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Inclusion complex of piroxicam with β-cyclodextrin and incorporation in cationic microemulsion. In vitro drug release and in vivo topical anti-inflammatory effect

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Abstract

Topical formulations of piroxicam were evaluated by determination of their in vitro release and in vivo anti-inflammatory effect. The in vitro release assay demonstrated that the microemulsion (ME) systems provided a reservoir effect for piroxicam release. However, the incorporation of the ME into carboxyvinilic gel provoked a greater reduction in the release of piroxicam than the ME system alone. Anti-inflammatory activity was carried out by the cotton pellet granuloma inhibition bioassay. Topical anti-inflammatory effect of the piroxicam inclusion complex/ME contained in carboxyvinilic gel showed significant inhibition of the inflammation process (36.9%, $P < 0.05$). Subcutaneous administration of the drug formulations showed a significant effect on the inhibition of inflammation, 68.8 and 70.5%, $P < 0.05$, when the piroxicam was incorporated in ME and in the combined system β-cyclodextrin (β-CD)/ME, respectively, relative to the buffered piroxicam (42.2%). These results demonstrated that the ME induced prolonged effects, providing inhibition of the inflammation for 9 days after a single dose administration. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Piroxicam; β-Cyclodextrin; Microemulsion; Anti-inflammatory effect; In vitro drug release

1. Introduction

The interaction of drugs with organized structures (e.g. micelles, microemulsions (ME), liposomes and β-cyclodextrin (β-CD)) is an important subject in pharmaceutical field since these system can bind drug compounds which modify the stability (Oliveira and Chaimovich, 1992; Oliveira et al., 1990, 1991; Oliveira, et al., 1997; Loftsson and Brewster, 1996; Scarpa et al., 2000) and bioavailability of the compounds (Reddy and

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Udupa, 1993; Reginster and Franchimont, 1993; Stella and Rajewski, 1997; Canto et al., 1999).

In a previous work, we demonstrated the interaction of piroxicam with β-CD, cationic ME and ME in the presence of β-CD in order to optimize the drug delivery (Canto et al., 1999; Dalmora and Oliveira, 1999).

Cyclodextrins and ME have been studied as drug delivery systems because they can increase the therapeutic effect and reduce side-effects (Otagiri et al., 1983a,b; Duchene and Wouessidjewe, 1993; Pattarino et al., 1993). Cyclodextrins are cyclic oligosaccharides, with a hydrophobic cavity that allows the formation of an inclusion complex with lipophilic molecules, modifying the physicochemical properties of the drugs. Molecular complexes with cyclodextrins have been used to increase the aqueous solubility of non-steroid anti-inflammatory drugs, and also to improve the therapeutic effect by increasing the absorption rate of the complexed substance (Duchene and Wouessidjewe, 1992; Rajewski and Stella, 1996; D'Souza et al., 1997; Ammar et al., 1997, 1998; Piel et al., 1997, 1998).

ME are clear and thermodynamically stable preparations, which, depending on their internal structure, can allow the incorporation of hydrophilic or lipophilic compounds (Friberg, 1990; Attwood, 1994). These aggregates have been described in the literature as reservoir systems, allowing slow release of drugs and providing a prolonged effect which avoids high concentrations in the blood (Gasco and Morel, 1990; Pattarino et al., 1993). Since ME contain surfactant compounds in its composition, the application on the skin surface usually produces an increase in the membrane permeability facilitating transdermic transport (Friberg, 1990; Osborne and Amann, 1990; Walters and Hadgraft, 1990; Tadros, 1992). The literature shows that ME can control release and bioavailability of many drug compounds (Gallarate et al., 1990; Gasco et al., 1990; Pattarino et al., 1993). Since O/W ME are able to incorporate lipophilic substances, they can be used to facilitate the administration of water insoluble drugs (Bhargava et al., 1987).

Several authors have described the use of ME for topical application in therapeutic systems (Martini et al., 1984; Trota et al., 1989; Attwood et al., 1992), especially when formulated with balanced concentrations of surfactant/alcohol and oils of pharmaceutical use (Fubini et al., 1988, 1989).

Piroxicam is a non-steroid anti-inflammatory compound with analgesic and anti-pyretic effects. It is one of the most important therapeutic compounds and can be administered by both oral and topical routes. It has been used for rheumatoid arthritis, osteoarthritis and traumatic contusion treatment. However, because piroxicam is associated with gastrointestinal side effects (Pandey et al., 1992; Monteiro-Riviere et al., 1993; Marks and Dykes, 1994), it is important to develop therapeutic systems that will minimize these effects. It would, therefore, be of great value to develop formulations for topical absorption to benefit from the biopharmaceutical advantages of this route of administration (Osborne and Amann, 1990; Walters and Hadgraft, 1990; Lister et al., 1993).

Different in vitro and in vivo methods for topical evaluation of drugs have been described (Barry, 1983). For example, the in vitro release of drugs from topical preparations can be examined using the diffusion method with continuous or static flow, combined with natural (human or animal skin) or synthetic membranes (cellulose acetate) (Babar et al., 1990; Loftsson et al., 1991; Boltri et al., 1994).

The anti-inflammatory activity can be assessed by several methods, including the croton oil-induced dermatitis in mice, ultra violet (UV)-induced erythema in guinea-pigs, rat paw oedema and cotton pellet granuloma (Meier et al., 1950; Dorfman and Dorfman, 1965; Niemegeers et al., 1975; López et al., 1990; Germano et al., 1993). The procedures have been used for the investigation of topical and percutaneous anti-inflammatory effect of piroxicam (Schiantarelli et al., 1982; Reddy and Udupa, 1993).

The aim of this work, therefore was to assess the in vitro release and the topical biological activity of (a) an inclusion complex of piroxicam with β-CD, (b) piroxicam incorporated in cationic ME and (c) piroxicam derived from a mixture of both formulations.

Table 1
Stock formulation of ME

Components	Composition (%)
HTAB	30.05
Ethyl alcohol	30.05
Isopropylmiristate	6.6
Phosphate buffer, pH 5.5	33.30

2. Experimental section

2.1. Materials

Piroxicam (lot f301), was kindly donated by (ANSA, Milano, Italy). Stock solutions of piroxicam were prepared daily, maintained at 4°C, and discarded after use. Analytical grade *N*-hexadecyl-*N*,*N*,*N*-trimethylammonium bromide (HTAB), citric acid, phosphoric acid, sodium hydroxide, ethanol, sodium chloride, Tris–(hidroxymethyl)aminomethane *N*,*N*-dimethylformamide (Merck S.A., Brazil), 3-[morpholine]propan-esulfonic acid (Sigma Chemical Company, St. Louis, MO, USA), Carbopol 940® (BF Goodrich Company, Cleveland, OH), hexadecane (Aldrich Chemical Company, Milwaukee, WI), acetyl alcohol (Henrifarma S.A., Brazil), β-CD (USP XXII, Roquete), butyl stearate (Aquatec S.A., Brazil), Isopropyl miristate (Aquatec S.A., Brazil), ampicillin (Fontoura-Wieth, Brazil), cotton pellets (Johnson and Johnson, Brazil) were used as received. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Inclusion complex preparation

Inclusion complex was prepared as described previously (Dalmora and Oliveira, 1999) and the compositions of formulations used were presented in Table 2.

2.2.2. Microemulsion preparation

Selected weights of ethanol were added to solid HTAB followed by isopropyl miristate and aqueous buffer. This addition sequence enabled the solubilization of the surfactant with gentle stirring. The mixture was left for 10 min in thermostatic chamber at 25 ± 0.1°C (Dalmora and Oliveira, 1999).

2.2.3. Formulation preparation

The composition of the ME formulation is shown in Table 1, as previously described (Dalmora and Oliveira, 1999). The formulations F1–F8 were described in Table 2 and were prepared by the following procedure, the required weights of piroxicam and β-CD were transferred to a volumetric flask and buffer at pH 5.5 or buffered ME was added and maintained at constant stirring for 1 h. Carbopol 940® was added slowly, 1 h before each test.

2.2.4. In vitro drug release

The cell diffusion model described in the USP-24, was used holding the temperature of the water-bath at 37 ± 2°C. The dissolution apparatus, adapted for semi-solid pharmaceutical drug dosage forms was set up with the following condi-

Table 2
Formulations for in vitro and in vivo assay (w/w)

Components	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Piroxicam	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
β-CD	–	0.50	–	0.50	–	0.50	–	0.50
ME q.s.p.[a]	–	–	100	100	–	–	100	100
Phosphate buffer pH 5.5 q.s.p.	100	100	–	–	100	100	–	–
Carbopol 940®	–	–	–	–	3.00	3.00	3.00	3.00

[a] See Table 1.

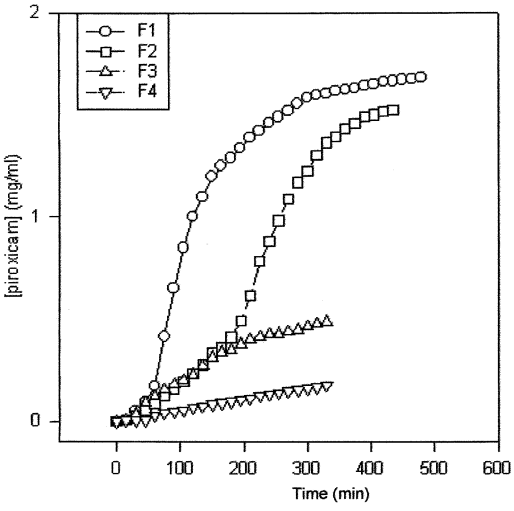


Fig. 1. In vitro release profiles of piroxicam through cellulose acetate membrane at 37°C, in phosphate buffer pH 5.5 (F1); complexed with β-CD in phosphate buffer (F2); in a ME (F3); complexed with β-CD in a ME (F4).

tions, the receptor (dissolution medium) compartment volume, 300 ml, 120 rpm; diffusion system with static cell. The receptor solution was 0.01 M phosphate buffer pH 7.4. The synthetic cellulose acetate membrane (7.54 cm), previously treated with distilled water 100°C, 5 min. and maintained at 4°C, was fixed the end of the glass cylinder of the diffusion cell. The experimental procedure was carried out using 2 ml of the preparations F1–F4 and 2.0 g of the formulations F5–F8. The analysis was performed with 2 ml samples withdrawn from the receptor phase at 15 min intervals. Piroxicam was analyzed quantitatively by UV–vis spectrophotometry at 350 nm.

2.2.5. In vivo anti-inflammatory effect

The anti-inflammatory effect of the formulations was assessed by the cotton pellet granuloma method, suggested by Meier et al. (1950), modified by Niemegeers et al. (1975), Germano et al. (1993). Wistar rats at 150–170 g were divided into groups of six animals. The animals were anaesthetized by ethyl ether inhalation. Sterilized cotton dental roll pellets weighing 40 mg each were implanted subcutaneously in four symmetrical positions at dorsal sites, which are housed individually. Piroxicam formulations F5–F8, corresponding to 4.0 mg/kg were applied topically on dorsal sites on the animals daily, for 6 consecutive days. On the seventh day, the granuloma weights were determined after drying in incubator at 60°C for 24 h. Control groups received Carbopol 940® in phosphate buffer pH 5.5.

The same procedure was followed with formulations F1–F4, detailed in the Table 2, using the diluted ME at 30% in phosphate buffer pH 5.5. The method was also used for the evaluation of the long lasting effect of the anti-inflammatory activity of formulations F1, F3 and F4. The cotton pellets were implanted in the abdominal region and a single dose of the formulations corresponding to 10 mg/kg was injected subcutaneously in each rat. The granuloma weights were determined on the days 3, 6 and 9. The results were analyzed by the Student's *t*-test at 5 and 10% significance level.

3. Results and discussion

The formulations F2 and F4 containing piroxicam–β-CD complex were prepared with a relation 1:1 (w/w) according to previous study (Dalmora and Oliveira, 1999). The results of the in vitro release assay (Fig. 1) show that the free piroxicam is released very quickly from the buffered formulation. The dissolution profile corresponds to saturation type curve, characteristic of these processes (Fig. 1). The formation of the piroxicam/β-CD inclusion complex modified the dissolution curve profile producing a little decrease in the release. This difference in the release can be explained because the complexation was obtained with a weight relation 1:1. Since the molecular weight of piroxicam is 331 and of the β-CD is 1135 the piroxicam fraction in the complex is 1/3.4, and to reflect the little observed effect. Analysis of the data shown in Figs. 1 and 2 indicate that the inclusion of carboxyvilinic polymer in the buffer system inhibits about 6-fold the release of free piroxicam in the same time. However, for the piroxicam/β-CD inclusion complex the addition of polymer inhibited about 26-fold the maximum release of piroxicam (Fig. 2).

This effect can be related to viscosity enhance by the polymer added in the system. In fact, in the presence of polymer the viscosity changed from 0.90/1.02 cP (F1–F4, without polymer) and to 18 020/18 200 (with polymer) causing the observed decrease in the piroxicam release.

The incorporation of piroxicam in the positively charged ME, although producing the same type of release profile, gave a maximum level about 3.6-fold lower than the control, over a period of about 300 min. Over the same time, the inclusion of carboxyvinilic polymer produced a reduction of approximately 55-fold in the release profile of piroxicam in the internal phase of the ME.

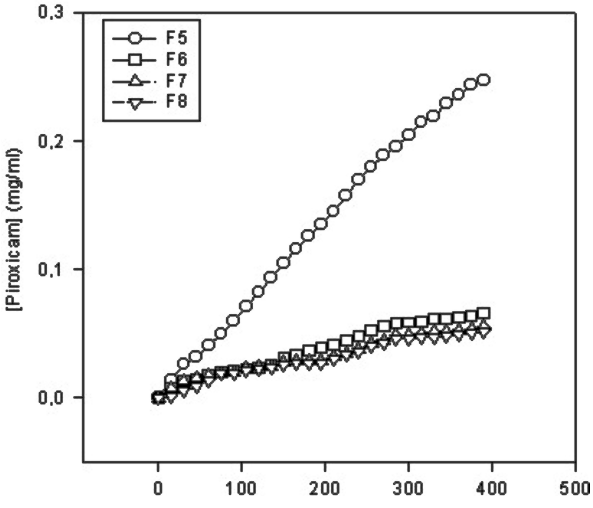
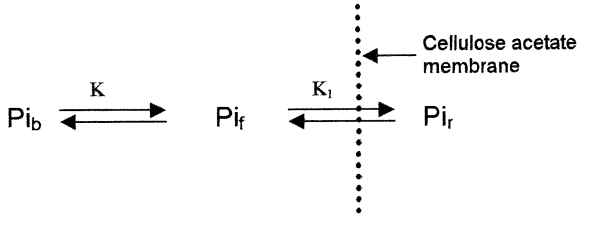


Fig. 2. In vitro release profiles of piroxicam in the presence of Carbopol 940® trough the cellulose acetate membrane at 37°C, in phosphate buffer pH 5.5 (F5); complexed with β-CD in phosphate buffer (F6); in a ME (F7); complexed with β-CD in a ME (F8).



Scheme 1.

The incorporation of piroxicam in the mixed system containing β-CD and the ME appeared to show the greatest effect on the release process. Analysis of the results shown in the Figs. 1 and 2, revealed that in addition to the piroxicam/β-CD complex affect in vitro release, the presence of ME produced additional inhibition of 2.8-fold relative to ME alone. So, the retention of piroxicam was of approximately ten times in relation to the control. More important characteristic observed in the systems containing ME was the retention capacity for piroxicam by the internal phase (oil) of the ME allowed a constant and regular release over a long time interval, when compared with the control. In all cases studied the inclusion of the carboxyvinilic polymer had a significant inhibition on the release profile, provoked by the increase of the viscosity of system.

This phenomena can be rationalized by the Scheme 1, were subscripts b, f and r represents the fraction of piroxicam associated with ME, free piroxicam and piroxicam released across the membrane, respectively. In the Scheme 1, if $K < K_1$, the free piroxicam do not accumulate in the membrane surface and the pass of drug release from ME can control all the process of drug delivery. In fact the presence of ME, produced a sustained in vitro release of piroxicam as a function of time, with an initial sharp rise in the slope of dissolution curve, followed by a non-discriminating (plateau) region. These results are important, since they can provide a basis on which to compare results of the in vivo evaluation.

For the assessment of the anti-inflammatory effect of the piroxicam formulations, the cotton pellet granuloma method, which is a chronic inflammation model, was applied (Germano et al., 1993). The method is robust and reproducible and the implanted pellet is capable of producing a large effect on the proliferate component of the inflammatory process (Swingle and Shideman, 1972). At the end of sixth day, the granuloma is characterized by the formation of a fibrous vascularized capsule containing fibroblast and mononuclear infiltrate cells (Bailey et al., 1982). Analysis of results is based on the fact that the reduction in granuloma mass is directly proportional to the sample.

Table 3
Effect of topical daily application of piroxicam formulations F5–F8, at a dose of 4 mg/kg for 6 days, on granuloma tissue formation[a]

Animals	Dry granuloma weight on seventh day (mg)				
	Groups				
	Control	F5	F6	F7	F8
1	372.0	244.0	231.0	284.0	256.8
2	410.9	223.0	254.0	281.1	248.0
3	400.0	228.3	268.3	267.00	270.2
4	397.3	213.0	229.1	230.0	229.0
5	409.0	218.5	222.0	260.0	246.1
6	352.4	214.0	235.0	248.7	227.7
Mean	390.3	223.4[b]	239.9[b]	261.8[b]	246.3[b,c]
S.E.	9.5	4.7	7.2	8.3	6.7
Inhibition (%)	–	42.7	38.5	32.9	36.9

[a] Control, carboxyvinilic polymer in phosphate buffer pH 5.5.
[b] ($P<0.05$), significantly different from control.
[c] ($P>0.05$), non-significantly different from F7.

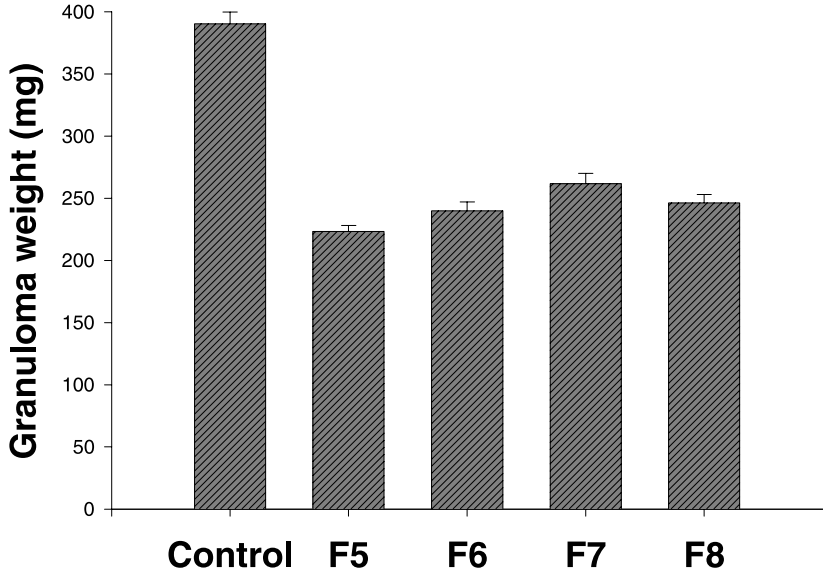


Fig. 3. Effect of daily topical application of piroxicam formulations F5–F8 (4 mg/kg) for 6 days, on the formation of granulomatous tissues. Control, carboxyvinilic polymer in phosphate buffer pH 5.5. The bar represents the mean ± S.E. of six rats.

The daily topical application of the piroxicam formulations in the dose of 4 mg/kg significantly inhibited granulomatous tissue formation, relative to control (Table 3 and Fig. 3).

The same procedure was carried out with F1–F4 (without carboxyvilinic polymer), administered by subcutaneous injection (4 mg/kg). The results in Table 4 and Fig. 4, showed significantly greater responses than that of the control ($P<0.05$). Interestingly, F3 and F4 inhibited the granuloma tissue formation to a similar extend ($P<0.05$) 68.8 and 70.5%, respectively, and were signifi-

cantly more potent than the other formulations. The data demonstrate that when the piroxicam is associated with ME system, the biological activity was similar to that of piroxicam–β-CD complex/ME system. However, the results of the in vitro dissolution assay, showed that F4 released about 6-fold less drug, thus providing the same effect 1/6 of dose. The process can be regarded as one in which a slow release rate is obtained when the drug is contained only in a reservoir region of ME (oil phase) provoking longer duration than F3. For the F3 preparation, a little fraction of the

Table 4
Effect of the daily subcutaneous injection of piroxicam formulations F1–F4, at a dose of 4 mg/kg for 6 days, on granulomatous tissue formation[a]

Animals	Dry granuloma weight on seventh day (mg)				
	Groups				
	Control	F1	F2	F3	F4
1	447.9	248.0	251.0	120.4	103.0
2	403.8	213.7	246.0	135.0	111.7
3	343.5	209.0	232.3	119.7	133.0
4	403.5	200.1	250.7	118.0	112.5
5	380.3	253.8	270.3	122.8	126.1
6	398.7	250.1	297.7	126.1	114.8
Mean	396.3	229.1[b]	258.0[b]	123.7[b]	116.9[b,c]
S.E.	13.9	9.8	9.4	2.5	4.4
Inhibition (%)	–	42.2	34.9	68.8	70.5

[a] Control, buffer phosphate pH 5.5.
[b] ($P<0.05$), significantly different from control.
[c] ($P>0.05$), non-significantly different from F3.

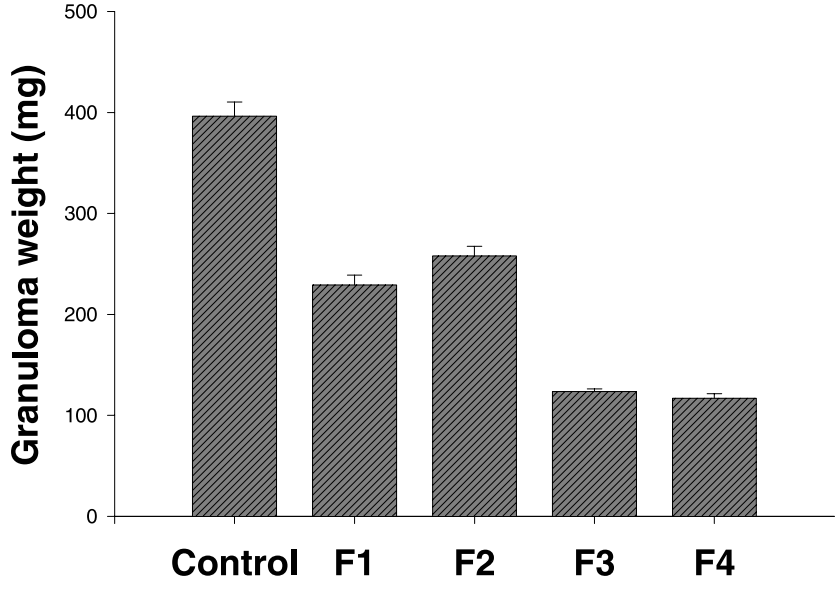


Fig. 4. Effect of the daily subcutaneous injection of piroxicam formulations F1–F4 (4 mg/kg), for 6 days, on granulomatous tissue formation. Control, phosphate buffer pH 5.5. The bar represents the mean ± S.E. of six rats.

Table 5
Effect of the subcutaneous injection of a single dose of piroxicam formulations F1, F3 and F4, corresponding to 10 mg/kg, on a granulomatous tissue formation[a]

Animals	Dry granuloma weight (mg)											
	Third day				Sixth day				Ninth day			
	Control	F1	F3	F4	Control	F1	F3	F4	Control	F1	F3	F4
1	267.1	200.0	170.0	190.3	375.4	330.0	158.30	155.3	424.6	387.5	336.0	298.2
2	286.5	187.5	146.3	160.5	360.8	360.3	170.4	180.0	466.6	459.4	272.0	310.3
3	232.4	190.0	131.2	163.4	400.0	280.5	190.2	128.9	459.3	442.3	311.7	270.5
4	270.4	220.3	142.0	191.4	390.2	295.8	130.4	139.0	480.3	406.6	249.0	296.3
5	251.0	201.0	150.5	180.3	305.0	300.3	145.5	170.0	438.0	379.0	308.8	350.5
6	240.1	211.5	144.3	144.7	355.0	290.3	128.4	171.3	457.0	429.0	245.3	330.5
Mean	257.9	201.7[b]	147.4[b,c]	171.8[b,c,d]	364.4	309.5[b]	154.0[b,c]	157.4[b,c]	454.3	417.3[b,f]	287.1[b,c]	309.4[b,c,e]
S.E.	8.31	5.1	5.2	7.6	13.5	12.2	9.8	8.2	8.2	12.9	15.1	11.5
Inhibition (%)	–	21.8	42.8	33.4	–	16.8	58.6	57.7	–	8.1	36.8	31.9

[a] Control, phosphate buffer, pH 5.5.
[b] ($P<0.05$), significantly different from control.
[c] ($P<0.05$), significantly different from F1.
[d] ($P<0.05$), significantly different from F3.
[e] ($P>0.05$), non-significantly different from F3.
[f] ($P>0.01$), non-significantly different from control.

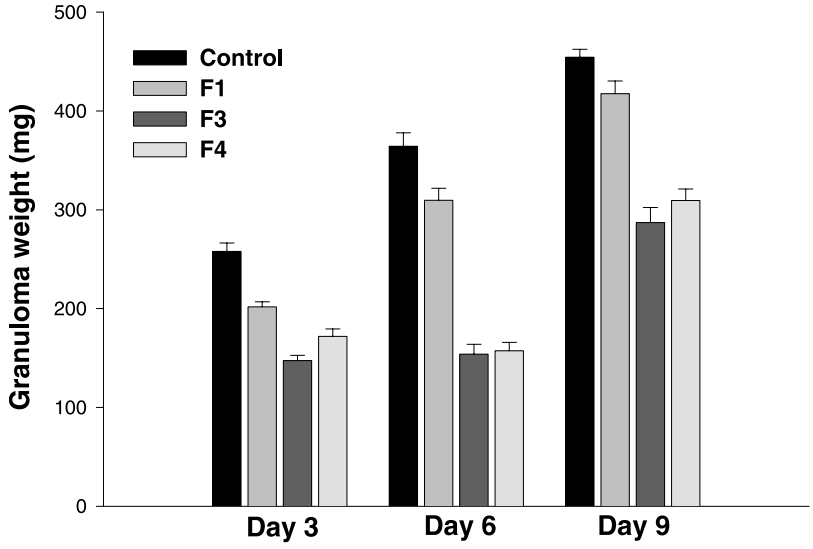


Fig. 5. Effect of a single subcutaneous injection of the formulations F1, F3 and F4 (10 mg/kg) on granulomatous tissue formation on days 3, 6 and 9. Control, phosphate buffer pH 5.5. The bar represents the mean ± S.E. of six rats.

piroxicam–β-CD complex, more soluble than free piroxicam, is located at oil/water interface of ME and can be slightly increase the bioavailability of the drug. However, both F3 and F4 formulations exhibited significant prolongation of pharmacological activity of piroxicam.

In order to investigate the duration of the anti-inflammatory effect F1, F3 and F4, was selected to assess the biological activity after administration (Fig. 5 and Table 5).

A single dose (10 mg/kg) of piroxicam formulation was injected subcutaneously and compared with buffered control. At the end of third day, all formulations exhibited anti-inflammatory activity significantly greater than the control group ($P < 0.05$), with F3 and F4 showing a greater effect than F1. After sixth day the formulations still maintained a more potent anti-inflammatory effect than control group. For example, as show in Table 5, the inhibitory activity of F3 and F4 increased from 42.8 and 33.4% on third day, to 58.6 and 57.7%, respectively, on sixth day. On the other hand, F1 on third day exhibited maximum activity of 21.8%, decreasing on sixth day to 16.8%. Similar results were also evident at the end of 9 days, when the inhibitory effect was not significantly relative to the control ($P > 0.01$). The results demonstrated the potential of the reservoir in vivo system following the use of a ME. The high degree of retention of the active substance can provide a means for modulating the anti-inflammatory effect, by greatly extending the release period relative to those formulations where the piroxicam is only dissolved or dispersed in a homogeneous aqueous medium. In conclusion, both ME and ME/β-CD can offer many promising features for their use as topical vehicle for piroxicam delivery.

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