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*Full Length Research Paper*

# Zerumbone improved immunoreactivity of neuropeptides in monosodium iodoacetate induced knee osteoarthritis in rat

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**The main objective of this investigation was to explore the improvement effect of oral administration of zerumbone on the density of protein gene product; calcitonin gene related peptide and neuropeptide Y immunoreactive nerve fibers against monosodium iodoacetate induced osteoarthritis changes in rat's knee synovial membrane. Prostaglandin (PG) $E_2$ and $F_2\alpha$ were determined to assess their role during osteoarthritis events and post zerumbone application. Forty rats were divided equally into four groups. Rats in the first and second groups were treated with two different concentrations of zerumbone. Rats in the third group received celecoxib (Celebrex®) and served as positive control; whereas those of the fourth group were given corn oil and served as the negative control. The results revealed lower pathology score beside an improvement of the immunoreactive nerve fibers densities in zerumbone treated groups compared with the negative control. Different prostaglandin levels were detected within the different treated groups. The data showed that, zerumbone had dose dependent plausible improvement effect against the depleted immunoreactive nerve fibers which occurred after monosodium iodoacetate injection. The prostaglandin $E_2$ but not $PGF_2\alpha$ showed distinct role during the osteoarthritis events and the post oral treatment with zerumbone.**

**Key words:** Osteoarthritis, neuropeptides, monosodium iodoacetate (MIA), zerumbone, rat.

## INTRODUCTION

Osteoarthritis (OA) is a major health burden in human because 10% of the world's population over 60 years of age suffers osteoarthritis pain (McDougall, 2006). In veterinary field, the disease caused health problems in different animal species such as horses, dogs and cats (Jouglin et al., 2000; Macphail, 2000; Taylor and Robertson, 2004). It is a chronic disease which affects articular cartilages and has been shown to cause damage to other structures such as subchondral bones, joint capsule and synovium. Synovitis play an important role in the pathogenesis of OA (Fiorito et al., 2005; Pearle et al., 2007) through formation of different catabolic and pro-inflammatory mediators such as nitric oxide, $PGE_2$, pro-inflammatory cytokines and several neuropeptides (Sutton et al., 2009).

Neuropeptides (NPs) are compounds of two or more amino acids linked together by peptide bonds. They are derived from large precursor's proteins and are present only in cell body and large dendrites of the neuron and their release performed at the axon terminals (Vander et al., 2001). They are released from a wide range of nerve fibers, in which they expose an attribute mode of localization within peripheral and central nervous system. It was thought that their release into the joint will inhibit the activation threshold of nociceptive nerve endings; thus

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enhance a pain response (McDougall et al., 2006).

Neuropeptides such as substance P (SP), calcitonin gene related peptide (CGRP), protein gene product (PGP) 9.5 and NPY were well investigated in inflamed joints in human and different animal species and their role and relationship were stated in this degenerative joint disease (Saxler et al., 2007; Tamura et al., 1998; Heppelmann et al., 1997).

In experimental animals, PGP 9.5 immunoreactive nerve fibers were identified mainly throughout the depth of the subintimal tissue either freely or around blood vessels and some of them extend to the intimal layer, but they were reduced at the OA joints due to their depletion. The depletion of both SP and CGRP was also detected in OA associated with chronic synovitis accompanied with lymphocytes and macrophages response in rabbit and rat synovial membranes (Michelle et al., 2004; Mapp et al., 1994).

Sympathetic NPY is often found together with SP and CGRP nerve fibers. Its action; to increase the production of interleukin (IL) -1β, IL-6 and tumor necrosis factor-alpha (TNF-α) was well identified. In another aspect, these cytokines are known to participate in OA development and progression which play an important role in the severity of inflamed joints (Hernanz et al., 2003; Medina et al., 2000).

Natural products from many medicinal plants that exert therapeutic effects against many diseases have been employed to cure and alleviate diseases such as OA. The premise is to attenuate inflammatory changes established in the structures of the joint and to improve immune-reactivity of implicated nerve fibers in their synovial membranes. Zerumbone, which is one of such candidates, has been studied extensively during the last decade due to its anti-inflammatory, anti-oxidant and anti-cancer properties (Chien et al., 2008; Ruslay et al., 2007). Recently, the improved effect of zerumbone on experimentally induced knee osteoarthritis was well stated by researchers (Al-Saffar et al., 2010)

The main objective of this investigation was to evaluate the improvement effect of zerumbone on the densities of PGP 9.5, CGRP and NPY immunoreactive nerve fibers in the synovial membranes (SMs) in monosodium iodoacetate (MIA) induced knee osteoarthritis in rats and to explore the PGs role during OA events and post oral treatment with zerumbone.

## MATERIALS AND METHODS

### Animals and induction of osteoarthritis

Forty adult male Sprague Dawley rats that weighed between 275 and 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. This investigation was implemented according to the guideline for animal handling and care regulations approved by Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia. Under ketamine/xylazine anesthesia, all rats were injected intraarticularly with 50 µl of 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).

### Preparation of zerumbone

The preparation of zerumbone was carried out according to the method of Murakami et al. (1999). The preparation and qualification was implemented at the Analytical Laboratory and Quality Assurance Programmed Technical Services Centre, MARDI, Malaysia. About 1.3 g zerumbone was obtained from each 1 kg fresh rhizomes. Fresh rhizomes of *Zingiber Zerumbet* Smith, purchased from local traditional herb suppliers, were thoroughly flushed and rinsed in multiple changes of water. The rhizomes were chopped into small pieces, minced well and then immersed onto a glass beaker filled with n-hexane. The mixture was stirred twice daily for three consecutive days. The rhizomes were extracted with n-hexane three times. The sample extract was transferred onto a rotary evaporator system to be concentrated in vacuum at 40°C until the sample became 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 zerumbone. The purity was confirmed with gas chromatography mass spectrometer.

### Protocol of treatments

Rats in each group received orally one of the suggested therapies using feeding catheter. The oral treatment started at day 16 after OA induction and lasted four weeks as follow: First group (ZI): 2 ml/kg body weight of 0.2% w/v of zerumbone diluted in corn oil; second group (ZII): 2 ml/kg body weight of 0.4% w/v of zerumbone diluted in corn oil; third group (CEL): celecoxib at a dosage of 30 mg/kg body weight diluted in 5% carboxyl methyl cellulose and served as positive control; fourth group (CO): corn oil at a dosage of 2 ml/kg body weight and served as the negative control.

### Preparation of synovial membranes

Immunohistochemistry (IHC) technique was processed to determine the densities of the NPs at the SMs. Synovial membrane samples were obtained immediately after joint dissection and were 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 isopentene, 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. 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 were cover-slipped in fluorescence mounting medium (Saxler et al., 2007). The densities of nerve fibers were expressed as nerve fibers/4 $mm^2$. For the 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 was implemented blindly by two of our team using image analyzer microscope (Olympus, BX 51).

**Table 1.** Histopathology score of synovial membranes of the rat's right knees of the treated groups with 0.2% zerumbone (ZI), 0.4% zerumbone (ZII), celecoxib (CEL) and corn oil (CO).

| Observations | Rat's groups (N=10) | | | | |
|---|---|---|---|---|---|
| | Score Grades | ZI * | ZII *¶ | CEL * | CO |
| Hyperplasia of intima layer | 0 | 5/10 | 7/10 | 0/10 | 0/10 |
| | + | 5/10 | 3/10 | 0/10 | 0/10 |
| | ++ | 0/10 | 0/10 | 8/10 | 6/10 |
| | +++ | 0/10 | 0/10 | 2/10 | 4/10 |
| Average pathology score | | 0.5 | 0.3 | 2.2 | 2.4 |
| Hypertrophy of intima layer | 0 | 9/10 | 10/10 | 0/10 | 0/10 |
| | + | 1/10 | 0/10 | 5/10 | 5/10 |
| | ++ | 0/10 | 0/10 | 5/10 | 5/10 |
| | +++ | 0/10 | 0/10 | 0/10 | 0/10 |
| Average pathology score | | 0.1 | 0 | 1.5 | 1.5 |
| Inflammatory cells infiltration in intima layer | 0 | 5/10 | 10/10 | 0/10 | 0/10 |
| | + | 5/10 | 0/10 | 0/10 | 0/10 |
| | ++ | 0/10 | 0/10 | 8/10 | 6/10 |
| | +++ | 0/10 | 0/10 | 2/10 | 4/10 |
| Average pathology score | | 0.5 | 0 | 2.2 | 2.4 |
| Hypergranulation of subintimal layer | 0 | 0/10 | 5/10 | 0/10 | 0/10 |
| | + | 6/10 | 5/10 | 0/10 | 0/10 |
| | ++ | 4/10 | 0/10 | 6/10 | 6/10 |
| | +++ | 0/10 | 0/10 | 4/10 | 4/10 |
| Average pathology score | | 1.4 | 0.5 | 2.4 | 2.4 |
| Hypervascularization of subintimal layer | 0 | 0/10 | 5/10 | 0/10 | 0/10 |
| | + | 6/10 | 5/10 | 0/10 | 0/10 |
| | ++ | 4/10 | 0/10 | 6/10 | 6/10 |
| | +++ | 0/10 | 0/10 | 4/10 | 4/10 |
| Average pathology score | | 1.4 | 0.5 | 2.4 | 2.4 |
| Inflammatory cells infiltration in subintimal layer | 0 | 4/10 | 10/10 | 0/10 | 0/10 |
| | + | 6/10 | 0/10 | 0/10 | 0/10 |
| | ++ | 0/10 | 0/10 | 5/10 | 5/10 |
| | +++ | 0/10 | 0/10 | 5/10 | 5/10 |
| Average pathology score | | 0.6 | 0 | 2.5 | 2.5 |
| Total average pathology score ± SEM | | 4.5±0.21 | 1.3±0.1 | 13.2±0.14 | 13.6±0.15 |

Data were analyzed using Kruskal-Wallis confirmed with Mann-Whitney *U* test. *Significantly ($P < 0.01$) lower score versus CO group; **Insignificantly ($P > 0.05$) lower score versus CO group; ¶significantly ($P < 0.05$) lower score versus ZI group.

**Assay of prostaglandins**

Blood was collected from all the rats to estimate the PGs ($PGE_2$ and $PGF_2\alpha$) concentrations at the three different periods, that is, before OA induction, end of OA induction and end of treatment periods. Under anesthesia, 5 ml of blood was collected from each rat via cardiac puncture and left for one hour was then centrifuged at 3000 rpm for 10 min. Sera were collected and stored at -20°C until further use. Enzyme immunoassay kits for $PGE_2$ and $PGF_2\alpha$ detection (Assay Design purchased from USA, Catalog # 900-001 and Catalog # 900-069, respectively) were used to perform the 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 was analyzed with Kruskal-Wallis for histopathological score, confirmed with Mann-Whitney U test and one way ANOVA for neuropeptides densities and hormone concentrations in serum were confirmed with the student's test (Tables1 to 3).

## RESULTS

### Histological examination of synovial membranes

Light microscopic findings of the normal (left) knee SMs revealed small round or oval synoviocytes arranged in 1 to 2 cell thicknesses constituting the superficial intimal layer. These synovial cells formed the boundary between

**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 [0.2% zerumbone (ZI), 0.4% zerumbone (ZII), celecoxib (CEL) and corn oil (CO)] and left normal non induced joints.

| Density mean of Immunoreactive nerve fibers ± SEM/ group | Rat's groups | | | | |
|---|---|---|---|---|---|
| | Normal left† | CO right | CEL right** | ZI right* | ZII right *¶ |
| PGP 9.5 nerve fibers | 53.3±2.4 | 4.7±0.55 | 5.2±0.6 | 17.1±0.6 | 27.4±1.5 |
| CGRP nerve fibers | 30.9±0.9 | 2.7±0.21 | 2.9±0.25 | 9.7±0.44 | 18.4±0.5 |
| NPY nerve fibers | 13.5±0.6 | 1.4±0.16 | 1.6±0.26 | 5.1±0.31 | 7.7±0.39 |

Nerve fibers density means ± SEM per each group (n=10). The density measured per each 4mm$^2$ of each synovial membrane. *Significantly ($P < 0.01$) higher nerve fibers densities versus CO group; **insignificantly ($P > 0.05$) higher nerve fibers densities versus CO group; ¶ significantly ($P < 0.05$) higher nerve fibers densities versus ZI group; † significantly ($P < 0.01$) higher nerve fibers densities versus other groups.

**Table 3.** Concentrations of prostaglandins (PGs) in serum of rats at three different periods and different treated groups: CO (corn oil), CEL (celecoxib), ZI (0.2% zerumbone) and ZII (0.4% zerumbone).

| Periods | PGE2 (pg/m) | $PGF_{2\alpha}$ (pg/ml) |
|---|---|---|
| First: Before OA induction | 17359.06 ±2122.9 | 26300.38±350.2 |
| Second: 15 days post OA induction | 29036.96± 2707.7* | 29753.35±535.6 * |
| Third: 28 days post treatment:- | | |
| CO (negative control) group | 34139.76±2403.6 | 29804.11±336.6 |
| CEL (positive control) group | 22845.05±750.4** | 27841.36±581.2† |
| ZI group | 23317.53±712.8** | 29611.87± 455.5† |
| ZII group | 19735.13±966.9** | 29543.86±646.27† |

Means of serum concentrations ± SEM of hormones were measured per each group (N=10). * Significant ($P < 0.001$) elevation of $PGE_2$ and $PGF_2\alpha$ levels versus before OA induction; ** significant ($P < 0.001$) reduction of $PGE_2$ level versus CO group; † insignificant ($P > 0.05$) reduction of $PGF_2\alpha$ level versus CO group.

the joint cavity and the deeper subintimal layer. The later is formed wide fibro-fatty stroma, with considerable number of blood vessels (Figure 1A).

Synovial membranes of the right osteoarthritis (OA) induced knee joints were well described and scored according to their groups (Table 1). Sections from the CO group showed apparent hyperplasia and to a lesser extent hypertrophy of the intima when compared with the left normal knee. Severe hypergranulation and hypervascularization were observed at all parts of the subintimal tissue with marked macrophage and lymphocytes infiltration (Figure 1B, C). Similarly, SMs from the right knees of the CEL group revealed mild-moderate hyperplasia and hypertrophy. Subintimal tissue suffered similar hypergranulation and hypervascularization with signs of inflammatory cells infiltration (Figure 1D).

Synovial membranes taken from the ZI and ZII groups revealed significantly lower histopathological changes than the earlier stated groups. In ZI, SMs showed mild intima hyperplasia and mild subintima granulation accompanied with mild increased number of blood vessels and very mild inflammatory cells infiltration (Figure 1E). In the ZII group, changes were mild hyperplasia and mild fibrous proliferation in the subintima accompanied with hypervascularization and subtle lack inflammatory cells infiltration (Figure 1F).

## Immunohistochemical study results

The normal left SMs revealed a high number of immunereactive nerve fibers running singly in an undulating course or seldom in a straight line but some nerve fibers were found running in bundles forming varicose (Figures 2 and 3). The PGP 9.5 immunoreactivity was found more abundant than those of the CGRP and NPY (Figure 2A, C). Protein gene product 9.5 nerve fibers were often localized within the subintimal tissue and were rarely found in the intima. Their densities in all the groups are summarized in Table 2.

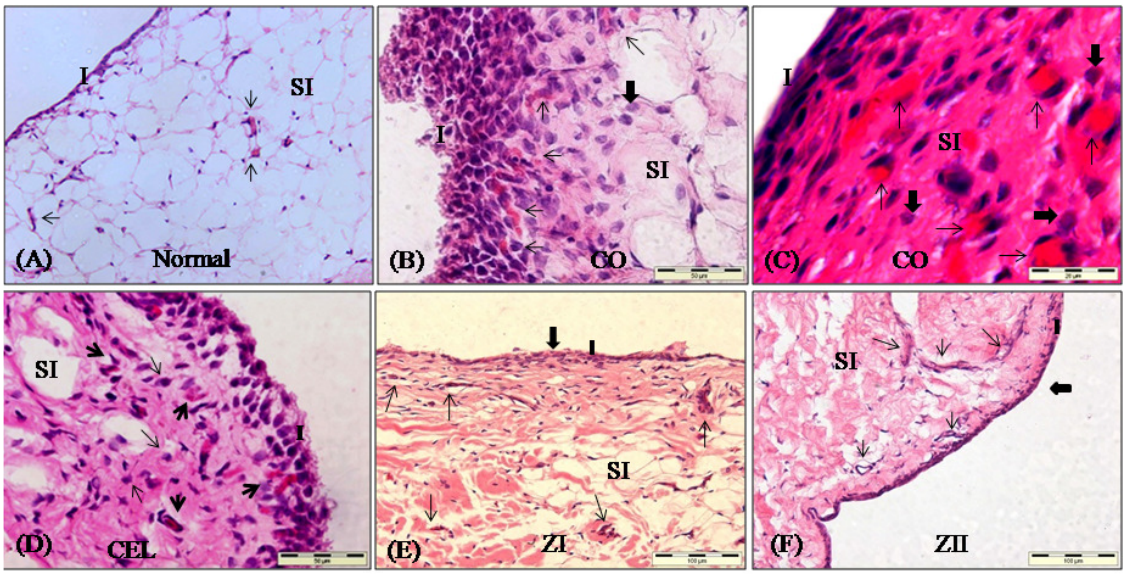


**Figure 1.** (A), Normal synovial membranes showed 1 to 2 cells thick synovial lining or intimal layer (I), fibro-fatty underlying subintimal tissue (SI), exposed considerable number of blood vessels (arrows), H and E, x200; (B, C), synovial membranes represent CO group (treated with 2 ml/kg body weight of corn oil) showed distinct hyperplasia of intimal layer (I) and hypergranulation of subintimal layer (SI), increased number of blood vessels (thin arrows) accompanied with infiltration of inflammatory cells such as macrophage (white thick arrow) and lymphocytes (black thick arrows), H and E, x400 and x1000, respectively; (D), synovial membranes represent CEL group (treated with celecoxib in a dose of 30 mg/kg body weight) showed hyperplasia of intimal layer (I) and proliferation of the collagen fibers in different parts of subintimal layer (SI), increased number of blood vessels (thick arrows) with distinct infiltration of inflammatory cells ( thin arrows), H and E, x400; (E), synovial membranes represent ZI group (treated with 2 ml/kg body weight of 0.2% w/v of zerumbone diluted in corn oil) showed mild hyperplasia of intimal layer (I) and proliferation of the collagen fibers in different parts of subintimal layer (SI), increased number of blood vessels (arrows) with mild infiltration of inflammatory cells, H and E, x200; (F), synovial membranes represent ZII group (treated with 2 ml/kg body weight of 0.4% w/v of zerumbone diluted in corn oil) showed milder changes and subtle lack of inflammatory cells infiltration, H and E, x200.

Marked depletion of PGP 9.5 immunoreactive nerve fibers was observed in the CO group. Similarly, celecoxib treated group showed poor density of such immuno-reactive nerve fiber at their SMs, indicating their poor effect on the osteoarthritis (OA) changes. The densities of these nerve fibers improved significantly ($P < 0.01$) in both the zerumbone treated groups compared with both the control groups.

The CGRP as well as the NPY immunoreactive nerve fibers were found distributed perivascularly throughout the subintimal tissue and some of them were present in the bundles forming varicose (Figure 2). Depletion of the CGRP and NPY nerve fibers was observed significantly in both the CO and CEL groups, whereas, they were improved significantly ($P < 0.01$) in the ZII group and in a lesser extent in the ZI group.

## Hormone assays

The results of the hormone assay revealed different concentrations of PGs at different periods and groups (Table 3). The concentration of $PGE_2$ was elevated significantly ($p < 0.01$) at day 15 following OA induction. However, the level of $PGE_2$ was significantly ($p < 0.001$) reduced after the oral treatment with 0.2 and 0.4% zerumbone. The positive control group showed significant ($p < 0.001$) reduction of $PGE_2$ too, whereas in the negative control group the level of $PGE_2$ remained significantly high ($p < 0.001$).

The basal level of $PGF_{2\alpha}$ was found to be higher than that of $PGE_2$. Following osteoarthritis (OA) induction for 15 days, the level was found to be elevated compared with the basal level. At the end of the treatment, in the zerumbone treated groups (ZI and ZII) the level of $PGF_{2\alpha}$ was found more or less similar to the level before the animals were treated. The changes seen in the level of $PGF_{2\alpha}$ were found to be insignificant.

## DISCUSSION

This study was intended to detect the improvement action of zerumbone on the densities of some OA related neuropeptides. In this study, OA was experimentally induced with the intraarticular injection of MIA into the

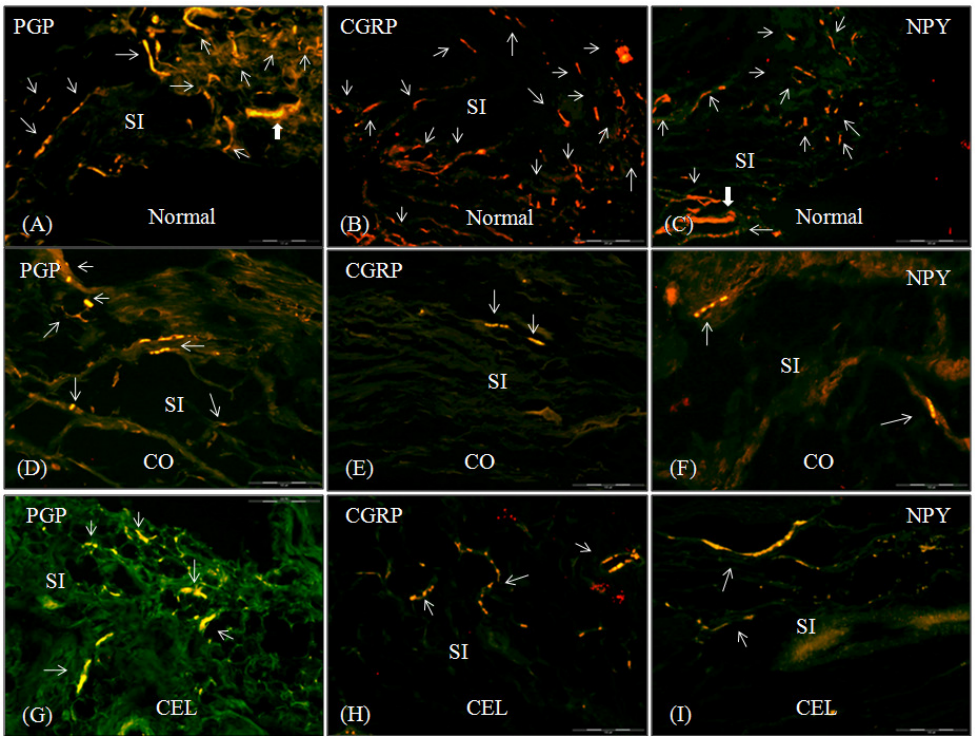


**Figure 2.** Upper panel (A-C), normal synovial membranes showed PGP 9.5, sensory CGRP and sympathetic NPY immunoreactive nerve fibers in the subintimal layer (SI) (thin arrows), some fibers are expanded forming varicosities, FITC Cy3, x200, x100 and x100, respectively; middle panel (D-F), synovial membranes of corn oil treated group showed severe depletion of PGP 9.5, CGRP and NPY immunoreactive nerve fibers in the subintimal layer (SI) (arrows), FITC Cy3, x200, x100 and x200, respectively; lower panel (G-I), synovial membranes of celecoxib treated group also exposed depletion of PGP 9.5, CGRP and NPY nerve fibers (arrows), Cy3, x200.

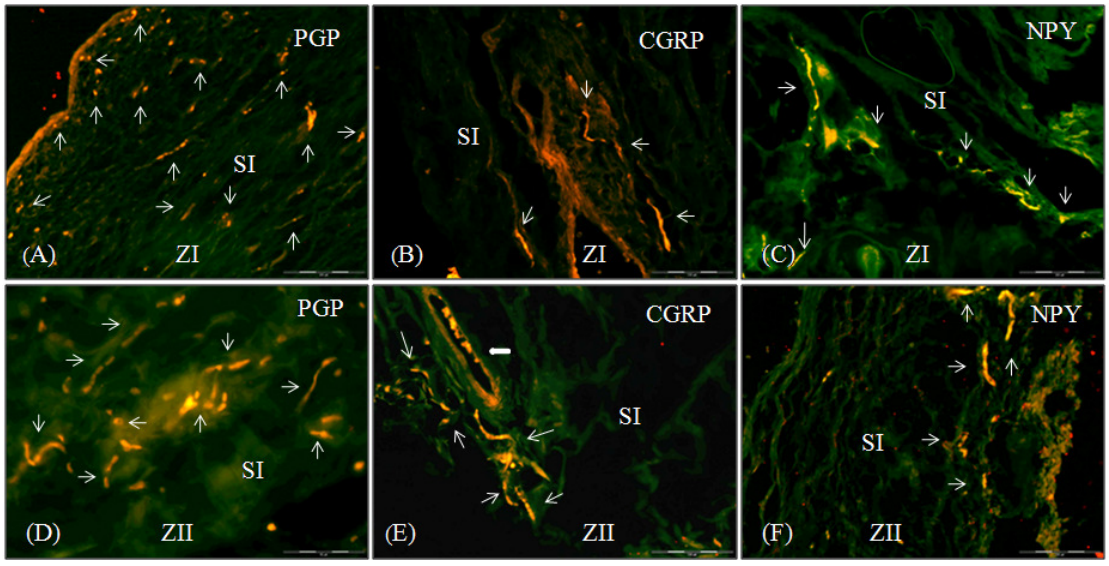


**Figure 3.** Upper panel (A), cryosections of synovial membranes from ZI group showed PGP 9.5 nerve fibers (signs of incomplete depletion of this neuropeptide) present in the structure of subintimal layer (SI) (arrows), FITC CY3, x100; (B, C), sparse CGRP and NPY nerve fibers were present in subintimal layer (SI) (arrows), FITC CY3, x200. Lower panel; (D), cryosections of synovial membranes from ZII group showed improved immunoreactivity of PGP 9.5 nerve fibers present in the subintimal layer (SI), CY3, x200; (E, F), plausible number of sensory (CGRP) and sympathetic (NPY) nerve fibers were present in subintimal layer (SI) of this group (arrows), FITC CY3, x400, x200 and x200, respectively.

right knee joints. Selection of MIA was based on its rapid onset of the disease (Al-Saffar et al., 2009) and its direct metabolic inhibitory effect on chondrocytes (Guzman et al., 2003). It is able to inhibit glyceraldehyde-3-phosphate dehydrogenase activity which leads to inhibition of glycolysis and subsequent degradation ended with cellular death (Cournil et al., 2001). Subsequent fragmentation of the degraded cartilage into the synovial fluid will triggers the inflammatory process by the production of pro-inflammatory mediators such as IL-1 and TNF-α, as well as recruitment of inflammatory cells into the synovium causing synovitis (Johnston, 1997). Inflamed synoviocytes, chondrocytes and inflammatory leukocytes can cause excessive release of cytokines, oxygen free species, PGs and NPs from the nerve fibers to a below detection level, showing signs of its depletion (Sutton et al., 2009).

The data of this study identified severe histopathological reaction at the CO group's SMs which was highly significant compared with the left normal one. Severe inflammation and marked depletion of PGP 9.5, CGRP and NPY immunoreactive nerve fibers was consistent to previous findings (Mapp et al., 1994). High serum level of pro-inflammatory mediator $PGE_2$ in the CO group indicated its role in osteoarthritis (OA) development and progression. Similarly, the histopathological and IHC results showed by the CEL group revealed low outcome of celecoxib treatment. It showed the poor effect of celecoxib to improve the immunoreactivity in the synovium. The effect of celecoxib was similar to the effect of other NSAIDs (Choi et al., 2002). The poor effect of celecoxib may be due to its low capacity to scavenge reactive species, exposing low or lack antioxidant activity (Bastos-Pereira et al., 2010).

Plausible reduction in the average pathology score occurred following the treatment with zerumbone (ZI and ZII groups). The anti-inflammatory action of zerumbone (Murakami et al., 2004) was apparent in the ZII group due to the subtle lack of inflammatory cell infiltration. In addition to that significant reduction of $PGE_2$ in zerumbone, the treated groups explored its important anti-inflammatory property.

In essence, zerumbone have dual actions: as an antioxidant (Ruslay et al., 2007) and as an anti-inflammatory (Chien et al., 2008).

It can suppress the production of free radicals at the affected joints which leads subsequently to synovitis and it suppresses cyclo-oxygenase (COX-2) expressions, which play a role in producing prostaglandins at the synovium (Tanaka et al., 2001). The effect of zerumbone was enhanced in the ZII group indicating the importance of its concentration and dosage regimen for future investigation.

This study exposed unambiguously that, zerumbone was able to inhibit at least the progression of the pathological events and improved the densities of the neuropeptides compared with those of the negative and positive control groups. However, the improvement of the immunoreactive nerve fibers densities remained significantly lower than those present at the normal left synovial membranes.

## Conclusions

The findings of this research showed the constant effect of the intra-articular injection of MIA on the densities of the PGP 9.5, CGRP and NPY nerve fibers, which were well correlated with inflammatory changes which occurred at the affected SMs. We suggest that, PGs do involve and play a role in osteoarthritis (OA) events and their concentrations at rat sera changed significantly at different periods and treatments. The treatment with celecoxib revealed low outcome on the OA changes present in the SMs. Dosage regimens of zerumbone applied in this research revealed different degrees of alleviation and improvement on the MIA induced changes. The data showed better improvement of the nerve fiber densities using higher concentration (0.4% w/v) than the lower concentration (0.2% w/v) of zerumbone daily for 4 weeks. Finally, it can be concluded that such medicinal herbal agent deserve more investigations. Future investigation for zerumbone therapeutic effect against OA should be conducted using higher doses or /and prolonged period of treatment.

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## Abbreviations

**CEL,** Celecoxib**; CGRP,** calcitonin gene related peptide; **CO,** corn oil; **COX,** cyclooxygenase; **FITC,** fluorescein isothiocyanate; **Gt XRb IgG Cy3,** goat anti-rabbit immunoglobulin G cyanine 3 ; **IHC,** immunohistochemistry; **IL,** interleukin; **MIA,** monosodium iodoacetate **; NPY,** neuropeptide Y; **NSAID,** non-steroidal anti-inflammatory drug; **OA,** osteoarthritis; **PBS,** phosphate buffer saline; **PG,** prostaglandin; **PGP 9.5,** protein gene product 9.5; **SM,** synovial membrane; **SP,** substance P; **TNF-α,** tumor necrosis factor -alpha; **Z,** zerumbone.

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