

Full Length Research Paper

## Calcium channel blockers attenuate chronic inflammation in rat knee joints

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Previous studies demonstrated therapeutic effects for calcium channel blockers (CCBs) in control of acute inflammation. There are little reports on the effect of these blockers on chronic inflammation. In this study, we investigated the effects of CCBs on chronic inflammation using complete Freund's adjuvant (CFA) induced inflammation in the rat knee joints. This experimental study was carried out on 80 male Wistar rats. Calcium channel blockers (verapamil and nifedipine) were used in doses of 100 and 800 µg/kg. For induction of chronic inflammation, CFA (0.2 ml) was injected into the right knee joint. The changes in blood flow, and local temperature of the animal's knee joint were measured by laser Doppler flowmetry and also the knee diameter was measured by a caliper, on 0, 7, 14, 21, and 28 days after CFA injection. CFA injection significantly increased blood flow from day 1 to 28 as compared to the day 0. Administration of both verapamil and nifedipine (100 and 800 µg/kg) significantly decreased the effect of CFA on increasing blood flow (all  $p < 0.05$ ). Administration of both verapamil and nifedipine (100 and 800 µg/kg) significantly inhibited the effect of CFA on increasing knee temperature (all  $p < 0.05$ ). Injection of CFA increased the knee joint diameter for all 28 days after injection. Both low and high doses of nifedipine and verapamil could inhibit the increased diameter that is induced by CFA injection (all  $p < 0.05$ ). The effects of CCBs on measured parameter were comparable with the effect of ibuprofen (which is a standard anti-inflammatory drug) on these parameters. The results revealed that CCBs inhibited the increased rat knee blood flow, diameter and temperature in chronic inflammation induced by CFA. Therefore, the underlying mechanisms for reduction of chronic inflammation possibly are modulated by these blockers.

**Key words:** Chronic inflammation, verapamil, nifedipine, complete Freund's adjuvant (CFA).

### INTRODUCTION

Rheumatoid arthritis is characterized as an inflammatory chronic disease with a prevalence of 1% and with various geographical distributions. Although, this disease is the focused area of research by several study groups worldwide, the new and effective therapeutic methods for rheumatoid arthritis are yet to be available (Badvi, 2000; Nkomo et al., 2010).

Vessels dilatation in parallel with increase in their blood flow is one of the complications induced by chronic

inflammation, which is among the main factors in raising temperature and inflammatory edema (Khaksari et al., 2002). The joint's cartilages nutrients are supplied by synovial fluid which originated from the joint's blood flow, thus, factors that regulate the joint's blood flow play a crucial role in maintenance of joint's tissues and the homeostasis environment. Changes in the normal mechanisms in regulation of blood flow in the synovium by inflammatory diseases of joints can interfere with the

damage of synovium (Najafipour and Niazmand, 2004). The constitutional mechanism for increasing blood flow in a chronic inflamed joint is yet to be clarified. Few reports showed that it could be due to the decreased sympathetic tonicity of the vessels (Boston et al., 2004), changes in beta-adrenergic receptors profile (Botrel et al., 1994), and the decrease in knee vessels in response to phenylephrine and overproduction of nitric oxide (NO). It has been revealed that this change is due to the altered secretion and release of local vessels relaxing mediators from the inflamed cells or sensory nerve ends, whereas calcitonin gene-related peptide (CGRP) and substance P that are released from the sensory nerves ends, caused the neurogenic dilatation of the vessels (Fahim et al., 1995). In addition, increase in the release of substance P, is possible. Local secretion of bradykinin and prostaglandins (especially prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>)) (Kaur and Halliwell, 1994), also can mediate the increase of blood flow.

Calcium ion participates in the many process, including release, activation, and effectiveness of many inflammatory mediators, such as activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>), increasing the production of arachidonic acid metabolites (Chawengsub et al., 2009), NO synthesis (Koya and King, 1998), interleukins releasing, mediators of exocytosis, and chemotactic responses (Lam and Ferrell, 1993). On the other hand, evidences showed that the voltage calcium channel blockers (CCBs) cause inhibition of NO production and the enzymes responsible for the synthesis of prostaglandins (Lam and Ferrell, 1992), and decrease the release of histamine, bradykinin, serotonin, arachidonic acid metabolites, and leukotrienes (Fogel et al., 2005).

Both acute and chronic inflammatory responses lead to rise in temperature (Leventhal et al., 2005), and swelling as the two important clinical signs of inflammation, thus compounds which are used to reduce swelling and temperature is suggested to have anti-inflammatory properties. One of the leading causes of the raised temperature is the increased joint blood flow accompanied by some of the chemical mediators that are released in the site of inflammation (Fahim et al., 1995). Therefore, the factors that reduce the blood flow of joint or inhibit the production and release of mediators down regulate the joint temperature in inflammation.

Calcium play key roles in production of the factors mentioned earlier, which are involved in the inflammation, so, in a way it can be suggested that calcium blockers may be used as useful tools for treatment of inflammation. In the previous studies, the effect of voltage calcium channels on acute (Kaur and Halliwell, 1994) and chronic (McDougall et al., 1994) inflammation has been demonstrated. Therefore, the present study was designed to examined, whether if inhibitory effect of these blockers in chronic inflammation of rat knee is mediated via decreasing blood flow, temperature, and the joint diameter.

## MATERIALS AND METHODS

### Animals

This study was carried out on 80 adult male Wistar rats weighing 200 to 250 g. Animals were housed in Rafsanjan Faculty of Medicine animal house at a temperature of 20 to 22°C and 12 h light/dark cycle with *ad libitum* access to food and water. The following protocol was similarly carried out on all groups. All protocols were approved by the institutional animal care and use committee of Rafsanjan University of Medical Sciences.

### Induction of chronic inflammation

To induce the chronic inflammation, after anesthesia by ether, 0.2 ml of complete Freund's adjuvant (CFA) was injected into the anterior space of the right knee joint by insulin syringe with 26 gage needles. This procedure is an accepted model for induction of chronic inflammations that produce rheumatoid arthritis like inflammatory reactions in human (Morgan et al., 1978; McDougall et al., 1995).

### Measurement of the effects of blocker on inflamed joint

The levels of the effects of blockers on inflamed joint were measured by the following three methods.

#### *Measurement of the changes in blood flow*

To measure the changes in blood flow in response to CFA and other drugs injection, animals were anesthetized by administration of 1.5 g/kg intraperitoneally (ip) urethane. Following complete anesthesia, a two channels laser Doppler machine (DRT4 model Moor instrument, England) was used to measure the changes in blood flow (Fahim et al., 1995). The probe of this device contacted with the interior capsules of the knee and from 20 point in 1 mm square area the blood flow was recorded, and then, the average of the recorded values were assigned as the blood flow level. The changes in blood flow was measured and compared on day zero (before injection) and it continued on days, 7, 14, 21, and 28 after injection of CFA and also after oral administration of CCBs. Finally, the animals were humanely killed by the high dose of urethane. The blood flow of biological zero level (BZL) of joint's tissue were recorded to subtract from the measured level during the experiment (McDougall et al., 1995).

#### *Measurement of the changes in temperature*

Temperature of inflamed joint was another index that was evaluated in this study. Temperature was measured by the same probe in parallel with blood flow on days 0, 7, 14, 21, and 28.

#### *Measurement of changes in joint diameter*

The measurement of intralateral knee joint's diameter is a criteria to evaluate the intensity of inflammation (McDougall et al., 1995). A caliper (Diamond, China) with the precision of 0.02 mm was used to measure the right knee joint diameter on day zero (before injection) and days 7, 14, 21, and 28 after injection in different groups. The diameter before injection was compared with the diameters after injection.

## Drugs and reagents

Verapamil and nifedipine (Rose Daruo Co, Iran), 100 and 800 µg/kg and ibuprofen (Sigma, UK) 15 mg/kg were administered orally by orogastric tube 7 days after injection of CFA (Biogen, Iran). These doses of drugs have shown inhibitory effect in previous studies (Najafipour and Ferrell, 1993). The drugs were dissolved in dimethyl sulfoxide (DMSO) (Merk, Germany) (Rawls et al., 2004a). The volume of oral solution was 1 ml/kg and was administered at the same time everyday from day 7 after CFA injection (onset of chronic inflammation) for 21 days forward. DMSO (as the solvent of drugs) was also administered orally in same volume. Urethane and ether for anesthetizing of animals were purchased from Merk (Merk, Germany).

## Experimental groups

Rats were divided into 8 groups randomly and 8 to 10 animals were allocated in each group. Group I: animals that had chronic inflammation of knee by injection of CFA, and the changes in blood flow, temperature, and the joint diameter were measured for 28 days. Group II: in this group, after CFA injection, animals received DMSO (the solvent of drugs) orally from the day 7 and as group I parameters, they were measured for 28 days. Group III (control): in this group, 0.2 ml (same volume as CFA) of normal saline were injected into the right knee and the alterations were measured like group I. Group IV received verapamil (100 µg/kg) for 21 days. Group V received nifedipine (100 µg/kg), group VI received 800 µg/kg of verapamil, group VII received 800 µg/kg of nifedipine, and group VIII were injected 15 mg/kg of ibuprofen for 21 days and the change in blood flow and other two parameters were measured as in group I.

## Statistical analysis

The statistical analysis was performed using excel and Statistical Package for Social Sciences (SPSS) softwares. All data are expressed as mean ± standard error of the mean (SEM). A *p* value of less than 0.05 has been considered as statistical significance. Repeated measurement on analysis of variance (ANOVA) was used to compare measured indices in different times. All post hoc comparisons were made using Tukey's post hoc test.

## RESULTS

### Changes in blood flow

The effects of CFA, saline, DMSO + CFA, and the low and high doses of verapamil and nifedipine on the knee joint blood flow in different days of study are shown in Table 1. CFA injection increased blood flow on days 7, 14, 21, and 28 as compared to the day zero (all *p* < 0.01). Despite the same blood flow on day zero in CFA, saline, and DMSO groups (99.9 ± 4.5), on the day 7 (after CFA injection), the blood flow in CFA group was significantly increased as compared to control group (229.8 ± 13.2 versus 102.1 ± 7.9, *p* < 0.001) and this was constantly maintained to the end of the study (day 28, 163.7 ± 12.4). On the other hand, DMSO did not significantly affected blood flow which was raised by CFA. Both low and high doses of verapamil and nifedipine (100 and 800 µg/kg) significantly decreased the blood flow which was raised

by CFA on days 14 and 21 (Table 1) (*p* < 0.001). While on day 28, both doses of nifedipine inhibited blood flow (*p* < 0.001), but only the low dose of verapamil had a significant effect. Ibuprofen significantly inhibited the elevation of blood flow by CFA on days 14, 21, and 28 (*p* < 0.001).

### Changes in knee joint temperature

The effect of CFA, saline, DMSO, and low and high doses of verapamil and nifedipine on the knee joint temperature are shown in Table 2. These results indicated that CFA injection elevated the temperature on days, 7, 14, 21, and 28 (*p* < 0.001). On day zero, not significant difference was observed between CFA, control, and DMSO groups (26.51 ± 0.58, 27.2 ± 0.4 and 27.5 ± 0.52°C respectively). Following 7 days of CFA injection, the temperature of knee joint in CFA group (35.17 ± 0.1°C) significantly increased as compared to the control (26.92 ± 0.32°C) (*p* < 0.001), and this was continued to the end of the study (*p* < 0.001). Although, DMSO had no significant inhibitory effect on temperature caused by CFA on day 14, but on days 21 (32.37 ± 0.29) and 28 (31.2 ± 0.19), the difference between DMSO and CFA group was significant (*P* < 0.001).

Low and high doses of both CCBs significantly inhibited the temperature by CFA on days 14, 21, and 28 (*p* < 0.001), there was a significant difference between low and high doses of nifedipine (*p* < 0.001) (Table 2). Indeed, on days 21 and 28, there was a significant difference between low and high doses of nifedipine and verapamil (*p* < 0.001), also on days 14 and 28, the difference in high dose of nifedipine with verapamil was significant (*p* < 0.001). Ibuprofen on days 14, 21, and 28 decreased the knee joint temperature as compared to CFA (*p* < 0.001) and there were significant differences between ibuprofen and low dose of verapamil (*p* < 0.05) and nifedipine (*p* < 0.05) on days 21 and between ibuprofen and low and high dose of nifedipine (*p* < 0.01) and verapamil (*p* < 0.001) on day 28.

### The effects of different drugs on knee diameter

The comparison of the effects of different drugs on increasing the knee diameter caused by CFA injection is shown in Table 3. The knee diameter on day zero were the same as CFA, saline and DMSO groups (9.99 ± 0.08, 10.1 ± 0.07, and 9.8 ± 0.08 mm, respectively), but on days 7, 14, 21, and 28, the knee diameter in CFA group was higher than saline group (*p* < 0.001); on the other hand, DMSO did not inhibit the increased diameter caused by CFA, the knee diameter decreased on day 14 by high dose of nifedipine (*p* < 0.001) and on day 21 by low and high doses of verapamil and high dose of nifedipine (*p* < 0.001). Indeed, on day 28, the low and high doses of nifedipine and low dose of verapamil inhibited the increased diameter caused by CFA (*p* < 0.01). Ibuprofen inhibited the increased diameter caused

**Table 1.** Comparison of the effects of drugs on the blood flow (arbitrary) of rats' knee joint in different groups and different days of study.

Group	Day				
	0	7	14	21	28
CFA	99.9 ± 4.5	229.8 ± 13.2	217.1 ± 11.5 <sup>b</sup>	244.8 ± 10.4 <sup>c</sup>	163.7 ± 11.4 <sup>d</sup>
Saline (control)	99.8 ± 3.7	102.1 ± 7.9 <sup>a</sup>	101.1 ± 6.1 <sup>a</sup>	93.3 ± 1.4 <sup>a</sup>	97.8 ± 5.6 <sup>a</sup>
DMSO	98.9 ± 4.6	230.5 ± 14.1	199.9 ± 13.7	232 ± 6	195.9 ± 16.9
N-100	-	240.9 ± 10.5	159.2 ± 10.9	121.3 ± 8.7	124.1 ± 6.9
V-100	-	239.4 ± 12.7	135.2 ± 21.4	112.1 ± 10.1	96.8 ± 5.1
N-800	-	230.5 ± 14.5	144.7 ± 13.7	132.7 ± 11.5	127.1 ± 8.7
V-800	-	232.8 ± 12.5	136.8 ± 14.1	148.1 ± 15	158.2 ± 10.4
Ibuprofen	-	235 ± 15.4	176.2 ± 9.5	108.1 ± 2.8	95.6 ± 5.8

Data are shown as mean SEM. CFA: complete Freund's adjuvant, DMSO: dimethyl sulfoxide, N-100 and N-800: 100 and 800 µg/kg of nifedipine, respectively; V-100 and V-800: 100 and 800 µg/kg of verapamil, respectively; ibuprofen: 15 mg/kg. <sup>a</sup>Significant difference ( $p < 0.001$ ) between saline group with CFA or DMSO group in days 7, 14, 21, and 28 of the study. <sup>b</sup>Significant difference ( $p < 0.001$ ) between CFA group with different doses of verapamil, nifedipine and ibuprofen on day 14. <sup>c</sup>Significant difference ( $p < 0.001$ ) between CFA group with different doses of verapamil, nifedipine, and ibuprofen on day 21. <sup>d</sup>Significant difference ( $p < 0.001$ ) between CFA group with low dose of verapamil and with different doses of nifedipine and also with ibuprofen on day 28.

**Table 2.** Comparing the effects of drugs on the temperature (°C) of rats' knee joint in different groups and different days of the study.

Group	Day				
	0	7	14	21	28
CFA	26.51 ± 0.58 <sup>a</sup>	35.17 ± 0.1	34.8 ± 0.35	34.16 ± 0.09	34.25 ± 0.25
Saline (control)	27.2 ± 0.4	26.92 ± 0.32 <sup>b</sup>	26.26 ± 0.17	26.29 ± 0.29	26.71 ± 0.31
DMSO	27.5 ± 0.52	35.2 ± 0.4	33.85 ± 0.35	32.37 ± 0.29 <sup>c</sup>	31.21 ± 0.19
N-100	-	35.3 ± 0.21	29.97 ± 0.21 <sup>d</sup>	30.19 ± 0.26	30.25 ± 0.24
V-100	-	34.91 ± 0.35	28.38 ± 0.66 <sup>e</sup>	28.96 ± 0.18	28.73 ± 0.14
N-800	-	35.2 ± 0.4	30.65 ± 0.39 <sup>f</sup>	29.27 ± 0.21	29.32 ± 0.38
V-800	-	35.5 ± 0.65	28.8 ± 0.24 <sup>g</sup>	29.63 ± 0.47	29.91 ± 0.31
Ibuprofen	-	35.5 ± 0.2	29.9 ± 0.24 <sup>h</sup>	29.4 ± 0.12	28.1 ± 0.4

Data are shown as mean SEM. CFA: Complete Freund's Adjuvant, DMSO: dimethyl sulfoxide, N-100 and N-800: 100 and 800 µg/kg of nifedipine, respectively, V-100 and V-800: 100 and 800 µg/kg of verapamil, respectively, ibuprofen: 15 mg/kg. <sup>a</sup>Significant difference ( $p < 0.001$ ) between all days of the study as compared to day zero in CFA group. <sup>b</sup>Significant difference ( $p < 0.001$ ) between CFA group with saline group on days 7, 14, 21, and 28. <sup>c</sup>Significant difference ( $p < 0.001$ ) between CFA group and DMSO group on days 21 and 28. <sup>d</sup>Significant difference ( $p < 0.001$ ) between CFA group and low dose of nifedipine on days 14, 21, and 28. <sup>e</sup>Significant difference ( $p < 0.001$ ) between CFA group and low dose of verapamil on days 14, 21, and 28. <sup>f</sup>Significant difference ( $p < 0.001$ ) between CFA group and high dose of nifedipine on days 14, 21, and 28. <sup>g</sup>Significant difference ( $p < 0.001$ ) between CFA group and high dose of verapamil on days 14, 21, and 28. <sup>h</sup>Significant difference ( $p < 0.001$ ) between ibuprofen and CFA group on days 14, 21, and 28.

by CFA, significantly during all the days of the study ( $p < 0.01$ ).

On day 21, the difference between low and high doses of nifedipine was significant ( $p < 0.05$ ). On day 14, there were significant differences between ibuprofen with low dose of nifedipine ( $p < 0.01$ ) and low and high doses of verapamil ( $p < 0.001$ ), whereas on day 28, there was only significant difference between ibuprofen and 800 µg/kg of verapamil ( $p < 0.01$ ).

## DISCUSSION

Previous studies reported the inhibitory role of CCBs in acute (Najafipour and Ferrell, 1993) and chronic (McDougall

et al., 1994) inflammation. One of the background causes leading to pathologic events is the increased blood flow in the inflamed joints (Khaksari et al., 2002), thus, in the present study, the effects of this inhibitions in blood flow, temperature, and the diameter of inflamed joints were evaluated.

Results of the current study showed that the injection of CFA into the joint caused significant increase in the blood flow of joint from day 1, whereas on day 7 after injection of CFA, the blood flow in the injected knee increased by  $121.7 \pm 6.1\%$  as compared to day zero, and then gradually decreased during the following days, but it did not reach to the initial level and it had significant difference with day zero until the end of the study ( $93.8 \pm$

**Table 3.** Comparison the effects of drugs on the joint diameter (mm) of rats' knee joint in different groups and different days of the study.

Group	Day				
	0	7	14	21	28
CFA	9.99 ± 0.8	13.1 ± 0.2 <sup>a</sup>	12.4 ± 0.26	12.09 ± 0.24 <sup>e</sup>	11.76 ± 0.18 <sup>f</sup>
Saline (control)	10.1 ± 0.07	9.81 ± 0.08	9.71 ± 0.06	9.7 ± 0.06	9.74 ± 0.07
DMSO	9.80 ± 0.08	12.6 ± 0.08 <sup>b</sup>	12.3 ± 0.4	11.8 ± 0.16	11.86 ± 0.27
N-100	-	13.4 ± 0.22	11.91 ± 0.14	11.12 ± 0.29	10.68 ± 0.2
V-100	-	12.89 ± 0.15	11.47 ± 0.16 <sup>d</sup>	10.74 ± 0.18	10.6 ± 0.2
N-800	-	12.8 ± 0.2	11.02 ± 0.11 <sup>c</sup>	10.07 ± 0.24	10.65 ± 0.18
V-800	-	12.9 ± 0.15	10.95 ± 0.17	10.84 ± 0.2	11.08 ± 0.19
Ibuprofen	-	13.2 ± 0.25	10.46 ± 0.27 <sup>g</sup>	10.87 ± 0.33	9.9 ± 0.14

Data are shown as mean SEM. CFA: complete Freund's adjuvant, DMSO: dimethyl sulfoxide, N-100 and N-800: 100 and 800 µg/kg of nifedipine, respectively, V-100 and V-800: 100 and 800 µg/kg of verapamil, respectively, ibuprofen: 15 mg/kg. <sup>a</sup>Significant difference ( $p < 0.001$ ) between saline and CFA group in all days of the study. <sup>b</sup>Significant difference ( $p < 0.01$ ) between DMSO and saline group on days 7, 14, 21, and 28. <sup>c</sup>Significant difference ( $p < 0.001$ ) between CFA group and high dose of nifedipine on day 14. <sup>d</sup>Significant difference ( $p < 0.001$ ) between CFA group and low dose of verapamil on day 14. <sup>e</sup>Significant difference ( $p < 0.001$ ) between CFA group with high dose nifedipine, and also with both doses of verapamil on day 21. <sup>f</sup>Significant difference ( $p < 0.001$ ) between CFA group with both doses of nifedipine, and also with low dose of verapamil on day 28. <sup>g</sup>Significant difference ( $p < 0.01$ ) between CFA group with ibuprofen in all days of the study.

15.5% on day 28). CFA probably lead to increase in blood flow of joint with chronic inflammation by increasing the level of prostaglandins production, aggregation of phagocytes in joints and free oxygen radical production and other materials effective on vessels' diameter (Rawls et al., 2004b). The oral consumption of both calcium channel inhibitors in CFA receiving group reduced the increased blood flow of knee joint caused by CFA (the low dose of verapamil on days 14, 21, and 28, and low dose of nifedipine on days 14, 21, and 28, decreased the blood flow). The high dose of verapamil and nifedipine also reduced knee joint blood flow on the mentioned days, whereas the most inhibitory effects of verapamil ( $34.6 \pm 6.6\%$ ) and nifedipine ( $42.9 \pm 3.8\%$ ) was on day 14. The inhibitory effects of these blockers on this inflammation index was comparable with ibuprofen, hence, the vasodilatation caused by CFA which occurred in chronic inflammation decreases significantly by CCBs, and this indicates the role of calcium in the vessel response. CCBs possibly by altering the production and release of CGRP and substance P from the sensory neurons' terminal (Fahim et al., 1995), inhibition of PLA<sub>2</sub> enzyme and following that, the reduction in PGE<sub>2</sub> and PGI<sub>2</sub> production (Lam and Ferrell, 1992, Kaur and Halliwell, 1994), inhibition of calcium dependent protein kinase C (PKC) and finally the inhibition of pathway that is activated by this enzyme (Khoshbaten and Ferrell, 1990), inhibition of release or function of chemical mediators like histamine, bradykinin, serotonin and leukotrienes (Lees et al., 1998), by reduction of free oxygen radical production and superoxide (Rezaie et al., 2005) have induced their inhibition effects.

Previous studies showed that the NO level was increased during inflammatory disorders (Botrel et al., 1994; Rawls et al., 2004a). The response of smooth muscle

of the vessels to the  $\alpha$ -receptors antagonist is influenced by the released agents from endothelium of vessels (Botrel et al., 1994), whereas NO decreased vasoconstriction response in inflamed joint, thus, the CCBs may in turn causes decrease in blood flow by alteration in the production of NO (Lam and Ferrell, 1993). It has also been reported that in acute (Rosen, 1989) and chronic (Morgan et al., 1978) inflammation in rat knee, the constriction response to the knee sympathetic stimulation has decreased, so probably CCBs, prevented the decrease in responsiveness by local changes in the inflamed joint.

The results of other part of this research indicated that the low dose of verapamil and nifedipine had the most inhibitory effects on the reduction of the inflamed joint temperature that was  $16.7 \pm 1.2$  and  $12.05 \pm 1.2\%$ , respectively. On day 14, with the high dose of nifedipine and verapamil, the greatest effect was observed on day 21,  $16.1 \pm 1.5$  and  $15.2 \pm 1.7\%$ , respectively. The CCBs possibly via reduction of NO (Mustafa and Olson, 1999) inhibited the release of neuromediators as gamma-aminobutyric acid (GABA) (Shirota et al., 1988), reduction of interleukins release (Sobal et al., 2001), prostaglandins (Arend and Dayer, 1995), or lessened the blood flow that was observed in the present study that caused the decrease in the joint temperature. The useful inhibiting effect of CCBs on reduction of temperature in this study is consistent with the reports of the useful effect of these drugs on decreasing the temperature in ovariectomized animals (Wirth et al., 1992), Reynald syndrome (Zeni and Ingegnoli, 2004), and inflamed paw (Arend and Dayer, 1995).

The results of the current study also indicated that CFA injection in the knee joint caused significant increase of knee diameter on day 3 and it reached to the maximum and from day 7 the increase was constant up to day 28

(end of study). These results are consistent with McDougall et al. (1995) who reported that, after knee joint CFA injection, the chronic inflammation occurred on day 7 (McDougall et al., 1995). CFA possibly run a chronic inflammation or arthritis like phenomenon via increasing of PGE<sub>2</sub> production, reduction of sulfhydryl (SH) group in serum, increase in blood glutathione (GSH) production, phagocytes aggregation in joint, and free oxygen radical and super oxide production (Rawls et al., 2004b).

Treatment of chronically inflamed animals with both CCBs reduced the knee joint diameter, and the most inhibitory effects of low dose of verapamil on day 21 was 11.2%, while its high dose was on days 14 was 11.38% and 21 was 10.41%, respectively. Also, the increase in knee joint diameter (8.7%) caused by CFA, inhibited low dose of nifedipine on day 28 and by high dose of this drug (11.6%) on day 21 as compared to day zero. The inhibitory effect of these CCBs was comparable to the effect of ibuprofen, especially on day 21, although, the inhibitory effect of ibuprofen on day 28 was greater than both doses of CCBs.

Overall, findings of the present study indicated that both verapamil and nifedipine exhibited considerable and powerful effect on the reduction of blood flow, temperature, and knee joint diameter in chronic inflammation. The anti-inflammatory effects possibly occurred via reduction in blood flow, decrease in the temperature and reduction of the diameter. This study also showed that calcium ion plays a role in the processes of rheumatoid arthritis, indicating that CCBs could probably be introduced as new therapeutic targets which are effective in this chronic disorder, and to confirm this claim, clinical trial studies should be done.

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