant different concentrations of PGE₂ but not PGF₂α were detected within studied groups. Both remedies significantly improved the immunoreactivity which appeared more apparent in the group treated with zerumbone. Prostaglandin E₂ has a role in osteoarthritis development and regulation.

Key words: *Channa striatus*, osteoarthritis, neuropeptides, zerumbone, monosodium iodoacetate, rat.

INTRODUCTION

Osteoarthritis (OA) is one of the common disabling chronic joint illness affecting humans and different domestic animals. It is primarily noted in the weight bearing joints that is (knee, hips), usually accompanied with subchondral responses (Bove et al., 2003) and

synovitis which contributes to its pathogenesis through formation of different catabolic and pro-inflammatory mediators such as nitric oxide, PGE₂, pro-inflammatory cytokines and several neuropeptides (NPs) (Sutton et al., 2009).

Neuropeptides are contained in and released from a wide range of nerve fibers, in which they exhibits a unique mode of localization within peripheral and central nervous systems (Brain and Cox, 2006). Mapp et al. (1994) identified protein gene product 9.5 (PGP 9.5)

*Corresponding author. E-mail: shanthi@vet.upm.edu.my. Tel: 006- 0122920056. Fax: 603-89468333.

nerve fibers throughout the depth of the normal rat's knee synovial membranes (SMs) either freely or surrounding vascular tissues, but, they were depleted in the OA induced joints accompanied with lymphocytes and macrophages infiltration in the synovium. Reduction of PGP 9.5, calcitonin gene related peptide (CGRP) and Substance peptide (SP) immunoreactive nerve fibers was identified in the arthritic joints in both experimental animals and human.

The reduction of these fibers may be due to their depletion or necrosis (Buma et al., 2000) or indirectly due to the production of oxygen free radicals during OA events and subsequent necrosis of these nerve fibers (Kontinen et al., 1992).

In domestic animals, the role and relationship of CGRP, SP and PGP 9.5 in OA development and progression had been well identified in sheep, dog, cat and rabbit knee joints (Tahmasebi-Sarvestani et al., 2001; Tamura et al., 1998; Heppelmann et al., 1997; Michelle et al., 2004). Generally, sensory NPs are participating in pain transmission, inflammation and immunological reactions through which stimulate immune cells to release inflammatory cytokines (Ahmed et al., 1998). Sympathetic NPY is often found together with SP and CGRP nerve fibers, and has been shown to increase the production of interleukin-1 β (IL-1 β), IL-6 and tumor necrosis factor- α (TNF- α) which participates in OA development and progression (Hernanz et al., 2003). They play an important role in the severity of inflamed joints in rats induced adjuvant arthritis (Levine et al., 1986).

Osteoarthritis is a major health burden in human and veterinary practice and the most current therapies in use are non steroidal anti-inflammatory drugs (NSAIDs) but they are not 100% safe because some complications may occur in their long term use (Williams, 2007; Bove et al., 2003; Taylor and Robertson, 2004; Fernihough et al., 2004).

Adverse side effects of OA regulations may be ameliorated by the use of botanical or animal extracts alternatives. *Channa striatus* (common name: Haruan) extract and zerumbone are such candidates.

C. striatus is a snakehead fish, widely consumed by the community of South East Asia, as a rich source of protein and for its alleged effect to alleviate injuries after caesarean procedure and relieve post operative pain (Mat Jais et al., 1994). The extract has potent anti-inflammatory and analgesic properties (Somchit et al., 2004; Zakaria et al., 2004; Zuraini et al., 2006) and its formulation with palm-oil improved healing and increased tensile strength of the skin wounded experimentally in rat (Baie and Sheikh, 2000).

Zerumbone is a monocyclic sesquiterpene that can be found abundantly in rhizomes from *Zingiber zerumbet* Smith (Szabolcs et al., 2007). It is a food phytochemical possessing great potential for use in chemoprevention and chemotherapy strategies against cancers and other disorders. So investigations on zerumbone during the last

decade were focused on its anti-oxidant (Ruslay et al., 2007; Murakami et al., 2002) anti-cancer (Abdelwahab et al., 2010; Huang et al., 2005) and anti-inflammatory properties (Chien et al., 2008; Murakami et al., 2003). To our knowledge no study was undertaken to study the effect of orally administered zerumbone on the immunoreactivity in osteoarthritic joints to date. Also for channa extract there is only one preliminary study that was done by one of the current team who studied only PGP 9.5 immunoreactivity in surgically-induced knee osteoarthritis in rabbit. Therefore this study was undertaken to identify and compare the improvement ability of these natural remedies on the immunoreactivity of the following OA related NPs: PGP 9.5; CGRP and NPY. Inflammatory PGs in the serum were assayed to explore their role and relationship during OA development and post oral treatment with both natural remedies.

MATERIALS AND METHODS

Animals and induction of osteoarthritis

Forty, adult male Sprague Dawley rats, weighing between 275 to 400 g, were distributed into four groups each of ten. The rats were housed in well air-conditioned animal room at 22°C (one rat per cage). The rats were given commercial pellet and tap water *ad libitum* and were left for 2 weeks for acclimation before used. The study was conducted in accordance with the ethical guidelines for investigations in laboratory animals and was approved by animal care and use committee (ACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia (Reference: UPM FPV/PS/3.2.1.551/AUP-R44). Under ketamine /xylazine anesthesia, all rats were injected intraarticularly with fifty microlitre of monosodium iodoacetate (MIA) diluted with saline at a concentration of 60 mg/ml (Sigma, USA) in their knee joints at day 0. The injection of MIA was performed once through the patellar ligament using a 27 gauge, 0.5 inch needle (Bove et al., 2003).

Protocol of treatments

Rats in each group were treated orally with one of the following treatments using feeding catheter. The treatment was started on day 16 after OA induction and continued for four weeks. First group (CS): 10 ml/kg body weight of 20% *C. striatus* (CS) extract diluted in normal saline. Second group (ZER): 2 ml/kg body weight of 0.4% w/v of zerumbone (ZER) diluted in corn oil. Third group (CEL): 30 mg/kg celecoxib (Celebrex[®]) diluted in 5% carboxyl methyl cellulose and served as positive control. Fourth group (NS): 10 ml/kg body weight normal saline (NS) and served as negative control.

Preparation of *C. striatus* extract

The extract was prepared from *C. striatus* fish fillet according to the method described by Mat Jais et al. (1997). Briefly fillet free of bones was prepared from fresh fish. The fish was cut into small pieces, placed on a stainless steel wire mesh mounted on a stainless steel tripod in the pressure cooker sets at 100°C for thirty minutes. Fresh distilled water was then added in volume ratios of 1:2. Water level was below the wire mesh so that the fillet was not submerged in the water. Extract was then obtained through steaming. At the end of extraction procedure, the fillet was discarded while the liquid extract was collected, filtered, centrifuged and stored at 4°C until use. This extract was diluted with

physiological saline to the required concentration (20%) and administered orally in a volume of 10 ml/kg.

Preparation of zerumbone

The preparation was implemented according to the method published (Murakami et al., 1999) with the aid of Analytical Laboratory and Quality Assurance Programmed Technical Services Centre, MARDI, P.O. Box 12301. General post office: 50774, Kuala Lumpur, Malaysia. It was isolated from the rhizomes of *Z. zerumbet* Smith. Rhizomes were thoroughly flushed and rinsed in multiple changes of water, then chopped into small pieces minced well and immersed onto a glass beaker filled with n-hexane. The mixture was stirred twice/daily for three consecutive days. The sample extract was transferred onto a rotary evaporator system to be concentrated in vacuum at 40°C until the sample becomes sticky liquid called slurry. The slurry was subjected to a silica gel column chromatography. Several crystallizations and column chromatography of the hexane slurry was carried out to obtain pure ZER. The purity was confirmed with gas chromatography mass spectrometer.

Preparation of synovial membranes

Rats of all groups were euthanized with intraperitoneal injection of 500 mg/kg sodium phenobarbital. Synovial membrane samples were obtained immediately after joint dissection and fixed in Zamboni fluid for 6 h, subsequently washed in 0.1 M phosphate buffer saline (PBS) (pH 7.4) then immersed in 15% sucrose for 2 days at 4°C. Then, they were snap frozen in isopentane cooled with liquid nitrogen and sectioned at 8 µm in a cryostat. The sections were incubated in the primary antisera: anti- PGP 9.5 (Ultraclone Cambridge Ltd. UK), anti-CGRP (Cambridge Research Biochemical, UK) and anti-NPY (Peninsula Laboratories, UK) for 24 h at 4°C. The sections were washed in PBS and then incubated for one hour in the secondary antiserum (Gt XRB IgG Cy3) at room temperature (dilution 1:400). Sections were washed in PBS and then cover-slipped in fluorescence mounting medium (Saxler et al., 2007). Densities of nerve fibers were expressed as nerve fibers/4 mm². For general morphological examination some SMs were fixed in 5% neutral buffered formalin, dehydrated, sectioned and stained with hematoxylin and eosin (H and E) then their OA changes were scored according to the method described by Kikuchi et al. (1998). Evaluation of OA changes had been implemented blindly by two of current team using image analyzer microscope (Olympus, BX 51).

Prostaglandins assay

Blood was collected from all rats to estimate PGs (PGE₂ and PGF_{2α}) concentrations at three different periods that is before OA induction, post 15 days of OA induction and post 4 weeks of treatment. Under anesthesia, 5 ml of blood was collected from each rat through cardiac puncture and left for one hour then centrifuged at 3000 rpm for 10 min. Sera were collected and stored at -20°C until further use. Enzyme immunoassay kits for PGE₂ and PGF_{2α} detection (Assay Design purchased from USA, Catalog # 900-001 and Catalog # 900-069, respectively) were used to perform hormone assays.

Statistical analysis

Statistical calculations were carried out with the SPSS 15.0 for Windows software package. Data was expressed as mean ± SEM and analyzed with Kruskal-Wallis (for non parametric data: histopathological score) confirmed with Mann-Whitney *U* test and

one way ANOVA (for parametric data: NPs densities and PGs concentrations in serum) confirmed with student *t*-test.

RESULTS

Histological examination of synovial membranes

Examination of the normal (left) knee SMs revealed small round or oval synoviocytes arranged in 1-2 cell thickness forming the superficial intimal layer which separates the joint cavity from the deeper subintimal layer. The later formed of wide fibro-fatty stroma, perfuse with blood vessels (Figure 1A).

Changes occurred at the SMs of the right OA induced knee joints have been well described according to their groups. Synovial membranes from NS group showed apparent hyperplasia and for a lesser extent hypertrophy of the synovial lining. Marked hypergranulation and hypervascularization within subintimal tissue stroma accompanied with severe infiltration of macrophage and lymphocytes (Figures 1B and C).

In CEL group, similar findings to the above group were observed such as hyperplasia of the synovial lining with sign of hypertrophy in some joints. Hypergranulation and hypervascularization was detected in the underlying stroma with signs of moderate inflammatory cells infiltration (Figures 1D and E).

Microscopic examination of the SMs belongs to CS group revealed moderate hyperplasia of the synovial lining with mild hypertrophy of some specimens. Underlying subintimal layer underwent mild to moderate hypergranulation and hypervascularization. Few members of this group showed mild inflammatory cell infiltration (Figures 1F and G).

Synovial membranes of ZER group exposed minor histological changes. It showed mild hyperplasia in some rat's synovial lining. Underlying subintimal layer revealed mild proliferation of collagen fibers in its deeper part with subtle lack of inflammatory cells infiltration (Figure 1H and I). Statistical analysis to the above data was well summarized in Table 1.

Immunohistochemical results

Normal left joint's SMs revealed plenty number of immunoreactive nerve fibers running singly in an undulating course or rarely in a straight line through its structures, but some nerve fibers runs as bundles forming varicose. The general neuronal marker PGP 9.5 nerve fibers existed more profuse than the other types, that is sensory CGRP and sympathetic NPY. Generally, these nerve fibers were often distributed through subintimal tissue and very sparsely at the intimal lining epithelium. Densities of these three different types of nerve fibers present in all treated groups (NS, CEL, CS and ZER) were summarized and compared with those non induced

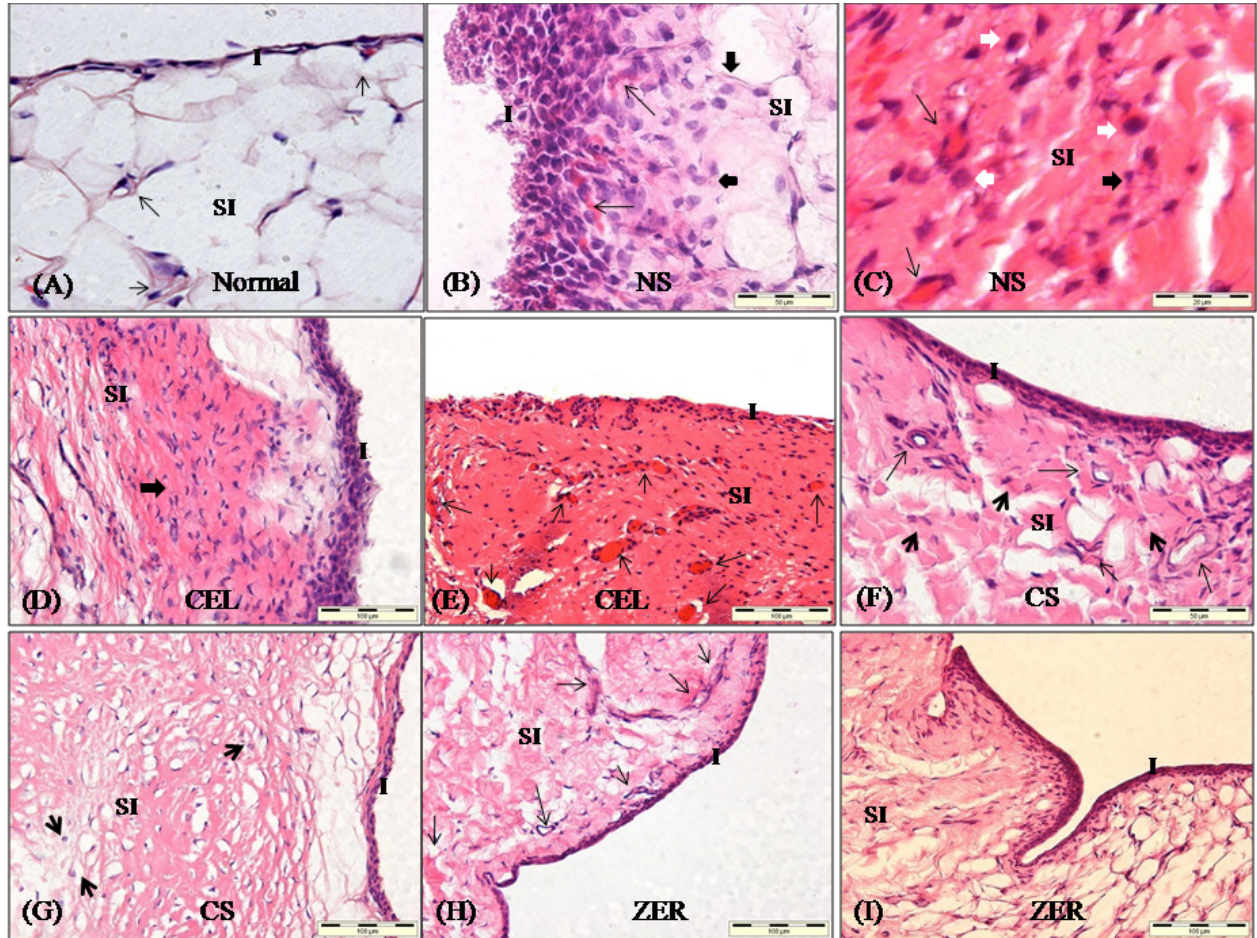


Figure 1. (A): Normal synovial membranes showed 1-2 cells thick intimal layer (I), fibro-fatty subintimal structures (SI) with considerable number of blood vessels (arrows), H and E, x400. (B, C): Synovial membranes represent NS group showed distinct hyperplasia of the intimal layer (I) and hypergranulation of the subintimal collagen fibers (SI), increased number of blood vessels (thin arrows) with infiltration of macrophage (white thick arrow) and lymphocytes (black thick arrows), H and E, x400 and x1000, respectively. (D, E): Synovial membranes of CEL group showed hyperplasia of the synovial lining of the intimal layer (I). Hypergranulation (thick arrows) of subintimal layer (SI) and an increased number of the blood vessels (thin arrows) accompanied with signs of inflammatory cells infiltration, H and E, x200 and x200. (F, G): Synovial membranes represent CS group revealed mild hyperplasia of the intimal layer (I), moderate hypergranulation and hypervascularization (thin arrows) of the subintimal tissue (SI) accompanied with mild infiltrations of inflammatory cells (thick arrows), H and E, x400 and x200, respectively. (H, I): Synovial membranes represent ZER group showed mild hyperplasia of intimal layer (I) and mild proliferation of the subintimal structures (SI), mild increased in number of blood vessels (arrows) with subtle lack of inflammatory cells infiltration, H and E, x200 and x200.

left normal one (Table 2). Numerous PGP 9.5 nerve fibers were found at the left normal SMs (Figure 2A) whereas, they were typically depleted at those rats treated with normal saline (Figure 2D). In the same way, CEL group showed poor density of such immunoreactive nerve fiber at their SMs, indicating their low outcome against OA changes (Figure 2G), while the densities were improved significantly at both CS and ZER groups in comparison with those of NS and CEL groups (Figures 3A and D).

Normally, CGRP as well as NPY immunoreactive nerve fibers were distributed throughout the subintimal layer

as free nerve fibers, mostly surrounding the blood vessels and some of them set in bundles forming varicose (Figures 2B and C). Depletion of these nerve fibers was obviously observed at both NS and CEL groups (Figures

2E, F, H and I), while, they were improved significantly in those of ZER group and for a lesser extent in CS group (Figures 2B, C, E and F). Analysis of IHC data with ANOVA revealed an improvement of the above NPs in both CS extract and ZER treated groups in comparison with NS group ($p < 0.05$ and $p < 0.001$, respectively), but they showed lower densities ($p < 0.01$) than those

Table 1. Microscopic evaluation score of the right rat's knees synovial membranes of the treated groups with normal saline (NS), celecoxib (CEL), CS extract (CS) and zerumbone (ZER).

Observations	Score			Rat's groups	
	Grades	NS	CEL **	CS *	ZER *†
Hyperplasia of intimal layer	0	0/10	0/10	0/10	7/10
	+	0/10	0/10	2/10	3/10
	++	6/10	8/10	8/10	0/10
	+++	4/10	2/10	2/10	0/10
Average pathology score		2.4	2.2	1.8	0.3
Hypertrophy of intimal layer	0	0/10	0/10	0/10	10/10
	+	5/10	5/10	3/10	0/10
	++	5/10	5/10	0/10	0/10
	+++	0/10	0/10	0/10	0/10
Average pathology score		1.5	1.5	0.3	0
Inflammatory cells infiltration in intimal layer	0	0/10	0/10	0/10	10/10
	+	0/10	0/10	2/10	0/10
	++	6/10	8/10	0/10	0/10
	+++	4/10	2/10	0/10	0/10
Average pathology score		2.4	2.2	0.2	0
Hypergranulation of subintimal layer	0	0/10	0/10	0/10	5/10
	+	0/10	0/10	2/10	5/10
	++	6/10	6/10	8/10	0/10
	+++	4/10	4/10	0/10	0/10
Average pathology score		2.4	2.4	1.8	0.5
Hypervascularization of subintimal layer	0	0/10	0/10	0/10	5/10
	+	0/10	0/10	2/10	5/10
	++	6/10	6/10	8/10	0/10
	+++	4/10	4/10	0/10	0/10
Average pathology score		2.4	2.4	1.8	0.5
Inflammatory cells infiltration in subintimal layer	0	0/10	0/10	0/10	10/10
	+	0/10	0/10	3/10	0/10
	++	5/10	5/10	0/10	0/10
	+++	5/10	5/10	0/10	0/10
Average pathology score		2.5	2.5	0.3	0
Total average pathology score ± SEM		13.6±.15	13.2±.14	6.2±0.34	1.3±0.10

Data were analyzed using Kruskal-Wallis confirmed with Mann-Whitney *U* test. All values were expressed as the mean ± SEM (n=10). *Significant (p < 0.001) lower score versus NS group, ** Non significant (p > 0.05) lower score versus NS group, † Significant (p < 0.01) lower score versus CS group.

recognized at the normal left one (Table 2).

Results of prostaglandins assay

Results of hormone assay revealed different concentrations of PGs at different periods and groups (Table 3).

The concentration of PGE₂ was elevated significantly (p < 0.01) at 15 days post OA induction. Following treatment with 0.4% ZER the level of PGE₂ was significantly (p < 0.001) reduced. Significantly (p < 0.001), the reduction of PGE₂ was also observed in the group that was treated with 20% CS extract. The positive control group (CEL) also showed significant (p < 0.001) reduction of PGE₂ but

Table 2. Density means of PGP 9.5, CGRP and NPY immunoreactive nerve fibers detected at the synovial membranes of the right knees for the treated groups [normal saline (NS), Celecoxib (CEL), CS extract (CS), zerumbone (ZER)] and left normal non induced joints.

Type of immunoreactive nerve fibers	Rat's Groups (n=10)				
	Normal* left joints	NS	CEL**	CS†	ZER ‡ #
PGP 9.5 nerve fiber	53.3±2.36	4.7±0.55	5.2±0.59	14.9±0.76	27.4±1.49
CGRP nerve fibers	30.9±0.87	2.7±0.21	2.9±0.25	8.7±0.49	18.4±0.54
NPY nerve fibers	13.5±0.58	1.4±0.16	1.6±0.26	4.3±0.20	7.7±0.39

Density means of the nerve fibers were measured per each 4mm² of the synovial membrane per each rat per each group (n=10). * Significantly ($P < 0.001$) higher nerve fibers density than in the other groups. **Insignificantly ($P > 0.05$) higher nerve fibers density than in the NS group. † Significantly ($P < 0.05$) higher nerve fibers density than in the NS group. ‡ Significantly ($P < 0.001$) higher nerve fibers density than in the NS group. # Significantly ($P < 0.01$) higher nerve fibers density than in the CS group

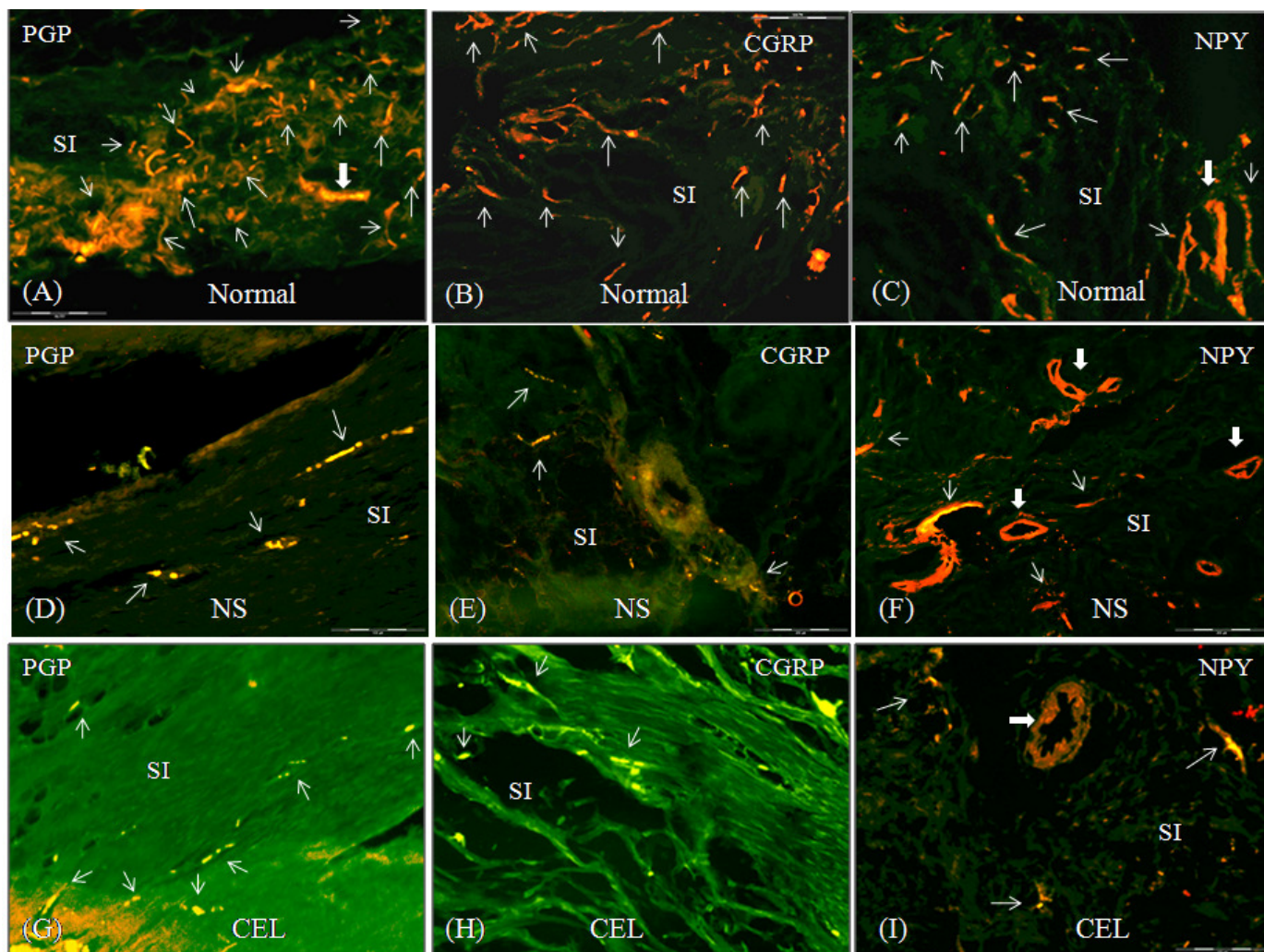


Figure 2. (A-C): Cryosections of synovial membranes showed normal distribution of PGP 9.5, CGRP and NPY immunoreactive nerve fibers in the subintimal structure (SI) (thin arrows), some of them expanded forming varicose (thick arrow), Cy3, x200, x100 and x100. (D-F): Synovial membranes of corn oil treated group showed severe depleted PGP 9.5, CGRP and NPY immunoreactive nerve fibers in the subintimal layer (SI) (thin arrows), with many blood vessels (thick arrows), Cy3, x200, x100 and x100. (G-I): Synovial membranes of Celecoxib treated group exposed depletion of PGP 9.5, CGRP and NPY immunoreactive nerve fibers in the subintimal layer (SI) (arrows), Cy3, x100, x200 and x200.

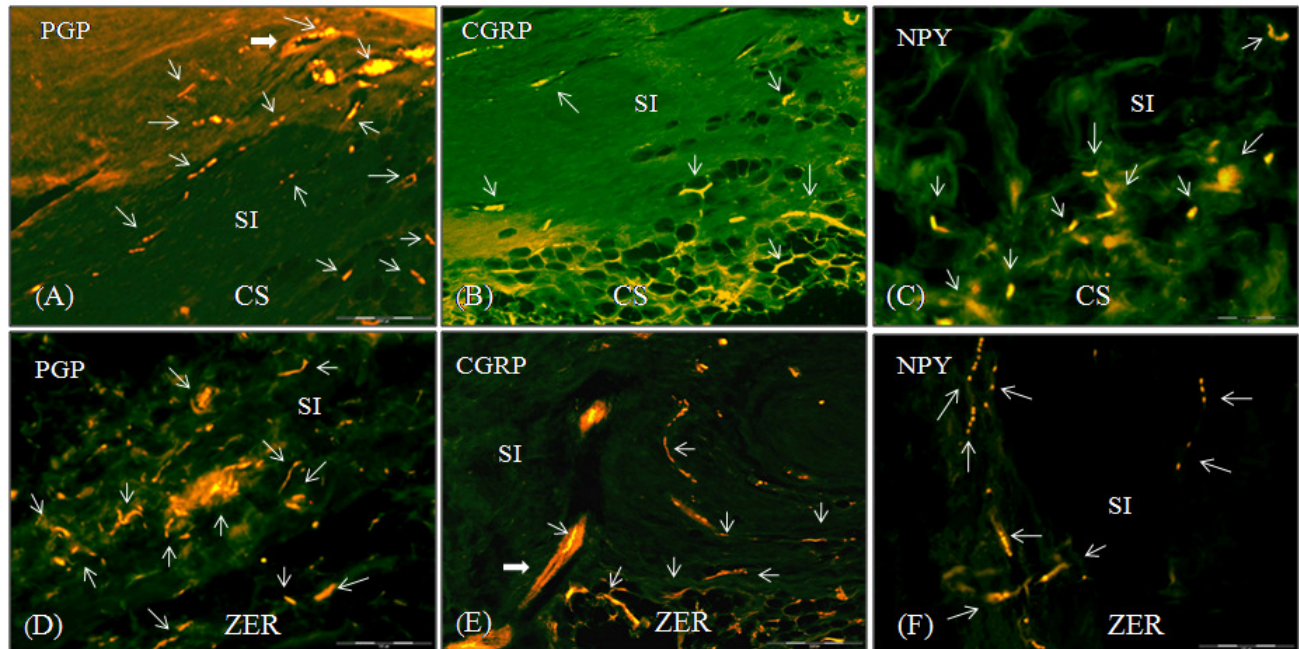


Figure 3. (A-C): Cryosections represent synovial membranes of the rat's right knees of CS group. It showed PGP 9.5, CGRP and NPY immunoreactive nerve fibers distributed all over the subintimal layer (SI) (thin arrows), some of them present nearby the blood vessels (thick arrows), Cy3, x100, x100 and x400. (D-F): Cryosections of synovial membranes from ZER group showed improved immunoreactivity of PGP 9.5, CGRP and NPY nerve fibers present in the subintimal layer (SI) (thin arrows), some of them present nearby the blood vessels (thick arrows), CY3, x200, x100 and x200.

Table 3. Concentrations of prostaglandins (PGs) in serum of rats at three different periods and different treated groups.

Periods	PGE ₂ (pg/ml)	PGF _{2α} (pg/ml)
First: Before OA induction	17359.06 ±2122.7	26300.38±350.21
Second: 15 days post OA induction	29036.96±2707.7*	29753.35±535.57*
Third: 28 days post treatment:-		
(1) NS (negative control) group	34012.02±2451.6	29789.11± 346.46
(2) CEL (positive control) group	22845.05±750.4**	27841.36±581.2***
(3) CS (CS extract treated) group	17823.21±468.5**	28524.16±467.76***
(4) ZER (zerumbone treated) group	19735.13± 966.19**	29543.86±646.27***

Serum concentrations of hormones were measured per each rat per each group. Data were analyzed using one-way ANOVA followed by student's *t*-test. All values were expressed as the mean ± SEM (n=10). *Significant ($p < 0.001$) elevation of PGE₂ and PGF_{2α} levels versus those before OA induction. **Significant ($p < 0.001$) reduction of PGE₂ level versus NS group. *** Non significant ($p > 0.05$) reduction of PGF_{2α} level versus NS group.

to a lesser extent compared to the treated groups. In the negative control group (NS) which was administrated with normal saline, the PGE₂ level was elevated significantly ($p < 0.001$). The basal level of PGF_{2α} was found to be higher than PGE₂. Following OA induction for 15 days the level was found to be elevated compared to the basal level. On day 16, the groups were treated with CS extract and ZER, the level of PGF_{2α} was found more or less similar to the level before the animals being treated. The changes seen in the level of PGF_{2α} was found to be insignificant.

DISCUSSION

The main aim of current investigation was to evaluate the effects of two important common natural remedies in local traditional medicine that is, CS extract and ZER against one of the common chronic disease in human live and different domestic animals. For such purpose we conducted an experimental induced knee OA in adult Sprague Dawley rats with intra-articular injection of MIA. The election of this metabolic inhibitory agent was based on its rapid onset of OA changes (Al-Saffar et al., 2009;

Guzman et al., 2003) due to its ability to inhibit glyceraldehyde-3-phosphate dehydrogenase activity of the articular cartilage chondrocytes, and then their eventual death (Cournil et al., 2001). Loss or reduction of chondrocytes which are the only cell type able to control vital activities of the articular cartilage will contribute and enhances matrix degradation (Lee et al., 2005).

The fragmentation of the degraded cartilage into the synovial fluid will triggers inflammatory cells and subsequently causes synovitis. Inflamed synoviocytes can produce some pro-inflammatory cytokines such as IL-1 and TNF- α (Johnston, 1997). These cytokines can stimulate again synoviocytes and inflammatory leukocytes to produce PGs and generate oxygen free radicals. Excessive production of free radical species may enhance NPs excretion leading to their exhaustion and subsequent depletion (Sutton et al., 2009).

Severe inflammatory reactions were observed in the SMs of the rats in the negative control group accompanied with significant elevation of PGE₂ in sera. Such results indicated two important processes in the OA development at this group: It explores MIA action on joints, manifested by higher histopathology score of their SMs and lower nerve fiber densities of the investigated NPs. Effect of MIA in the this group is in consistent with previous findings (Al-Saffar et al., 2009; Guzman et al., 2003). Depletion of immunoreactive nerve fibers in this group was in good agreement with previous findings too (Michelle et al., 2004; Mapp et al., 1994; Hukkanen et al., 1992). Also elevated PGE₂ concentrations in the rat's sera of this group indicate the action of this pro-inflammatory mediator in occurrence and progression of OA in the affected joints. These findings are corresponding to those of Inoue et al. (2001) who postulated that inflamed synoviocytes can produce PGE₂ and free radical into the joint and participate in the pathogenesis of chronic inflammatory conditions. Similarly, celecoxib treated group showed low outcome effect in their SMs which may be due to the poor capacity of celecoxib to scavenge reactive species and exposed lack antioxidant activity (Bastos-Pereira et al., 2010). Low densities of the NPs and severe pathological reactions at the SMs of this group were parallel to previous findings of other NSAIDs effects against OA joints (Choi et al., 2002).

Rats treated with either CS extract or ZER showed significantly lower scores of pathological reactions compared to those of negative control group. The score was noticeably lower at ZER group than in CS group ($p < 0.01$). These findings were accompanied with significant inhibition of concentrations of the pro-inflammatory hormone (PGE₂) in the sera of both groups and such anti-inflammatory action of these remedies is steady with previous findings (Chien et al., 2008; Somchit et al., 2004). Histological findings revealed subtle lack of inflammatory cells infiltration in the SMs of ZER group whereas it revealed mild inflammatory cells infiltration in

case of CS group. These different effects may be due to the facts that CS extract possess anti-inflammatory property only (Somchit et al., 2004) whereas ZER have antioxidant, anti-inflammatory properties (Ruslay et al., 2007; Chien et al., 2008). Zerumbone possess dual actions. It can suppress the production of free radicals at the affected joints thus inhibit further OA changes or progression (Murakami et al., 2002). This property of ZER is due to its ability to suppress the expression of inducible nitric oxide synthase (Murakami et al., 2003). In addition to that ZER is able to suppress cyclooxygenase-2 (COX-2) expression (Tanaka et al., 2001). This enzyme is very important in producing pro-inflammatory prostaglandins, thus inhibit inflammatory cells infiltration and subsequent further inflammatory reactions in the affected joints. Immunohistochemical results were interesting. It revealed increased nerve fibers densities of the NPs in the groups treated with either CS extract or ZER, with marked improved immunoreactivity in the later compared to negative control group. However, nerve fibers densities remain lower significantly than those detected before OA induction.

Current findings revealed significant changes of PGE₂ levels in serum in different treated groups before and following OA induction. Its level raised significantly post 15 days of OA induction. Elevation of this pro-inflammatory mediator is due to synovitis and subsequent stimulation of inflammatory cytokines (TNF- α , IL-1 β) which in turn lead to their excessive production (Sutton et al., 2009). Levels of PGE₂ reduced significantly post treatment with celecoxib, CS extract or ZER but not in the negative control group. Reduction of this inflammatory hormone post 4 weeks of treatment may be due to the inhibition cyclo-oxygenase expression which is an essential enzyme in catalyzing arachidonic acid and subsequent formation of this prostaglandin (Brodie et al., 1980). Levels of PGF₂ α concentrations in the serum have not changed following the treatment period. Therefore PGF₂ α have poor response during OA regulation with these treatments against MIA-induced OA.

Conclusions

The current findings exposed the role of the two administered natural remedies in lowering some important events occurred at the exaggerated joints. They revealed curative effect within the dosage regimen and course of treatment designed. We expected that curative effect of CS extract was due its anti-inflammatory property whereas the effect of ZER due to its antioxidant as well as anti-inflammatory activities manifested with higher improvement of the NPs densities at the group treated with it. Prostaglandin E₂ but not PGF₂ α play a role in OA progress and subsequent regulation and treatment. Prostaglandin E₂ concentration at different periods gave a useful interpret to those different events occurred at the

SMs. This hormone ought to be put in consideration in the future research concerned OA and methods of its management and regulations.

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Abbreviations: **OA**, osteoarthritis; **MIA**, monosodium iodoacetate; **NP**, neuropeptide; **PGP 9.5**, protein gene product 9.5; **CGRP**, calcitonin gene related peptide; **SP**, substance P; **CS**, *Channa striatus*; **ZER**, zerumbone; **CEL**, celecoxib; **NSAID**, Non-steroidal anti-inflammatory drug; **TNF- α** , tumor necrosis factor-alpha; **IL-1 β** , interleukine-1 beta; **Ns**, normal saline; **Gt XRb IgG Cy3**, goat anti-rabbit immunoglobulin G Cyanine 3; **SM**, synovial membranes; **FITC**, fluorescein isothiocyanate; **COX**, cyclooxygenase; **IHC**, Immunohistochemical.

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