Anti-inflammatory Activity of Curcumin in Gel Carriers on Mice with Atrial Edema

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Abstract: Curcumin is a bioactive compound with proven antioxidant and anti-inflammatory activities, but has low water solubility and dermal absorption. The inflammatory process is considered as the biological response to damage induced by various stimuli. If this process fails to self-regulate, it becomes a potential risk of cancer. The objective of this work was to evaluate the anti-inflammatory activity of curcumin administered to mice with induced atrial edema using two topical vehicles: organogels and O/W-type nanogels at pH 7. Organogels and O/W-type nanogels at pH 7 were prepared, characterized and the anti-inflammatory activity was assessed. A histopathological analysis of mouse ears was performed and two gel formulations were selected. Thermograms of organogels indicated that increasing the gelling agent improved the stability of the system. Deformation sweeps confirmed a viscoelastic behavior characteristic of gels in both systems. During the anti-inflammatory activity evaluations, the nanogels demonstrated greater activity (61.8 %) than organogels; Diclofenac[®] (2-(2,6-dichloranilino) phenylacetic acid), used as a control medication achieved the highest inhibition (85.4%); however, the drug produced the death of 2 (40%) of the study subjects caused by secondary adverse events. Histopathological analysis confirmed the data.

Key words: curcumin, nanogel, organogel, bioactivity, anti-inflammatory

1 Introduction

Inflammation is defined as a biological response to damage induced by certain stimuli, an essential mechanism for the body to maintain a homeostatic state. Although it is normally regulated, in some cases the process fails to function adequately leading to acute inflammation and becomes a potential risk to the organism. When an inflammatory response persists, a chronic inflammation diagnosis can be established, which may function as a trigger for several chronic pathologies¹⁾. The consumption and application of medicinal plants as treatments against regular or chronic inflammation has been widely used since ancient times, with reported beneficial effects. It is now known that specific bioactive compounds are responsible for the attributed health enhancing properties of these plants^{2, 3)}.

A bioactive compound is a product that provides health benefits when consumed, that may include anti-inflammatory, antioxidant activity, ROS and RNS scavenging, specific cytotoxicity, among others. These properties may aid in the prevention and/or mitigation of some chronic diseases⁴⁾. Curcumin, known as the "Indian gold", has been used to alleviate arrhythmia, and as an aid in the treatment of various illnesses including Alzheimer's, asthma, liver disease, and also as antioxidant, anti-inflammatory, cardio-protective, anticarcinogenic and chemopreventive agent⁵⁻⁷⁾. Curcumin has been reported to interfere in several mechanisms that control inflammatory responses, among which are: modulation of arachidonic acid metabolism⁸⁾, inhibition of cyclooxygenase 2 (COX-2) and lipoxygenase (LOX), two enzymes involved in inflammation. COX-2 induces cyto-

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kines that transform arachidonic acid in prostaglandins and thromboxanes during episodes of inflammation and platelet aggregation; whereas LOX transforms arachidonic acid into leukotrienes, which are involved in the recruitment of leukocytes by endothelial cells and their mobilization from the vasculature to damaged tissues⁶⁾. Curcumin is also able to inhibit COX activity by suppressing the activation of NF- κ B factor; this factor regulates the expression of COX-2 and inhibits the expression of the pro-inflammatory enzyme LOX-5, inflammatory cytokines, chemokines, nitric oxide synthase, and reduces the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF α)^{9, 10)}. This cytokine regulates the expression of pro-inflammatory genes¹¹⁾, and the expression of several inflammatory cytokines such as IL-1, and IL6, involved in inflammatory responses and further progression towards cancer¹²⁾. It is also a potent inhibitor of the production of free oxygen radicals, thereby reducing inflammation¹³⁾. Despite all the previously reported beneficial properties, curcumin has as a major drawback its low solubility in aqueous media, of only 0.6 μ g/mL. When 10-12 g/mL were orally administered to humans, curcumin serum levels did not exceed 50 ng/mL. This amount was below the suggested necessary concentration required to achieve significant therapeutic effects¹⁴⁾.

On the other hand, curcumin has a rapid biological degradation rate and is susceptible to breakdown in alkaline media. These traits characterize curcumin as a bioactive compound with low bioavailability, resulting in a suboptimal concentration in blood, not sufficient for reaching health-enhancing effects¹⁵⁾. Because of this limitation, the design of carriers capable of increasing its concentration in the body, solving the solubility aspect and protect this hydrophobic material, have been pursued^{16, 17)}. A gel system is highly biocompatible and biodegradable, and thus accumulation in the body is prevented. The gel preparation process is relatively easy since it does not require toxic reagents or catalysts nor generate byproducts, which makes them appropriate and favorable for biomedical applications.

2 Chemicals and Reagents

2.1 Materials and reagents

All chemical compounds and solvents used were analytical or HPLC grade; soy lecithin (98% purity, Shenyang, Liaoning, China) was selected as emulsifier. For nanoemulsions, curcumin (98% purity, LKT Laboratories, St. Paul, MN) was used; reactive grade glycerol (JT Baker, Mexico City). For gel formulations, nanogels required Carbopol 940[®] (Polyacrylic acid) (Lubrizol, Mexico City), whereas Phospholipon[®] 90 H (Phosphatidylcholine, hydrogenated), Lipoid, Ludwigshafen, Germany) was employed for organo-

gels as gelling agents; Diclofenac[®] (2-(2,6-dichloranilino) phenylacetic acid) was used as an anti-inflammatory drug. 12-O-tetradecanoylphorbol 13-acetate (TPA, Sigma-Aldrich, Mexico City) was employed to induce a localized inflammation on the ear.

2.2 Methods

2.2.1 Formulation of Curcumin nanoemulsions (CNEs)

CNEs were prepared using a dispersed:dispersant phase proportion of 5:95. The dispersed phase was prepared as follows: 25 mg/g NE of curcumin, 1 mL ethanol and 5% of commercial medium chain triglycerides (MCT, Original Thin Oil[®], Sound Nutrition, Dover, ID); this phase was placed in an orbital shaker (Thermo Scientific MaxQ 4450) for 30 minutes at 300 rpm and 45°C. The dispersant phase consisted of 25% glycerol, 5% phosphatidylcholine (PC) and water, manually mixed for 5 minutes. Both phases were sonicated in an Aquawave 9376 (Barnstead/Labline) bath for one minute and incorporated to homogenize for 3 minutes at 20,000 rpm in a T25 digital ULTRA-TURRAX homogenizer (IKA Works, Inc., Wilmington NC), producing a coarse emulsion. Immediately, ultrasonic emulsification was performed at 20% amplitude, using a Branson Digital S-450D sonicator (Emerson Electric Co., St. Louis MO), fitted with a 13 mm diameter probe for 3 minutes to obtain the nanometric droplets.

Droplet size was measured in a Nano-Zs90 dynamic light scattering device (Malvern Instruments Inc. Worcestershire, UK) with a 90° fixed angle at 25°C; samples were diluted at a rate of 1:200 with deionized water to avoid multiple scattering effects.

2.2.2 Formulation of Curcumin organogels (COGs)

To prepare organogels, Phospholipon 90, and curcumin were mixed with MCT in a 1.5 cm diameter tube. The samples were prepared by dissolving all components using a drying oven at 110°C, until completely fused. Then, the homogeneous mixture was stirred using a vortex and returned to the stove to melt and achieve complete homogenization; it was then set to cool at room temperature (25°C) and finally stored in a cooling chamber at 15°C¹⁸⁾.

2.2.3 Formulation of Curcumin nanogels (CNGs) at pH 7

In order to obtain formulated O/W- nanogels, Carbopol 940[®] was dispersed at a concentration of 0.5, 1 and 1.5%, and set to stand for 6, 12 and 24 hours, for a gel to develop^{19, 20)}. The resulting mixture was stirred at room temperature and neutralized with triethanolamine to attain a nanogel with a pH = 7²¹⁾.

2.2.4 Assessment of gel formation

The inverted tube method is the most commonly used to confirm gel formation. In this method, the gel was placed in a test tube; which was then carefully heated to 110°C for a complete melt. The tube was set to cool for 3 hours in a chamber set at 15°C and then inverted for one hour at room temperature^{22, 23)}. After the inversion, the absence of

flow indicated the formation of a gel, assuming that the sample formed the cross-linked network that prevents the flow of liquid trapped in the matrix²⁴⁾. The nanogels were also analyzed using this test.

2.2.5 Rheological Characterization

The rheology of the organogels was analyzed using a Discovery HR2 rheometer (TA Instruments, New Castle, DE) with a cone:plate geometry of 40 mm in diameter, and a 2° cone angle, equipped with a Peltier temperature control. To obtain the rheological measurements, the initial position of the orifice was set at 500 µm. However, according to the thickness of each organogel, the width of the gap was varied in order to use a normal force of zero during the measurements. The deformation sweep was carried out between 0.1 and 1000%, at 25°C and a fixed frequency of 1 Hz. Three stages were established: first, heating from 25°C to 110°C at a rate of 5°C/min; second, cooling from 110°C to 15°C, at the same rate; and, third, again from 15°C to 110°C. The formed nanogels were evaluated using the described methodology with some modifications; no changes in temperature were made.

2.2.6 Bioactivity of curcumin NGs

For *in vivo* studies, 35 mice (strain cd1) of 8 weeks of age (22–25 g) were kept under constant temperature (25 ± 0.5°C), 60% humidity, 12/12 light/dark periods and *ad libitum* fodder (Harlan Teklad Global 18% protein rodent diet 2018S) and water. Animal maintenance and handling were performed according to the NOM-062-ZOO-1999 and the NRC Guide for the Care and Use of Laboratory Animals (8th Ed., 2011). Seven groups were randomly formed (n = 5): group (A) as a healthy control; group (B) only TPA was applied as an edema control; group (C) administered with Diclofenac®; group (D) free curcumin in ethanol (FC) (10 mL); group (E) administered with CNGs; group (F) administered with COGs; group (G) administered with CHGs.

All test groups, except A, were subjected to an ear edema protocol in accordance to Stanley²⁵⁾, using multiple topical applications of TPA; maximum ear thickness was reached on the fifth day. The FC, COGs, CHGs and CNGs groups received the corresponding treatments in each TPA topic application. Mice were euthanized by dorsal dislocation six hours after the last treatment application.

2.2.7 Histological analysis

After euthanasia, dissection of swollen ear samples was performed by means of a punch; samples were preserved in 10% formaldehyde in preparation for histological analysis of all groups. A macroscopic description was made and three slices were taken from each sample, the slices were encapsulated in histocassettes and immersed in 10% formaldehyde. Subsequently, an automatic tissue processor was employed following a standard protocol that uses ethanol at different concentrations (70% 80% 90% and 100%); to achieve cell detachment; samples were rinsed with xylol.

Samples were submitted to inclusion by placing in a bath

of liquid paraffin at 60°C; tissue was removed from the histocassette and deposited on a mold where liquid paraffin was added; samples were fixed to the mold using manual pressure; additional paraffin was added and then placed on a surface at 4°C to remove the paraffin and proceed to histological cut in a microtome. Once the desired cuts were obtained, samples were observed in an Olympus BX51 microscope with an attached camera using the 10x and 40x lenses; cuts were examined in duplicate.

2.3 Statistical analysis

Data from the experimental designs were analyzed by means of ANOVA and comparison of means by Tukey's test, to identify pairwise differences between the different treatments, with differences indicated by different superscript letters, with a level of significance of $p < 0.05$ using the MINITAB statistical software v17 (Minitab, Inc. College Station, PA).

3 Results and Discussion

3.1 Development of COGs and inverted tube test

Developed COGs displayed positive results during the inverted tube test (Fig. 1A). Gel formation is caused by micellar level transitions to a low viscosity Newtonian liquid consisting of inverse lecithin micelles in the oil. A solid phase forms a rigid network interconnected with sub-micron size pores that trap and immobilize a liquid phase and resulted in the formation of a three-dimensional network. This is achieved by the presence of small amounts of water in non-aqueous solutions with soy lecithin, which causes a sharp increase in viscosity²⁶⁾.

3.2 Rheology of COGs

Table 1 depicts results obtained for the logarithm of G' (storage modulus), expressed in Pascals, as a function of the activation time and the concentration of Phospholipon 90 H, where G' had higher values at high concentrations of the gelling agent. When comparing the different formulations, it was determined that 25% of Phospholipon 90 H with 25 mg of curcumin and 25% of Phospholipon 90 H with 15 mg of curcumin, were the most elastic COGs obtained and were significantly different from the rest.

These findings suggest that even at low concentrations of crosslinker (Phospholipon 90H) a desired system can be obtained, as confirmed in Fig. 1B, where it can be noted that G' is over one order of magnitude greater than G'' .

3.3 Effect of formulation of curcumin NGs on their pH values

NGs had a translucent, yellow coloring with smooth appearance; depending on the amount of polymer present; higher amounts of polymer resulted in less softness and

Fig. 1 (A) Organogels developed during the inverted tube test according to the experimental design %phospholipon 90H/mg Curcumin, a) 25/15, b) 25/25 c) 35/5 d) 35/15 e) 35/25. (B) Curcumin organogel rheogram, where a lineal viscoelastic region can be observed, also G'' loss modulus and G' storage modulus can be observed. C) Nanogels formulated with different concentrations of curcumin, a) 1.5%, b) 1% and c) 0.5% developed during the inverted tube test (D) Nanogel deformation sweep, the linear viscoelastic interval is indicated in the selected region.

Table 1 Logarithm of the storage module of the different concentrations of gelling agent in the organogels developed and shown in Fig.1 % phospholipon 90H/mg curcumin, a) 25/15, b) 25/25, c) 35/5, d) 35/15, e) 35/25.

% Phospholipon 90 H/ mg Curcumin	$\ln G'$ (Pa)
25/25	13.8 ^A
25/15	12.3 ^B
35/25	11.2 ^C
35/15	11.2 ^C
35/5	9.0 ^D

Entries that do not share the same letter are significantly different.

smaller Pa values (Fig. 1C); pH values were determined using a pH meter; all formulations had pH values ranging from 6.9 to 7.5, close to that reported by Phatak and Chaudhari²⁷; these values are considered acceptable to avoid the risk of irritation after skin application. At a pH value closer to 6, a gel using Carbopol 940[®] had a higher viscosity (lower Pa values) and bio-adhesiveness, most likely caused by a complete ionization of the polymeric main chain. This condition produces a repulsion between the native charges that in turn increases polymer swelling up to 1000 times its original volume, which results in stronger gels. These results are similar to those reported by

Chaudhary and co-workers²⁸, who developed a transdermal curcumin gel using Carbopol 940[®] with and without penetration enhancer, and performed *in vitro* permeation studies through skin cell lines. The above authors succeeded in developing the transdermal curcumin gels using Carbopol 940[®] as a release control polymer; these systems showed promising results.

3.4 Rheological characterization

Gel elasticity of curcumin NGs prepared by Carbopol 940 was examined. Then, gel elasticity (higher Pa values) increased with higher polymer concentration as shown in Table 2. Activation time was not considered for the Tukey analysis depicted in the table, because significant differences were observed in preliminary tests. This information concurs with the report by Islam and co-workers²⁹ who formulated gels by mixing Diclofenac[®] with curcumin. In Fig. 1D the loss modulus (G'') can be observed; this value represented less than 20% of the elastic modulus, indicating complete elasticity in the system. As deformation increased, elastic structures of the nanogels decreased abruptly. This behavior is consistent with another study²⁹ that performed a rheological characterization of gels containing Carbopol 940[®] as a crosslinker. As indicated by the author, smaller spheres lead to a smaller rupture tension; similarly, it could be observed, as Fig. 1 on this work indicates, before G' crossed with G'' , the loss modulus exhibited the characteristic waviness of viscoelastic systems.

Based on the results obtained in the development of the

Table 2 Storage module for formulated CNGs formulated with different concentrations of carbopol 940® a) 1.5%, b) 1%, and c) 0.5% with 6 hours of activation time.

Treatments	G' (Pa)
1.5%	24 h 528.6 ^A
	12 h 479.6 ^B
	6 h 518.6 ^A
1%	24 h 445.1 ^C
	12 h 477.0 ^B
	6 h 461.1 ^{BC}
0.5%	24 h 279.1 ^E
	12 h 257.5 ^{EF}
	6 h 339.9 ^D

Entries that do not share the same letter are significantly different.

Fig. 2 Percentage of auricular edema inhibition from the treatments applied on the murine model. Bars that do not share a letter are significantly different.

CNGs and their rheological analysis, the CNG with the lowest concentration of Carbopol 940® was chosen among all the formulations because of the texture obtained once the system was neutralized, allowing a better application since it was less elastic. Higher concentrations of Carbopol 940® resulted in systems that were difficult to manipulate because of their hardness (higher Pa values); also, as there were no significant differences with respect to time, the CNG with the shortest activation time was employed for the *in vivo* assessment. The final Carbopol 940® concentration used for the system that was applied to the mice was in agreement with that used in a previous study where vitamin A palmitate-carrying hydrogels (unlike the CNG hydrogels have no nanometric particles, and contain only water, carbopol and curcumin) were developed, obtaining viscous systems³⁰⁾.

Table 3 Weight (mg) and Tukey analysis of dissected mouse ears, to which treatments were applied.

Group	Weight (mg)
Healthy control	9.90 ± 0.7 ^a
2-(2,6-dichloranilino) phenylacetic acid	11.2 ± 0.8 ^{ab}
Nanogel (CNGs)	13.3 ± 0.6 ^{bc}
Organogel (COGs)	14.7 ± 0.8 ^{cd}
Free Curcumin (FC)	15.3 ± 0.6 ^d
Hydrogel (CHGs)	16.3 ± 0.8 ^{de}
Edema control	18.9 ± 0.6 ^e

Means that do not share the same letter are significantly different.

3.5 Bioactivity of curcumin NG

To assess the effects of the various treatments (CNGs, COGs, Diclofenac®, CHGs and FC) on atrial edema; this condition was induced by multiple applications of TPA to produce a localized inflammation in the ear. The anti-inflammatory response was determined by the degree of swelling of the treated ears compared to ears with no treatment³¹⁾. The results are expressed as the weight of the ear and as a percentage of inhibition, in contrast to the control group that did not receive any treatment. Comparison of the weight means of the ears from different treatments was carried out (Fig. 2). Healthy mice that were only given Diclofenac® had the smallest means, and showed the smallest edema. On the other hand, mice treated with the CNGs were the only subjects that did not have significant differences to the reference drug. In the case of the COGs and FC, the response of the mean values was in an intermediate scale, resulting in statistical similarity. However, mice treated with the CHGs had the highest mean value of the treatments, and were no significantly different to mice treated only with TPA. When analyzing the percent inhibition of established treatments, we observed that CNGs had a higher anti-inflammatory activity (61.8%) than COGs (45.9%), FC (33.2%) and CHGs (28.1%). Diclofenac® accomplished the highest inhibition (85.4%); however, it caused the death of 2 (40%) of the animals. The lethal effect of Diclofenac® has been associated with up to 40% of increased probability of cardiovascular risks, as it has been reported in several studies^{32, 33)}.

Also, it is highly recommended to use the drug only for short periods of time and, if possible, avoid it. **Table 3** shows the weights and percent inhibition of the different treatments. The results obtained are comparable to a study conducted by Sun and co-workers³⁴⁾ who investigated the effect of a transdermal curcumin gel in a model of atrial edema induced by TPA in K14-VEGF transgenic mice. The increase in bone thickness was employed to indicate the extent of inflammation. As in our study, on days 2-4 after

the start of treatment with TPA, ear skin started to display signs of thickening and erythema development with increased severity that peaked on days 6-10.

H&E staining analysis results from ear skin samples treated with TPA showed thickened subcutaneous tissue and mild epidermal enlargement compared to the control group (Fig. 3A, B). The increase in thickness was attributed to the rise in the number of inflammatory cells in the subcutaneous layer. In another study³⁵, the anti-inflammatory activity of curcumin was evaluated *in vitro* using a septic shock model induced by LPS. Exosomes (30-100 nm nanoparticles secreted by cells in the extracellular environment) formed a complex with curcumin. Exosomal curcumin potentiates apoptosis in CD11b + Gr-1 + cells and regulates the activity in several crucial transcription factors in inflammation, including NF- κ B, STAT3 and Nrf2; therefore, additional biological effects on cell proliferation, apoptosis, induction of cytokines and an antioxidant effect can also contribute to the prevention of induced septic shock³⁵.

3.6 Histopathological analysis

To verify our data, histological sections of the ears were

prepared and examined using an optical microscope. The group treated with TPA showed a chronic and acute inflammation with moderate ulceration, i.e., loss of continuity of the epithelium by chemical effects, clear evidence of epidermal hyperplasia, edema, and dermal inflammatory cell infiltration along with possible disruption of connective tissue (Fig. 3A, B); these observations concur with those of Paul & Kang³⁶. It is possible to notice a disarrangement in cellular structures with the presence of multinucleated cells through microscopical observation³⁷. Ear portions obtained from the group treated with Diclofenac[®] (Fig. 3C, D) showed similar cellular structure to the healthy control group. Despite the fact that better results were observed compared to the other treatments, and as mentioned above, 40% of the animals of this group died before the end of the experiment. Their demise could have been caused by the adverse effects of the drug, as previously mentioned. In a meta-analysis work it was concluded that Diclofenac[®] increased the risk of cardiovascular complications when compared with a placebo. In view of well-established damage and duration of treatment, intermittent, short-term use, a dose of less than 75 mg/day was recommended. Diclofenac[®] has been associated with a statistical-

Fig. 3 Histological analysis of the mouse ear stained with H&E: (A,B) Sick control group at 10x and 40x; (C,D) Healthy control group at 10x and 40x; (E) Nanogel treated group at 10x; (F) Organogels treated group at 10x; (G) Free curcumin treated group at 10x; (H) Hydrogel treated group at 10x. Epidermis(1), Dermis(2), Elastic cartilage(3), Striated muscle(4), Sebaceous glands(5), Hair follicles(6).

ly significant 22% increase in risk at low doses³⁸). The group treated with the CNGs displayed the best results out of the proposed treatments, with only an incipient acute inflammation in the dermis.

This result may be attributed to the CNGs ability to increase curcumin release and the data is similar to that of Al-Rohaimi³⁹, who evaluated the anti-inflammatory effects of curcumin in a nanogel, and compared with a Diclofenac[®] gel in a plantar edema induced by carrageenan in rats. After its application, the inhibition % of the nanogel did not show significant difference with the commercial gel (78.54% and 81.13%, respectively). In this study it was also proven that curcumin had permeability problems if particles had a larger size; so, the nanogel increased permeability and effectiveness of the bioactive compound. The diagnosis proposed that this group had a mild chronic inflammation (Fig. 3E). Although the inhibition was 61%, it was smaller than values reported by Wang and co-workers⁴⁰, in which curcumin was administered through a nanoemulsion and its effect was tested on atrial edema induced by TPA, and obtained an 85% decrease in ear weight.

The COGs produced a slight loss of inflammation; when analyzing the histological section, severe chronic and acute inflammation could still be observed (Fig. 3F), with a clear increase in cellularity in the stroma, which was secondary to the presence of an inflammatory infiltrate and foci, thus increasing the sub-epithelial inflammatory infiltrate. The 45% inhibition of auricular edema produced in this study contrasts with the results obtained by Esposito and co-workers⁴¹, who compared the preventive effect of applying a curcumin organogel and an aqueous dispersion of monoolein on skin to be later exposed to UV radiation and measured the degree of erythema. The results showed that the organogel inhibited 50% of skin damage in comparison to 10% by the aqueous monoolein dispersion.

Huang and co-workers⁴², demonstrated that the application of 1 µL of curcumin before a single application of TPA inhibited the induction of edema by 99%. Additionally, Yadav and co-workers⁴³ applied topical curcumin, which significantly inhibited the inflammation induced by TPA, and resulted in reductions in ear redness. Ears treated with FC had a decline in atrial edema, which exhibited significant differences compared to the group with edema. However, acute and chronic inflammation was also observed, as well as micro-abscesses, as shown in Fig. 3G.

Finally, the group treated with the CHGs displayed severe inflammation, as well as abscesses that could be distinguished in the epidermis and corneal layer. An acute predominant inflammatory infiltrate was identified in the thickness of the dermis, which extended to the muscular layer (Fig. 3H). This group did not show significant differences with the group treated only with TPA, because the system showed a high viscosity and did not manage to

remain in the damaged site or achieve the desired effects. This result is lower than that reported by Al-Rohaimi³⁹, who used a hydrogel in a plantar edema induced by carrageenan and achieved an inflammation inhibition of 40%; however, this work was preventive, whereas our multiple application model of TPA was focused as curative.

4 Conclusions

Successful formulation of organogels and nanogels was achieved and used as vehicles to carry curcumin for topical application on a model mouse ear edema. Thermograms displayed a linear growth in enthalpy with respect to the concentration of the gelling agent used in COGs. By means of the deformation sweeps, both viscous and elastic behavior were confirmed in both systems. CNGs had a greater anti-inflammatory activity (61.8%) than the COGs (45.9%), FC (33.2%) and CHGs (28.1%). Diclofenac[®] achieved the highest edema inhibition (85.4%); however, 40% of the study subjects in this group died from exposure to the drug.

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