Implantable delivery device of EUDRAGIT/PCL-T/ Sodium diclofenac: mechanical characterization, drug release and in vivo anti-inflammatory activity

*Dispositivo implantável de EUDRAGIR/PCL-T/ Diclofenaco de sódio: caracterização mecânica, liberação do fármaco e atividade anti-inflamatória*in vivo

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ABSTRACT

Study design: Experimental study. **Objective:** The aim of this study was the characterization and evaluation of the anti-inflammatory activity of an implantable polymer system containing sodium diclofenacin carrageenan-induced acute inflammation in a rat kneemodel. **Methodology:** An implantable device made of Eudragit RS 100 and PCL-T containing 3.0 mg of sodium diclofenac was produced by casting. The device showed similar mechanical properties to elastomeric polymer products. The mechanism of sodium diclofenac release from the Eudragit and PLC-T matrix showed two stages: first, a rapid release and then a slow release with a zero-order kinetic behavior. The devices were implanted in the articular joint of the posterior area of the arthritis-induced knees of rats. **Results:** Within 6 hours and on day 7 after arthritis induction, the knee edema was evaluated, and the inflammatory mediators, myeloperoxidase (MPO) and nitric oxide were analyzed. The results were compared with oral administration of 30 mg/kg of sodium diclofenac. **Conclusion:** The proposed implantable device was able to produce similar anti-inflammatory results in carrageenan-induced arthritis compared with oral treatment, but at lower doses.

Keywords: Osteoarthritis. Drug Delivery Systems, Implantable. PCL-T. Inflammation. Diclofenac / Sodium. Rats.

RESUMO

Modelo do estudo: experimental. **Objetivo do estudo:** produzir, descrever e avaliar a atividade anti-inflamatória de um sistema polimérico implantável contendo diclofenaco de sódio na inflamação aguda induzida por carragenina em um modelo de artrite emjoelho de ratos. **Metodologia:** Um dispositivo implantável de Eudragit RS 100 e PCL-T (EUDPCL-T) contendo 3.0 mg de diclofenaco de sódio foi produzido. Os dispositivos foram implantados na região peri-articular posterior dos joelhos de ratos induzidos a artrite. **Resultados:** Após 6 horas e no dia 7 após a indução de artrite, o edema do joelho foi avaliado, e os mediadores inflamatórios, mieloperoxidase (MPO) e óxido nítrico foram analisados. Os resultados foram comparados com a administração oral de 30 mg / kg de diclofenaco de sódio. **Conclusões:** O dispositivo implantável foi capaz de produzir os mesmos resultados anti-inflamatórios na artrite induzida por carragenina quando comparadoscom o tratamento por via oral, entretanto com doses mais baixas.

Palavras-chave: Osteoartrite. Sistemas Implantáveis de Liberação de Medicamentos. PCL-T. Inflamação. Diclofenaco/ Sódio . Ratos.

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Introduction

In recent decades, the search for new technologies to provide controlled release of medications had a big boost. Research seeking materials that can lead to constant drug release over an extended period was achieved with the use of various chemical composition systems that can provide controlled drug delivery.[1,2]

Controlled drug-delivery systems have the advantage of improved drug efficacy, reducing toxicity and increasing comfort and adherence to treatment. Studies have demonstrated a superior efficacy compared with conventional methods of administering drugs, but few products are currently available for clinical use. Many of these systems are in early stages of development, and a number of questions are yet to be answered.[1-7]

The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly consumed medications in the world. They are used to decrease pain and inflammation caused by various diseases, such as in the treatment of arthritis.[8]However, the continuous use of NSAIDs is directly associated with gastrointestinal, cardiovascular and renal complications. NSAID-related morbidity and mortality are well known in the scientific community. For that reason, there is a recommendation to use these drugs in the lowest effective dose and for the shortest possible time.[8-11]

This study was aimed at producing and assessing anti-inflammatory activity of an implantable system for sodium diclofenacdelivery in an articular joint to promote drug activity at lower doses than those used orally, thus reducing unwanted side effects.

Materials and methods

Production of implantable device

To produce the implantable device, 1350 mg of Eudragit-L (Evonik Industries, Essen, Germany), 600 mg of PCL-T (Aldrich Chemical Co. Inc., St. Louis, USA) and 860 mg of sodium diclofenac (SA Galena, São Paulo, Brazil) were previously dissolved in 35 ml of ethanol. The solution was poured into a Teflon plate measuring 26.34 cm^2, and maintained in fume hood at 25 ± 5°C until completely dry. The produced films containing 32.6 mg/cm^2sodium diclofenac were kept in a desiccator for further characterization. Devices measuring 1 mm wide and 11 mm long were molded for *in vivo* testing.

Mechanical analysis

A dynamic-mechanical analysis equipment Model DMA Q800 from TA Instruments was used to examine the device. The tests were conducted at a constant temperature of 37°C and 1N/min loading rate up to a maximum force of 18 N. Assessment of the responses encompassed the elastic modulus strain in the range between 0.2% and 0.4%, maximum stress (MPa) and breaking strain (%) (N > 3).

Release studies

The release studies were performed using dissolution tester Model *Etica*, with six baskets and one cell release. The cell was immersed in 350 ml of phosphate buffer solution (pH = 7.4) under continuous stirring at 120 rpm, at 37°C ± 1.0°C. Solution samples were taken over an 11-day period, and the concentration of sodium diclofenac released was determined by UV spectroscopy (Hitachi 2010, Japan) in lmax 272 nm, and the tests were performed in triplicate. After each sample collection, an equal volume of phosphate buffer was added to the dissolution medium to maintain constant volume and sink conditions. The concentration of sodium diclofenac released from the films was calculated using the equation of a straight line derived from the calibration curve of diclofenac obtained from the analytical method validation. The validated method showed r = 0.99838, a linear coefficient of 0.0046, and a slope of 32.9342. The coefficient of variation between tests was 2.5% (precision) and accuracy of the presence of formulation components was 98.7%.

Assessment of the anti-inflammatory activity

The *in vivo* anti-inflammatory activity of the devices were compared with the same drug administered orally in carrageenan-induced arthritis rats models. Fifty-six male Wistar rats (*Rattus norvegicus albinus*) 60 days of age, obtained from UNIVALI vivarium, weighing 150-200 g, maintained under controlled conditions of luminosity (12-hour light-dark cycle), temperature (22 ± 2%C), receiving water and balanced chow ad libitum,were used for the

experiments. The research project was approved by the UNISUL Ethics Committee on Animal Experimentation (number 12.013.4.01 IV). The rats were randomly divided into four groups named DICL (sodium diclofenac), EUDPCL-T, CRGN (carrageenan) and Control, each group containing 14 animals. On the first day, the rats were anesthetized, weighed and had the thickness of the right knee measured using an InSize Digital Micrometer, Model 3109. Rats from the DICL group received 7.5 mg (corresponding to 30 mg/kg) of sodium diclofenac via a gastric tube inserted orally once daily for 11 days.[12,13] Rats from the EUDPCL-T group were implanted with devices containing 3.0 mgof sodium diclofenac(the highest possible concentration being incorporated into the matrix of EUD/PCL -T) in the popliteal region, medially to the biceps femoris tendon and laterally to the semitendinosus muscle along the posterior joint capsule of the right knee. Rats from the CRGN and CONTROL groups did not receive any anti-inflammatory drug.

The animals were anesthetized for the surgical procedures with an intraperitoneal injection of ketamine hydrochloride (40mg/Kg of body weight) combined with Xylazine (16 mg/Kg of body weight).[14]

On the 4th day after initiation of treatment, the rats were anesthetized again. Arthritis was induced in the right knee of rats from the DICL, EUDPCL-T and CRGN groups by intra-articular injection (0.4 mL carrageenan (Sigma chemical CO, USA), 3% saline). The right knee joint of rats in the control group was injected 0.4 ml saline intra-articularly.

Six hours after arthritis induction, 7 randomly chosen rats in each group were anesthetized for knee swelling measurement, followed by euthanasia for resection of the right knee. The soft tissues of the knee were collected for myeloperoxidase (MPO) and nitrite/nitrate (NO) assay. Seven days after arthritis induction, the remaining 7 rats in each group underwent the same procedure. The diameter of the knee joint was defined as the distance between the regions of the medial and lateral collateral ligament measured with a digital micrometer with the knee in extension. The variation in the knee diameter after carrageenan administration was determined based on the initial measured diameter.[15]

MPO and NO assay in the joint capsules

The joint capsules and periarticular tendon structures of the sacrificed rats were removed and homogenized in 0.3 mL saline, pH 7.4 with Turrax homogenizer for 10 seconds. The homogenate was placed in a freezer at -20°C for later analysis.[16]

On the day of the experiment, the samples were removed from the freezer and heated for 2 h at 60°C in an oven for inactivation of endogenous catalase activity. After heating, they were centrifuged at 12,000 g for 2 min. This centrifuged material was used for the assessment of MPO activity and nitrite concentrations.[17]

For the assessment of the MPO activity, 10 ìL of the supernatant was pipetted and added to 200 ìL of a buffer solution of potassium phosphate (pH=6) containing 0.164 mg/mL of o-dianisidinedihydrochloride and 0.0005% hydrogen peroxide (H_2O_2).[18]H_2O_2 was broken down by MPO released from tissue homogenates with0.5% hexadecyltrimethylammonium bromide detergent. The resulting oxygen radical combined with the o-dianisidine was converted into a colored compound. The formation of this compound over time was measured using a Hitachi 2000 spectrophotometer at 650 nm at 37ºC. Data were expressed as mU per mg of protein.[19]

Nitric oxide (NO) was quantified by detecting its stable metabolites, nitrate (NO_3) and nitrite (NO_2). The samples were deproteinized by adding 250 uL ofzinc sulfate (10%) and 350 ìL of sodium hydroxide (2.5 N). Then, 300 ìL from washed of the knee soft tissues were diluted in a 30 ìLsolution of ammonium formate (150mM) and 30 ìLsodium phosphate (50mM). The solution was incubated for 2h at 37°C, and then centrifuged (15000g, for 5 min). About 250 ìL of the supernatant was collected in a cuvette, and the same volume of Griess solution (sulfanilamide (1%) (w:v), phosphoric acid (5%) (v/v) and N-(1-naphthyl) ethylenediamine (0.1%) (w:v) was added and incubated for 10 minutes at room temperature. NO_2 reaction produced a pinkish tinge, which was quantified by measuring absorbance using a Hitachi 2000 spectrophotometer with a wavelength of 540 nm. Quantification of NO3 and NO2 expressed in ìM was determined by reference to a standard curve with previously known concentrations (0-150 ìM).[20]

Statistical anlaysis

The quantitative evaluation of knee swelling in the rats, and MPO and nitrite/nitrate levels were compared using analysis of variance (ANOVA), followed by Tukey's test for comparison of means, with a 95% confidence level.

Results and discussion

Eudragit/PCL-T 900 blend is transparent when pure (Figure 1). This feature can be altered by the presence of sodium diclofenacin the matrix. Two possible behaviors are expected from the addition of sodium diclofenac in the matrix: (a) crystallization of the drug during the production process, which would produce opaque films; or (b) solubilization of the drug in the polymeric matrix yielding transparent films.

It can be stated that the opacity of the matrix indicates the non-solubilization of sodium diclofenac in the polymer mixture and possible crystallization of the drug in the EUDPCL-T matrix.

Mechanical properties

The application of polymeric blends in pharmaceutical systems is dependent on their mechanical properties. For example, for transdermal therapeutic systems, highly elastomeric polymeric films are necessary to provide good skin coverage and adhesion. Furthermore, in the case of implantable devices, the ability to adapt to the implant site is essential.[21] Pure polymer films of EUDRAGIT RS 100 exhibit a plastic mechanical behavior, which is attributed to their semi-crystalline property. Although they are good candidates for the production of films, they are quite hard and require plasticizers to improve their mechanical properties.[22]

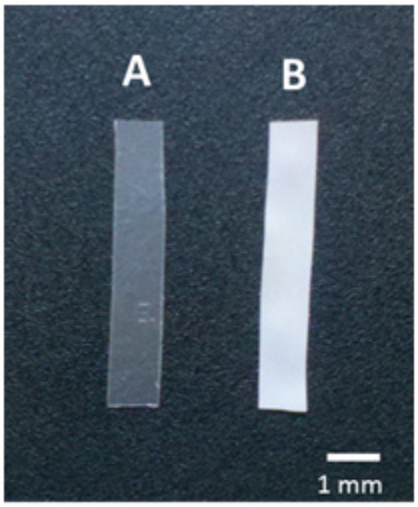

Figure 1: Photos from EUD/PCL-T (A) and EUD/PCL-T/Diclofenac implantable matrix.

Figure 2 shows the stress-strain curves obtained from DMA analysis at 37ºC form EUDPCL-T and EUD/PCL-T/Diclofenac devices measuring 11 mm x 1 mm.

The result shows that the EUD/PCL-T/Diclofenac device has a progressive elongation varia-

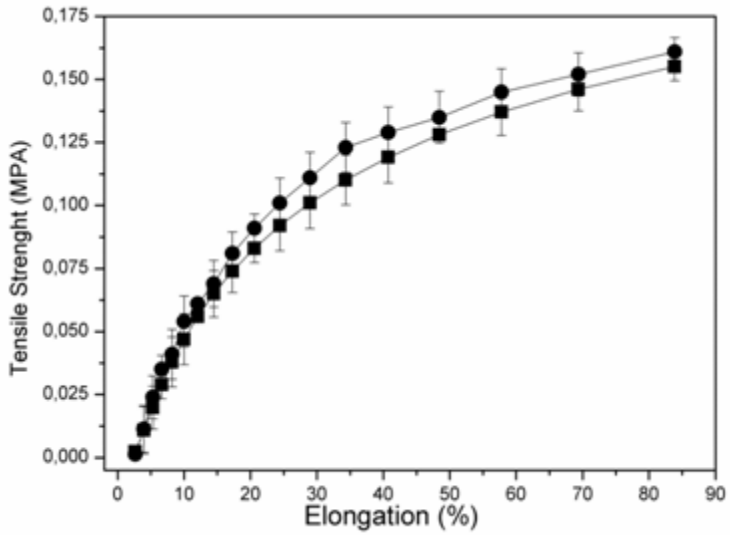

Figure 2 - Stress-strain curves obtained from DMA analysis at 37ºC form EUD/PCL-T/Diclofenac(%) and EUD/PCL-T (%) devices measuring 11 mm x 1 mm.

tion with increasing maximum tensile strength. This behavior is different from that described in the literature for pure Eudragit RS films, a semi-crystalline polymer. Pure Eudragit RS films show a maximum tensile elongation of 6% and have a maximum tensile strength of 0.150 MPa.[23]The EUD/PCL-T device showed a maximum tensile strength of 0.156 MPa, elasticity modulus of 0.86 MPa, and average capacity deformation up to 75% compared with the initial size of the specimen. No statistical difference ($p<0.05$) was observed between EUD/PCL-T/Diclofenac and EUD/PCL-T, showing that the Diclofenac content in the polymeric matrix did not change the mechanical properties.

Studies show that the PCL-T can provide a plasticizing effect on polymer materials.[24]This behavior is associated with reduced molar weight and the large number of hydroxyl groups in its chemical structure favoring the intercalation within the polymer chains, and the formation of chemical bonds between them. The chemical structure of Eudragit RS shows a large number of carbonyls available to make hydrogen bonds with the hydroxyl groups of PCL-T, which explains the plasticizing effect.[25]The deformation capacity of this material is essential for producing a device that will be implanted in the lateral region of the knee, a site of constant muscle movement.

Drug release

Figure 3 shows the release profile of sodium diclofenac from the EUDPCL-T films over a 25-day period.

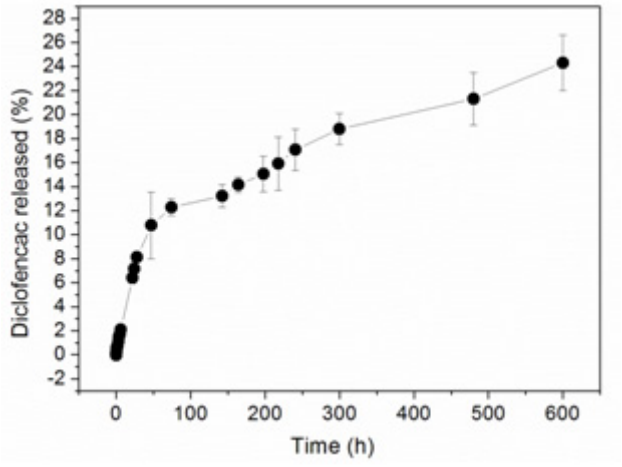

Figure 3: Release profile of sodium diclofenacfrom the EUDPCL-T devices at 37ºC

Drug delivery occurred in two phases. First, an accelerated phase that lasted about 48 hours with a mean release of 13%, which was associated with the dissolution and release of diclofenac available on the matrix surface. Second, a phase that showed a linear release behavior trend ($r = .99148$), with a constant release of 0.022%/h.

That diltiazem hydrochloride release of polyethylene-co-methyl acrylate matrices containing PCL-T increase when the study was performed at a temperature of 37ºC.[26]This was explained by the ability of PCL-T fusion at this temperature, a fact that favors the simultaneous diffusion of the drug and the PCL-T matrix. If the release of sodium diclofenac from the matrix was solely dependent on diffusion through the polymer matrix, the release would probably follow a Fickian behavior, which would cause a reduction in the amount of drug released due to time constraints. However, given that the assay with EUDPCL-T device was conducted at body temperature (37 ± 1.5ºC), the PCL-T melting and diffusion phenomena from the Eudragit matrix associated with the dissolution and diffusion of diclofenac favored the diclofenac release and provided a release mechanism after 48 hours following zero-order kinetics, then promoting the controlled drug release.

Anti-inflammatory activity of implanted device

Carrageenan-induced inflammation is acute, non-immune and biphasic. In the initial phase (0-1 h) the release of histamine, serotonin and bradykinin occurs. The subsequent phase (1-6 h) is correlated with increased production of prostaglandins, activation of COX-2 and nitric oxide release.[27] Carrageenan-induced arthritis is dose dependent and can produce a long-lasting effect in animal models. Intra-articular injection of 3 mg carrageenan reduces the latency of the mechanical withdrawal threshold, so as to heat significantly in 4 hours, and lasts for 7 weeks, whereas the effect of 1 mg dose lasts for 3 weeks and a dose of 0.3 mg lasts for 24 h.[28] The induced arthritis produces edema in the affected joint that can be measured by the joint diameter before and after the injection. In this model, the diameter of the joint injected with carrageenan reaches the maximum level within 8 hours after injection, and gradually decreases depending on the injected dose.[15,28]

Figure 4 shows the edema variation within 6 hours after carrageenan-induced arthritis in animals previously treated for 4 days with sodium diclofenac both orally and by the implanted devices.

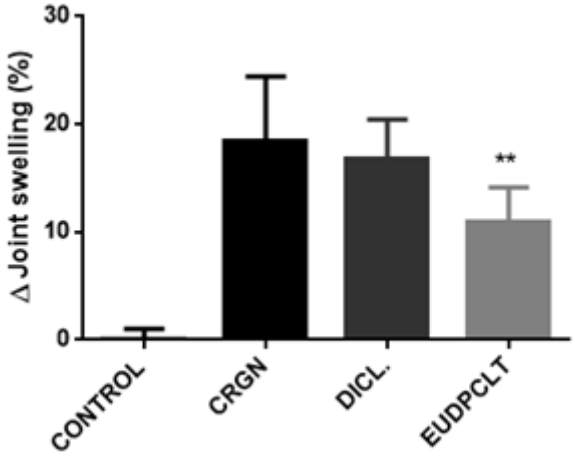

Figure 4: Variation in the carrageenan-induced knee edema in the DICL group ofWistar rats treated for 4 days with sodium diclofenac (30 mg/kg/day), EUDPCL-T group (implant containing 3.0 mg), CRGN and control groups 6 hours after arthritis induction. (N = 6, ** '' significant in relation to the CRGN group at p <0.05)

Figure 4 shows that DICL groups tended to have edema reduction when compared with CRGN groups, but no statistically significant difference was found (p <0.05). The EUDPCL-T group showed a statistically significant edema reduction compared with the CRGN group. The dose of sodium diclofenac administered orally was not enough to reduce significantly the edema at a stage in which arthritis induced by a high dose of carrageenan was very close to its peak.

Figure 5 shows the variation in the edema after 7 days of the induction of arthritis observed in animals treated for 11 days with sodium diclofenac orally and via the implanted device.

When measurements of the knee circumference were taken on day 7 after induction of arthritis, all treated groups showed statistically significant reduction in the edema compared with the group that received only carrageenan. This arthritis-induced model reaches an edema peak within 8 hours after injection, and gradually decreases. The statistically significant response to treatment with oral sodium diclofenac can be explained by the continuous administration of the drug.[28]

Based on these results, we may state that the anti-inflammatory effect of EUDPCL-T device may have been favored by the controlled release of the drug to the joint, favoring the diffusion to the joint capsule, and thence to the membrane and synovial fluid, where there is known affinity with sodium diclofenac and where the site of the drug action is located.[29]

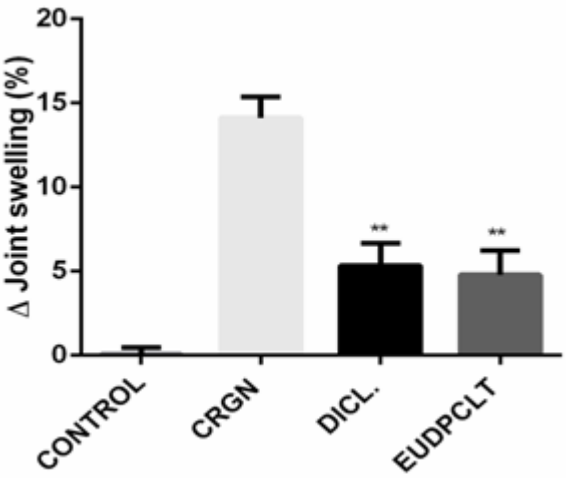

Figure 5: Variation in the carrageenan-induced knee edema in the DICL group of Wistar rats treated for 11 days with sodium diclofenac (30 mg/kg/day), EUDPCL-T group (implant containing 3.0 mg), CRGN and control groups 7 days after arthritis induction. (N =6, ** '' significant in relation to the CRGN group at p <0.05).

Table I shows the mean and standard deviation of the variation in MPO activity and nitrate/nitrite levels observed in animals at 6-hour and 7-day intervals after arthritis induction.

Six hours after arthritis induction, oral administration of diclofenac did not cause a significant reduction in MPO levels; however, a statistically significant reduction was observed in the EUDPCL-T group when compared with the CRGN group ($p<0.05$). Seven days after arthritis induction, both the DICL and EUDPCL-T groups showed statistically significant edema reduction compared with the CRGN group in decreased MPO activity. These results confirm what was observed in the evaluation of knee edema, indicating a higher activity of EUDPCL-T device when compared with oral administration of diclofenac.

Six hours after carrageenan-induced arthritis, the search for nitrates and nitrites in the DICL group showed no statistical difference when compared with the CRGN group. The EUDPCL-T group had a reduction in nitrate/nitrite levels, a statistically significant difference when compared with the CRGN group ($p<0.05$). On the seventh day after the arthritis induction, which is a late phase of the inflammatory process when these metabolites are already at low levels, there was no difference in the concentration of nitrates and nitrites between the groups. This was an expected behavior because the nitric oxide is identified as a mediator and regulator of inflammatory responses associated with vasodilation that occurs in the early stages of the inflammatory process.[20]

Conclusion

The polymeric device made of Eudragit RS, PCL-T and sodium diclofenac showed similar mechanical properties to elastomeric polymer products, which is associated with the plasticizing effect of PCL-T. The mechanism of sodium diclofenac release from the Eudragit and PLC-T matrix showed two release stages, the first rapid and the second with a zero-order kinetic behavior. The proposed implantable device was able to produce anti-inflammatory activity in the carrageenan-induced arthritis similar to that obtained with oral treatment, but with lower doses of the drug.

Table 1: Variation in MPO activity and nitrate/nitrite levels at 6-hour and 7 day after arthritis induction

	MPO (mg/g protein) ± sd			
Group	**Control**	**CRGN**	**DICL**	**EUDPCL-T**
6 hours	0.0079 ± 0.0011[a]	0.0240 ± 0.0078[b]	0.0183 ± 0.0022[b,c]	0.0153 ± 0.0029[c]
7 days	0.0079 ± 0.0011[a]	0.0209 ± 0.0041[b]	0.0137 ± 0.0012[c,d]	0.0109 ± 0.0013[a,d]
	Nitrates / Nitrites (units) ± sd			
	Control	**Carrageenan**	**Diclofenac**	**EUDPCL-T**
6 hours	0.0177 ± 0.0020[a]	0.0289 ± 0.0043[b]	0.0245 ± 0.0020[b,c]	0.0221 ± 0.0013[c]
7 days	0.0201 ± 0.0026[a,b,d]	0.0277 ± 0.0049[d,b]	0.0313 ± 0.0071[d,b,c]	0.0252 ± 0.0026[d,c]

Same letters meaning no statistical differences $p<0.05$

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