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RESEARCH ARTICLE

Novel Skin Drug Delivery Technology

Self-assembling Organogels Loaded with Tenoxicam for Local Intensive Pain and Inflammation Cure: *In Vitro* and *In Vivo* Correlation

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Abstract

Due to tenoxicam (TX)'s poor aqueous solubility (0.072 mg/ml), it is poorly absorbable in the GIT, and the long-term oral administration of TX may cause severe GIT disturbances. Topical administration of TX can help in bypassing the GIT adverse effects. Therefore, in the present work, we constructed different pluronic/lecithin organogels (PLOs) for topical delivery of TX. PLO was constructed simply via direct mixing of an aqueous pluronic solution with lecithin solution. The prepared PLO formulations were characterized for their physicochemical properties including pH, drug content, visual inspection, viscosity, and spreadability. Also, the *in vitro* release and kinetic studies were carried out to investigate the mechanism of drug release. Moreover, the *in vivo* studies were carried out by investigating the anti-inflammatory and analgesic activities using albino male rats. The results showed that the modified PLOs have good physicochemical properties. The viscosity of the modified gels is a direct proportionality with both lecithin and pluronic concentrations. Also, subsequently, the drug release rate is directly proportional to gel viscosity. Moreover, the *in vivo* studies showed that the modified PLOs (F19) showed a significant ($<0.05\%$) paw edema inhibition and pain analgesia compared with other investigated groups. Also, the results indicated that the increase in dose is accompanied by higher activity and a longer duration of action which extended to 12 h. Hence, the modified PLOs are promising safe candidates or vehicles for effective TX loading with sustained delivery behavior.

Keywords analgesia · anti-inflammatory · lecithin · PLO · pluronic · tenoxicam · *in vitro* · *in vivo* · viscosity

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Introduction

Tenoxicam (TX) is one of the non-steroidal anti-inflammatory medications that belongs to the oxicam class of non-selective cyclooxygenase (COX) inhibitors, as illustrated in Fig. 1 [1]. It is commonly used to treat rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout, extra-articular diseases, tendonitis, and other non-articular rheumatic problems [1]. It has high permeability and poor aqueous solubility (0.072 mg/mL). Long-term oral administration, on the other hand, causes mild and reversible GIT disturbances, peptic ulcers, GIT bleeding, renal impairment, and hepatic impairment [2]. Other routes of administration, such as topical administration of the medicine onto or through the skin, might solve these issues. To attain this goal, several types of topical formulations have been developed.

The use of transparent gels has extended and may be classified into hydrogels and organogels based on the kind (aqueous or organic) of the external liquid component or vehicle. For hydrogels, the vehicle or external liquid component is water, while in the case of organogels, it is a non-polar solvent such as isopropyl myristate (IPM). Because of their ease of production and inherent long-term stability, organogel-based products are becoming more popular. Moreover, organogels have the benefit of improved pharmaceutical ingredient delivery via multiple routes such as oral, parenteral, topical, and transdermal [3–5]. Figure 1 illustrates the chemical structures of lecithin, pluronic F127, and IPM together with TX.

Lecithins are yellow-brownish amphiphilic fatty substances extracted mainly from egg yolk and soybeans [6, 7]. They possess both hydrophilic and hydrophobic properties. They were utilized for the emulsification and homogenization of immiscible liquids. Although they have low water solubility, they are considered excellent emulsifiers and surfactants since, in an aqueous solution, their phospholipids can form micelles or liposomes depending on the temperature and the extent of hydration [8, 9]. Interestingly, It was reported that the addition of water (trace amounts) into lecithin solution led to a huge increase in the system viscosity (˃ 10,000-fold), producing a sol–gel transition (i.e., formation of 3D networks of entangled cylindrical micelles, immobilizing the continuous phase, and thus convert from liquid to viscous gel) [10–12]. This phenomenon was utilized for the construction of lecithin organogels (LOs) in more than one article [13, 14].

LOs provide a higher extent of drug penetration through the skin compared with other hydrophilic vehicles (hydrogels). It was reported that the skin penetration rate of aceclofenac from LO increases is higher (two times) than that from Carbopol-940 hydrogel [15]. Moreover, it was stated that the penetration ability increased with the increase of lecithin content in the modified gel [16].

Figure 2a illustrates the mechanism of hydrogel formation from lecithin in an organic solvent such as IPM (isopropyl myristate, as illustrated in Fig. 1). Pluronic F127 is a triblock copolymer of polyethylene oxide (PEO)-polypropylene oxide (PPO)-polyethylene oxide (PEO-PPO-PEO). Pluronic was utilized together with other polymers such as chitosan in the formation of hydrogels [17–20]. It was reported that pluronic could form 3D thermo-reversible networks in concentrations between 15 and 30% w/v [21]. Interestingly, pluronic F127 exists in a liquid state at 4°C and forms a gel at room temperature. Pluronic alone was utilized for

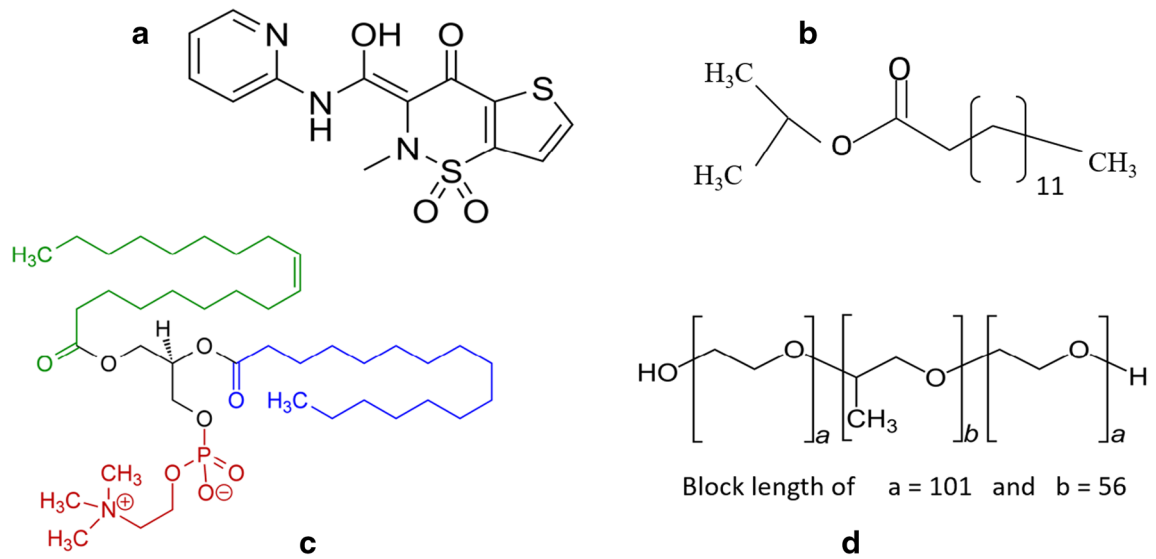

Fig. 1 Chemical structures of **a** tenoxicam (TX), **b** isopropyl myristate (IPM), **c** lecithin, and **d** pluronic F127

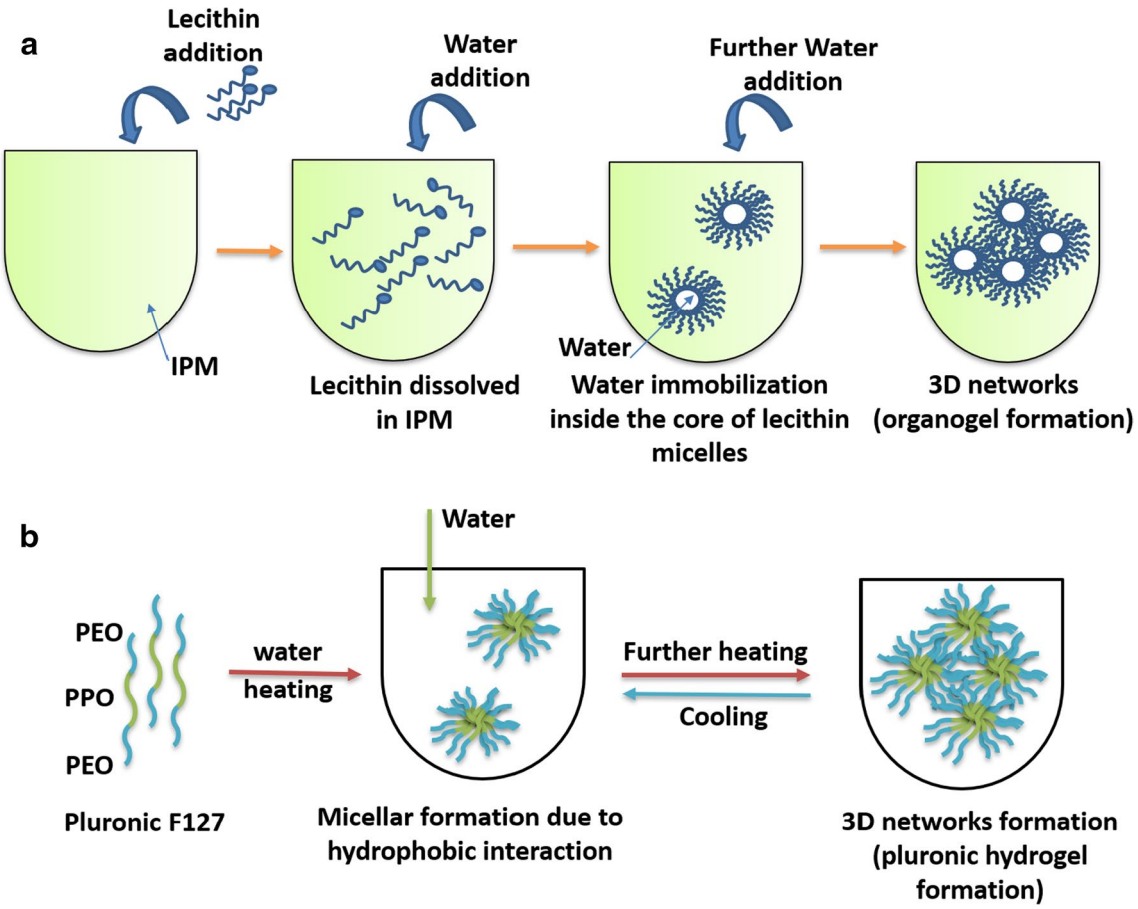

Fig. 2 A schematic representation of the technical steps for the construction of the organogels of our investigations. **a** Arrangement of lecithin molecules in micellar systems and gel formation upon addition of aqueous solution; and **b** the mechanism of thermosensitive pluronic F127 hydrogel formation via temperature elevation

the delivery of several kinds of drugs [15, 16, 19, 22, 23]. Figure 2b illustrates the mechanism of hydrogel formation from pluronic F127 in an aqueous medium.

In 2001, Crandall and coworkers [24] described for the first time and published a new patent for the formulation and application of pluronic-lecithin organogel (PLO) system as an effective delivery system for anti-psoriatic agents. The modified formula is composed of a suitable amount of pluronic solution and lecithin solution (in IPM or IPP).

Pluronic lecithin organogel (PLO) is a lipid-based organogel formulation that disturbs the stratum corneum's lipid layers without harming them [10]. PLO gel is a microemulsion-based gel composed of an oil phase (lecithin dissolved in oil as IPP or IPM) and an aqueous phase (pluronic F127 aqueous solution with or without short-chain alcohol) [25]. The advantages of such gel systems include their biocompatibility, thermodynamic stability, and viscoelastic behavior. In addition, they possess selective and localized therapeutic efficacy. Because of their coexistent organic and aqueous phases by micellar networks of phospholipids, large interfacial area, ability to entrap different solutes within the gel matrix, long-term stability, organized microstructural matrix, and super-solubilizing capacity, PLOs are considered excellent carriers for both hydrophilic and lipophilic drug molecules [25, 26]. Moreover, due to the existence of lecithin in the PLO composition, it improves the skin penetration and transport of drugs into or across the skin. PLO was utilized for topical administration of local anesthetics and nonsteroidal anti-inflammatory medications (NSAIDs) [27]. Also, the topical administration of diclofenac and ketoprofen in the form of PLOs was reported to relieve pain in a particular region [28, 29].

Accordingly, the objective of this research was to develop and evaluate PLOs loaded with tenoxicam to reduce their undesirable side effects and improve their topical activity. The investigation was expanded to investigate the effect of lecithin concentration, oil phase composition, pluronic F127 concentration, release medium pH, drug concentration, and penetration enhancers on TX release. Moreover, the anti-inflammatory and analgesic effects of the formed organogels were investigated.

Materials and Methods

Materials

Tenoxicam (TX) was generously donated by the Global Napi Pharmaceutical Co. (Cairo, Egypt). Lecithin, pluronic F127, and carrageenan type I were provided by Sigma-Aldrich Co. (St. Louis, USA). Methylparaben, isopropyl myristate (IPM), and polyethylene glycol 300 Da (PEG 300) were obtained from Merck Schuchardt OHG (Hohenbrunn, Bavaria, Germany). Polysorbate 80 (Tween 80), oleic acid, sodium chloride, and standard cellulose membranes (MWCO 14 kDa) were provided from Carl Roth GmbH Co. (Herrenberg, Germany).

Methodology

Preparation of Pluronic Lecithin Organogel (PLO) Containing Tenoxicam

In the present study, different PLO formulations were constructed, as listed in Table I, Generally, PLO consists of two phases: an oil phase (lecithin in IPM) and an aqueous phase (distilled water containing pluronic, PEG300, and penetration enhancers) [30, 31]. Typically, the aqueous pluronic solutions were prepared in ice-cold water at various concentrations of pluronic F127, agitated constantly, and refrigerated overnight (at 4°C) for full pluronic F127 dissolution. As a preservative, 0.2% w/v methylparaben is added. On the other hand, the oil phase was prepared by dissolving lecithin at several concentrations in IPM. The prepared organic phase was stored overnight for full lecithin dissolution. The PLO gel was constructed by combining both the oil and aqueous phases via dropwise addition of pluronic solution to lecithin with continuous gentle stirring to avoid air incorporation. The temperature should be kept between 18 and 22°C [30, 31]. The loading of TX was achieved via simple drug incorporation into the constructed PLOs. The schematic representation of TX pluronic lecithin organogels formulation is illustrated in Fig. 2a and b.

Table I The ingredients in g% w/w utilized in PLO formulations

Code	TX (g%)	PEG (g%)	Lec./IPM (Oil phase g%)		Pluronic F127solution (g%)			(g%)		
			Lec	IPM	PL1	PL2	PL3	Tween 80	Oleic acid	PG
F1	0.5	4	12.65	30	70					
F2	0.5	4	22.65	50	50					
F3	0.5	4	3	30	70					
F4	0.5	4	5	30	70					
F5	0.5	4	7	30	70					
F6	0.5	4	9	30	70					
F7	0.5	4	3	30		70				
F8	0.5	4	3	30			70			
F9	0.5	4	5	50	50					
F10	0.5	4	7.5	50	50					
F11	0.5	4	10	50	50					
F12	0.5	4	12.5	50	50					
F13	0.5	4	15	50	50					
F14	0.5	25	3	50	50					
F15	0.5	25	5	50	50					
F16	0.5	25	7	50	50					
F17	0.5	25	3	50	50			5		
F18	0.5	25	3	50	50					5
F19	0.5	25	3	50	50				5	
F20	1	25	3	50	50				5	
F21	3	25	3	50	50				5	

TX tenoxicam, *PEG300* polyethylene glycol 300 Da, *IPM* isopropyl myristate, *PG* polypropylene glycol, *Lec* lecithin; PL1, PL2, and PL3 mean pluronic 127 at different concentrations 20 g%, 25 g%, and 30 g% w/v, respectively

Organogel Characterization

Physical Appearance

The physical properties of the TX organogels, such as clarity, fluidity, homogeneity, and phase separation, were visually investigated.

Drug Content

Drug content determination was carried out to ensure gel homogeneity. Typically, 0.5 g PLO samples ($n = 3$) was dissolved in 100 mL methanol. Then, the obtained solution was sonicated for complete drug dissolution. The resulting solution was filtered, and diluted with distilled water, and the drug concentration was measured spectrophotometrically at 370 nm [32, 33].

pH Determination

A digital pH meter (Ama Co., Germany) was used to determine the pH of TX organogel formulations. The measurement was accomplished by allowing the probe of the pH meter to be in contact with the examined samples ($n = 3$).

Spreadability and Extrudability

The organogel's spreadability measurement was assessed using the previously reported procedure [30]. Typically, 0.5 g gel was placed on a glass plate (inside the pre-marked circle of 1-cm diameter) and over which a second glass plate was laid. For a period of 5 min, a 500-g weight was laid on the upper plate. The extent of spreadability accounted for the increase in the diameter of gel distribution.

Also, the extrudability test can be simply performed by filling collapsible aluminum tubes with the formulations. Then, the tubes were pressed, with the aid of two fingers, allowing 0.5-cm ribbon gel to be extruded. The consumed time (seconds) for the process of gel release from each tube was noted and recorded.

Viscosity Measurement

DV-III ultra-viscometer (Brookfield Co., USA) was used to study the organogel rheological properties, including gel viscosity, at room temperature using spindle T-D 96 at different rates of shear (5, 10, 20, 50, and 100 rpm). Each experiment was repeated ($n = 3$), and the results were shown as the mean ± SD.

In Vitro Release Study

The drug release studies were performed to evaluate the release pattern of TX from different modified PLO formulations. Practically, 0.5 g of PLO samples, containing 0.5% w/w of TX (2.5 mg), was deposited over a semi-permeable cellulosic cellophane membrane (MWCO 3.5 kDa) pre-soaked in phosphate buffer pH 7.4 for 4 h. Then, the loaded membrane was wrapped around one end of a clear cylindrical glass tube (6.5 cm^2). A cotton thread was used to securely clip the membrane over the tube end. Perforated aluminum foil was used to cover the top of the tube. Then, the tubes were hung above the release medium (300 mL phosphate buffer pH 7.4) to the height which allowed them to be just 1 cm immersed into the surface of the release medium. The cells were incubated in a thermostat shaker which was fixed at 32 ± 0.5°C and a 50-rpm shaking rate. Five-milliliter aliquots of the receptor medium were withdrawn at scheduled time intervals (30, 60, 90, 120, 150, 180, 240, and 360 min) and promptly replaced with an equivalent volume of fresh buffer [34, 35]. The amount of released drug was then quantified spectrophotometrically at 370 nm [34, 35]. The cumulative percentage of the released drug was plotted against time (h). Each experiment was carried out in triplicate, and the results were presented as the means ± SD.

The effect of the oil/aqueous ratio of PLO on the release of TX was investigated since we studied two ratios, namely, (30:70 **v/v**) and (50:50 **v/v**) ratios. In addition, the effect of the oil phase composition on the TX release was studied using three different compositions of the oil phase as follows. Firstly, we fixed the volume of the oil phase at 30 g% v/v of the total organogel base volume with different lecithin concentrations (F1, F3, F4, F5, and F6). Then, we increased the fixed volume of the oil phase to be 50 g% as presented in the PLO formula (F9, F10, F11, F12, F13, and F2) comprising varying concentrations of lecithin (5, 7.5, 10, 12.5, 15, and 22.65% w/v) with a fixed concentration (4% w/v) of PEG 300 as a co-surfactant.

Also, we tried to investigate the effect of PEG300 concentration by increasing its concentration to 25% w/v comprising varying concentrations of lecithin (3, 5, and 7% w/v). In addition, the effect of pluronic F127 concentration on the TX release was explored. The impact of release medium pH was explored at three different release media (distilled water pH 7, phosphate buffer pH 5.5, and phosphate buffer pH 7.4). Moreover, the influence of penetration enhancers (propylene glycol, oleic acid, and Tween 80) on TX release was studied. Finally, the effect of TX concentration on the release profile was evaluated by using three different drug concentrations (0.5, 1, and 3 g% w/v).

Table II Different release model equations

Model	Equations	Reference
Zero order	$M_t = M_0 + K_0 t$	[41, 42]
First order	$\log M_t = \log M_0 + K_0\, t/2.303$	[32, 33]
Higuchi	$M_t = M_0 + K_H\, t^{1/2}$	[34, 35]
Korsmeyer-Peppas	$M_t/M_{inf} = K_{KP}\, t^n$	[36]

M_0 is the initial amount of drug released in the medium (most times, $M_0 = 0$); M_t is the amount of drug released at time t; K is the order release constant; M_t/M_{inf} is the fractional drug release; and n is the release exponent, indicative of the drug-release mechanism

Table III The analysis and interpretation of diffusional release mechanism and the effect of n values [43]

Exponent (n)	Drug release mechanism	Release rate
0.5	Fickian diffusion (follow Fick's law)	$(t^{-0.5})$
0.5^{-1}	Non-Fickian diffusion (anomalous transport)	$(t^{-0.5})$
1	Non-Fickian diffusion (case II transport) (time independent)	Zero order
> 1	Non-Fickian diffusion (super case II transport)	(t^{n-1})

In Vitro Release Mechanism (Mathematical Kinetic Models)

Now, the drug release rate is considered a basic parameter of a pharmaceutical dosage form. The release of drugs from the constructed pharmaceutical dosage forms has been described by different kinetic models in which the amount of drug released (M) is a function of the time (t) or can be written as $M = f(t)$ [36]. To analyze the drug-release mechanism, the following mathematical expressions, summarized in Tables II and III, were used. The obtained release data of the prepared organogels formulae were analyzed according to the zero-order, first-order, and Higuchi diffusion and Korsmeyer-Peppas model [37–39]. The best-fitted model can be accounted for the regression coefficient values (R) values were calculated for all the models [40–43].

Zero-order kinetics is applied in most cases, including tablets, capsules, or prolonged release forms, transdermal systems, matrix tablets with low-soluble drugs, coated forms, and osmotic systems [37, 44, 45]. In this model, the same amount of loaded drug will be released by time interval. So, it will be the ideal method to achieve a pharmacologically prolonged action. The release can be affected by different factors including the kind of drug, its particle size, crystallinity, solubility, and the drug concentration in the respected dosage form. Regarding first-order model, it was first introduced by Gibaldi and Feldman [46] and Wagner [47] for describing the mechanism of drug dissolution and release. In this context, the plot of the decimal logarithm of the drug released against time will be linear. In this model, there is a relationship between the amount of drug released at each time interval and the remaining amount of drug inside the dosage form. The dosage forms such as those containing water-soluble drugs in porous matrices follow first-order model.

Higuchi [48, 49] developed several theoretical models to study the release of water-soluble and low-soluble drugs incorporated in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. Higuchi describes drug release as a diffusion process based on Fick's law, square root time dependent. This model is the most widely used to describe the drug release from several pharmaceutical matrices, including matrix tablets with water-soluble drugs, and some transdermal systems. Korsmeyer *et al.* [50, 51] developed a simple, semi-empirical model, relating exponentially the drug release to the elapsed time (t). Peppas [43] used this n value to characterize different release mechanisms, as presented in Table III. When $n = 0.5$ (for a slab), the drug diffuses through and is released from the matrix with a quasi-Fickian diffusion mechanism. For $n > 0.5$, an anomalous, non-Fickian drug diffusion occurs. When $n = 1$, a non-Fickian, case II, or zero-order release kinetics could be observed. For the determination of the exponent n, the portion of the release curve should only be used where $M_t/M_{inf} < 0.6$. To use this equation, it is also necessary that release occurs in a one-dimensional way and that the system width/thickness or length/thickness relation be at least 10, such as in TTS cases. This model is generally used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena could be involved [43].

In Vivo Studies

Acute Anti-inflammatory Activity

Based on the *in vitro* results, the organogel formulations that released the highest quantity of drug were chosen and assessed for anti-inflammatory activity using carrageenan-induced hind paw edema technique devised by Winter *et al.* [52]. The most effective PLO formulations (F14 and F19) were employed. For comparison, the oral standard indomethacin® capsule and the topical standard Feldene® gel were utilized, white male albino rats, weighing 200 ± 15 g, were chosen for the anti-inflammatory activity tests by measuring edema size before and after local carrageenan injection. In this context, the animals were divided into five groups, four animals in each. Group 1 served as the control group, receiving no treatment. As an oral standard, group 2 was given indomethacin® capsules (50 mg/kg). As a topical standard, group 3 was given Feldene® gel. Group 4 was given F14

PLO with 0.5% w/v TX. F19 PLO containing 0.5% w/v TX and oleic acid (5%) w/v as a penetration enhancer were used in group 5.

Edema Size Measurement

The anti-inflammatory test was carried out according to the previously established procedure [31, 53]. Firstly, the specified amount of gel was applied topically to the right hind paw of the rats. Subsequently, the animals have injected in the planter area of the right hind paw with 100 µl of 1% w/v freshly prepared carrageenan solution in saline [31, 54]. The thickness of the right hind paw was monitored and measured using a micrometer caliper from ventral to dorsal surfaces immediately before and 1, 2, 3, 4, 5, 8, and 12 h after the sub-planter injection. The percentage of edema inhibition was calculated using the following equation [55, 56].

$$\% \text{ Inhibition} = \left[1 - \frac{(T_t)}{(T_0)}\right] \times 100$$

where T_0 represents the thickness of edema at zero time and T_t is the thickness at a specific time interval.

Dose–Response Relationship

Three different concentrations of TX (0.5, 1, and 3% w/w) were introduced into the specified PLO formula to evaluate the dose–response relationship. The edema-inhibition (%) was measured and noted as previously mentioned.

Analgesic Activity Test

The best anti-inflammatory organogel formulations were chosen and evaluated for analgesic effectiveness using an acetic acid–induced writhing technique which was introduced by Koster [57] and modified by Dambisya *et al*. [58]. White male/female albino rats weighing 200 ± 15 g were used for analgesic activity testing. All animals were subjected to fasting for 12 h before the experiment. The rats were divided into five groups ($n = 6$). Group 1 functioned as the control group, receiving no medication. As a topical control, group 2 was given Feldene® gel. Group 3 was given 0.5% tenoxicam PLO (F19). Group 4 was given 1% w/v TX-PLO (F20). Group 5 was given 3% w/v TX-PLO (F21). All animal groups were intraperitoneally injected with 0.6% glacial acetic acid saline solution (10 mL/kg) 1 h after applying different kinds of treatment to generate discomfort, characterized by abdominal constrictions or writhes. For 30 min, the number of writhes witnessed in each animal was recorded. The analgesic activity of the investigated animals was monitored by calculating the percentage of abdominal writhing inhibition using the following equation [59]:

$$\% \text{ Writhing inhibition} = \frac{\text{Wc} - \text{Wt}}{\text{Wc}} \times 100$$

where W_C represents the number of abdominal constrictions or writhing for the control group and W_t represents the number of abdominal constrictions or writhing for in treated groups.

Skin Irritation Study

A skin irritation study was performed in rats. Practically, 2 g of the selected medicated hydrogel formulae (F21) were applied onto the shaved dorsal skin of the rats by uniform spreading over an area of 9 cm^2 and occluded with gauze and bandage. Then, the applied formulation was removed after 24 h, and the score of erythema was recorded as in the following order: 0 for no erythema; 1 for mild erythema (barely perceptible light pink); 2 for moderate erythema (dark pink); and 3 for moderate to severe erythema (light red) [60].

Statistical Analysis

The obtained results or data all over the study are presented as mean ± standard deviation. To compare the responses of all investigated groups, one-way and two-way ANOVA analyses followed by post hoc test were conducted on SPSS software. The probability values are considered as an indicator for the degree of significance. When P value is less than 0.05, this means it is statistically significant.

Results and Discussion

Preparation of Pluronic Lecithin Organogels Containing 0.5% w/w TX

The 0.5% w/w TX concentration was chosen to approximate the same concentration as the marketed Piroxicam® (Feldene gel 0.5% w/w) of the same oxicam group. By combining the oil and aqueous phases, PLOs, containing TX, were effectively formed. Surprisingly, organogel does not develop in formulations from F9 to F13 since they just behave as viscous liquids (no 3D networks). This finding may be attributed to the existence of lower concentrations of lecithin and PEG 300. Therefore, these formulae were excluded from further investigations.

Physicochemical Properties of TX Organogels

Physical Appearance

Organogel formulations were yellowish-white viscous preparations with a smooth homogeneous texture.

Drug Content

The content of TX in the formulated PLOs was investigated to ensure that TX distribution was consistent inside the gel matrix. The results showed that the TX content of all PLO formulations was in the range of 95–101%, as listed in Table IV. These findings are deemed adequate to warrant further investigation.

pH Determination

Also, Table IV displays the pH values of the developed PLO formulations. It was observed that the formulations' pH ranged from 5.9 to 6.4, which is close to skin pH [31]. This result suggested that the formulations under consideration were suitable for cutaneous application.

Spreadability and Extrudability Measurements

Spreadability is a key mechanical feature of topical formulations in terms of patient compliance. The greater the spreadability, the easier the application and the greater the accessible surface area for cutaneous application. All formulae were tested for spreadability. The spreadability of the modified PLOs was calculated by measuring the mean diameters (± SD) of the spreading circles. The results showed that the spreadability values for all formulated PLOs ranged from 2.8 to 4.4 cm, which is regarded as enough for spreading the gel over the skin (Table IV and Fig. 3). Noteworthy, it was observed that the spreadability values (cm) increased with the decrease of gel viscosity. Previously, similar findings were achieved and published [30]. In terms of extrudability (the release of gel formulation in a continuous manner from the tube nozzles), all the developed formulations extruded 0.5 cm of PLO ribbon within 5–10 s by pushing the

Table IV Physicochemical properties, including pH, spreadability, extrudability, and mean drug content of the modified PLO formulations. The results are represented as the mean ± SD

Code	Spreadability (cm/5 min)	Extrudability (s)	pH	Drug content (%)	Homogeneity
F1	3.1 ± 0.17	6 ± 0.057	6.3 ± 0.47	102 ± 0.57	Homogenous
F2	2.8 ± 0.05	9.2 ± 0.11	6.2 ± 0.1	96.2 ± 2.1	Homogenous
F3	3.9 ± 0.11	7.9 ± 0.12	5.9 ± 0.1	95.9 ± 3.1	Homogenous
F4	3.7 ± 0.07	8.3 ± 0.13	6.3 ± 0.1	100.3 ± 1.1	Homogenous
F5	3.4 ± 0.10	6.1 ± 0.51	6.1 ± 0.57	102.1 ± 0.57	Homogenous
F6	3.1 ± 0.17	7.3 ± 0.57	6.3 ± 0.57	97.3 ± 0.97	Homogenous
F7	2.9 ± 0.11	7.4 ± 0.22	6.4 ± 0.1	101.4 ± 2.1	Homogenous
F8	2.9 ± 0.10	8.4 ± 0.33	6.4 ± 0.1	99.4 ± 2.1	Homogenous
F14	4.3 ± 0.12	6.9 ± 0.61	5.9 ± 0.1	98.9 ± 3.1	Homogenous
F15	4.2 ± 0.15	7.9 ± 0.52	5.9 ± 0.1	100.9 ± 1.4	Homogenous
F16	4.1 ± 0.21	8.3 ± 0.71	6.2 ± 0.57	97.5 ± 2.27	Homogenous
F17	4.1 ± 0.10	7.3 ± 0.54	6.3 ± 0.37	97.9 ± 2.58	Homogenous
F18	4.4 ± 0.16	8.4 ± 0.15	6.4 ± 0.1	100.4 ± 1.6	Homogenous
F19	4.3 ± 0.56	7.3 ± 0.37	6.3 ± 0.51	99.3 ± 1.57	Homogenous
F 20	4.3 ± 0.57	8.2 ± 0.47	6.2 ± 0.57	100.2 ± 0.87	Homogenous
F21	4.4 ± 0.21	8.7 ± 0.17	6.2 ± 0.50	97.2 ± 0.7	Homogenous

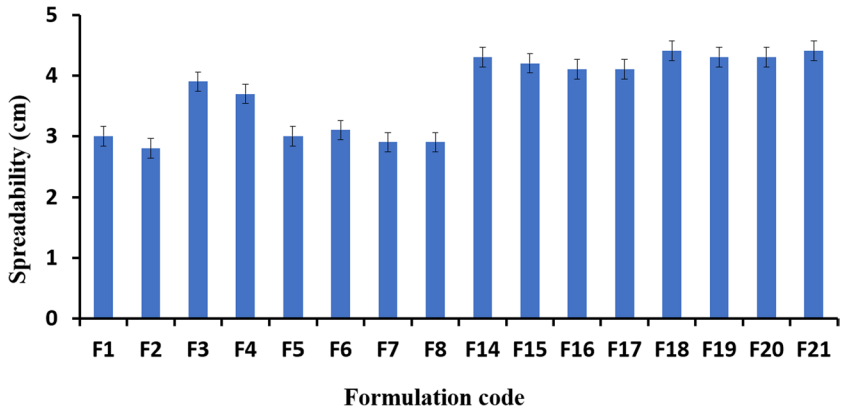

Fig. 3 Histogram of the spreadability of PLO formulations

collapsible tubes with two fingers. The obtained results indicated that the formulae were easily and smoothly removed from the tubes. The extrudability of the more viscous gel is lower than that of the lower viscous formula. Similar findings were observed and reported before [30, 31].

Rheological Study

The viscosity of the gel matrix is an important factor to consider because it influences both the drug release characteristics and the ease of use. The viscosity of organogel was tested using various concentrations of lecithin, Pluronic F127, and PEG300 as a surfactant. The findings of rheological investigations of TX PLO gel at various rpm are listed in Table V and illustrated in Figs. 4 and 5.

The downward sloping curves suggested that all formulations displayed non-Newtonian behavior with pseudoplastic flow shear thinning. This outcome might be attributable to the distortion of tightly packed particle arrangements and, finally, the breakdown of aggregates. The formation of a complex network may explain the rise in viscosity with increasing lecithin and pluronic concentrations [30].

This finding is consistent with the findings of Bonam, who investigated the rheological characteristics of PLO containing ricinolic acid for transdermal application [61]. The viscosity of the organogel was studied as a function pluronic solution/oil phase ratio of PLO (Fig. 5a). The results showed that the viscosity of PLO increased by the increase of pluronic solution/oil solution ratio. F1 which is composed of a 50/50 ratio exhibited a significantly lower viscosity at all applied shear rates (rpm) when compared with those obtained in the case of F2 which was formulated from a 70/30 ratio. For example, at a 5-rpm shear rate, the obtained viscosity values for both F1 and F2 were 4500 Poise, and 3400 Poise, respectively.

The obtained results may be attributed to the existence of a high concentration of pluronic in F2 (70/30 ratio) compared with F1 (50/50). Additionally, it was observed that the viscosity values of the formulated PLO rose as the concentration of lecithin increased 2840, 2950, 3070, 3200, 3400, and 4500 Poise at lecithin concentrations of 3, 5, 7, 9, 12.5, and 22.5% w/v, respectively (Fig. 4b). Also, it was noted that by making the concentration of lecithin fixed, the viscosity will increase with the increase of the aqueous phase ratio. This may be due to the existence of a higher concentration of pluronic polymer in the aqueous phase. For example, at a fixed concentration of lecithin (3), F3 which is composed of 70% aqueous phase/30% oil phase had a higher viscosity (2840 Poise) when compared with the value (760 Poise) of F14 which composed of 50% aqueous phase /50% oil phase.

Upon using a lower ratio of pluronic solution/oil phase (50/50 ratio), the existence of high conc of either lecithin (not less than 22%) or PEG300 (not less than 25%) is required for gel formation. Otherwise, using low conc of lecithin less than 22% or PEG300 less than 25% will not produce a 3D gel. For example, in the cases of PLO of formula F9 (4% PEG300 and 5% lecithin), F10 (4% PEG300 and 7.5% lecithin), F11 (4% PEG300 and 10% lecithin), F12 (4% PEG300 and 12.5% lecithin), and F13 (4% PEG300 and 15% lecithin), no gel was formed. Consequently, these formulations will be excluded from further investigations within the current study. Concerning the effect of different lecithin concentrations on organogel viscosity, it was revealed that as lecithin concentrations

Table V The viscosity at different rpm of different organogel formulae as a function of shear (rpm). The results are represented as the mean ± SD

Code	Viscosity (Poise) as a function of shear rate (rpm)				
	5	10	20	50	100
F1	3402 ± 50	1700 ± 41.4	869 ± 2.52	440 ± 31.16	223 ± 11.15
F2	4503 ± 76.64	2800 ± 42.58	2020 ± 34	1546 ± 15.5	783 ± 25.52
F3	2834 ± 35.5	1450 ± 33.1	795. ± 2.5	352 ± 18.85	192 ± 12.27
F4	2950 ± 55.1	1480 ± 31.1	805 ± 1.85	365 ± 18.81	204 ± 23.3
F5	3072 ± 25.52	1521 ± 15.53	822 ± 1.53	372 ± 18.81	206 ± 16.6
F6	3200 ± 27.75	1552 ± 25.5	833 ± 1.31	385 ± 18.88	212 ± 1.45
F7	3922 ± 25.52	2002 ± 27.75	1143 ± 1.42	516 ± 7.71	280 ± 8.85
F8	4420 ± 22	2502 ± 16.6	1373 ± 1.63	700 ± 17.71	380 ± 12.21
F14	766 ± 8.85	453 ± 15.53	245 ± 1.3	112 ± 7.75	68 ± 2.23
F15	813 ± 6.69	484 ± 12.2	274 ± 0.95	140 ± 7.75	75 ± 1.16
F16	842 ± 15.5	525 ± 25.5	305 ± 1.53	160 ± 6.61	85 ± 3.35
F17	770 ± 11.1	465 ± 8.8	257 ± 1.2	119 ± 11.1	75 ± 6.65
F18	756 ± 10.1	442 ± 8.8	254 ± 1.1	110 ± 7.75	65 ± 6.65
F19	754 ± 15.5	437 ± 11.1	235 ± 0.97	100 ± 9.95	63 ± 2.25
F 20	762 ± 14.4	459 ± 12.2	245 ± 1.5	112 ± 9.96	68 ± 1.12
F21	765 ± 13.1	456 ± 9.95	247 ± 0.95	112 ± 6.65	68 ± 1.07

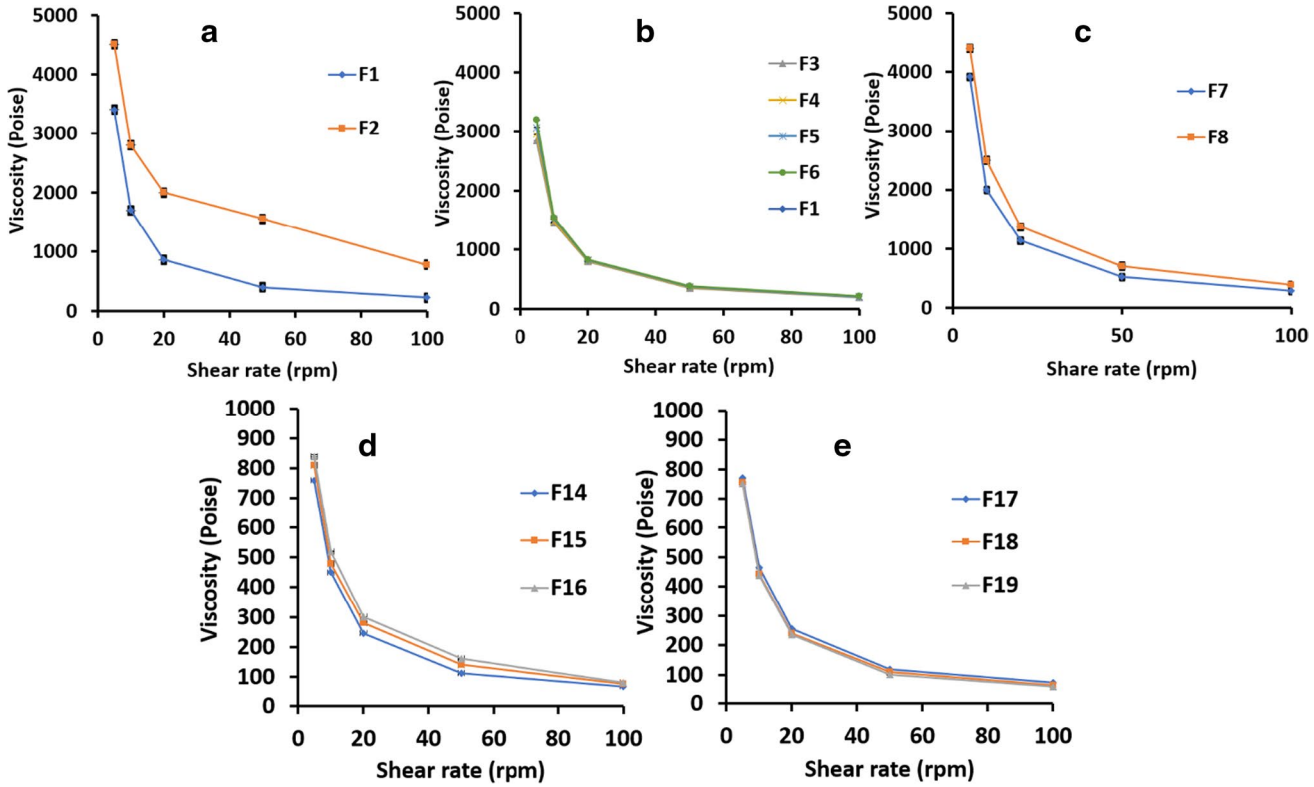

Fig. 4 **a** Effect of oil/aqueous phase ratio on the viscosity of organogel formulations F1 oil/aqueous (30/70), F2 oil/aqueous (50/50). **b** Effect of different concentrations of lecithin (oil phase 30% w/w) on the viscosity of the organogels at PEG300 (4% w/w) F1 lecithin (22.5% w/w), F3 lecithin (3% w/w), F4 lecithin (5% w/w), F5 lecithin (7% w/w), F6 lecithin (9% w/w). **c** Effect of pluronic concentration on the viscosity of the organogel. F7 25% w/w pluronic, F8 30% w/w pluronic. **d** Effect of lecithin concentration (oil phase 50% w/w and PEG300 25% w/w) on the viscosity of the organogels at PEG300 (25% w/w), F14 lecithin (3% w/w), F15 lecithin (5% w/w), F16 lecithin (7% w/w). **e** Effect of penetration enhancers on the viscosity of the organogel, F17 Tween 80, F18 propylene glycol, F19 oleic acid

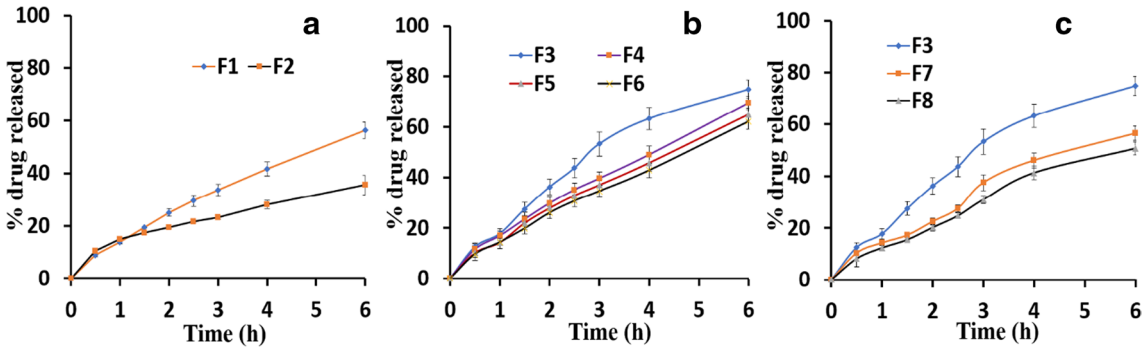

Fig. 5 **a** Effect of oil/aqueous phase ratio on the TX release since F1 composed of (30:70 w/w), and F2 composed of (50:50 w/w). **b** Effect of lecithin concentration on TX release from organogels base that contains 30/70 oil/aqueous phase ratio. **c** Effect of pluronic F 127 concentration F3 (20%), F7 (25%), F8 (30%) on the release rate of TX from PLO

increased, organogel viscosity increased. The obtained higher viscosity may be due to the higher crosslinking points which formed from the entanglement of long cylindrical micelles with each other, forming 3D networks with high viscosity [61] (Table 5 and Fig. 4b). Similar results were observed upon the investigation of pluronic concentration on the viscosity of PLO. The results revealed that the viscosity of PLO increased linearly with the increase of pluronic concentration for the same reason as lecithin. The viscosity values of PLO upon using 20%, 25%, and 30% pluronic were 3400, 3920, and 4400 Poise, as illustrated in Fig. 4c. As a conclusion, the viscosity of

the organogel formulations is organized as in the following descending manner:

$F2 > F8 > F7 > F1 > F6 > F5 > F4 > F3.$

Regarding the impact of different pluronic concentrations on the viscosity of the organogels, it was noticed that the viscosity of the organogel rose as the concentration of pluronic increased. This proportionate rise in viscosity with increasing polymer concentration can be ascribed to greater cross-linking in the polymer as polymer concentration increases (Table V and Fig. 4c). The viscosity is arranged in the following order: F8 > F7 > F1. In formulations F14, F15, and F16, both the percent of IPM and lecithin are decreased, and the percent of PEG300 is increased to 25% w/w that allow organogel formation. In contrast, without the addition of 25% PEG300, the organogels will not form due to the lower concentration of lecithin in these formulations (3% w/w in F14, 5% w/w in F15, and 7% w/w in F16). This composition decreased the viscosity of these formulations when compared to F2 which contains 22.65% (w/w) lecithin (Fig. 4d). Thus, the viscosity of the organogel formulations is arranged in the following order:

$(F16 > F15 > F14) < F2$

Regarding the influence of penetration enhancers on organogel viscosity, it was found that Tween 80 (F17) increases viscosity, while oleic acid (F18) and PG (F19) decrease the viscosity (Table 5 and Fig. 4e). The viscosity of the organogel formulations is organized as follows:

$F17 > F18 > F19.$

In vitro Drug Release Studies

Figures 6, 7, and 8 depict the graphical release profiles of TX from PLO organogels. The results showed that the release varied according to various factors such as the oil/aqueous ratio; the proportion of lecithin, pluronic, and PEG300; the change in pH of the release medium; and the presence of penetration enhancers in the formulations. Regarding the effect of the oil phase/aqueous phases ratio, the drug release rate from PLO-F1 was significantly greater than from F2 ($P > 0.05$). This behavior may be attributed to the greater viscosity of F2 (4500 Poise), compared with that of F1 (3400 Poise). The higher viscosity of F2 is caused by the higher concentration of lecithin in F2 (22.5%) compared with F1 (12.65%) (Table V and Fig. 5a). Hence, as the organic oil phase increases, the release of the drug will be retarded due to the higher affinity of the drug to the base which did not facilitate the release of the drug to the aqueous medium.

Regarding the impact of oil phase composition, Fig. 5b depicts the influence of varying lecithin concentrations on

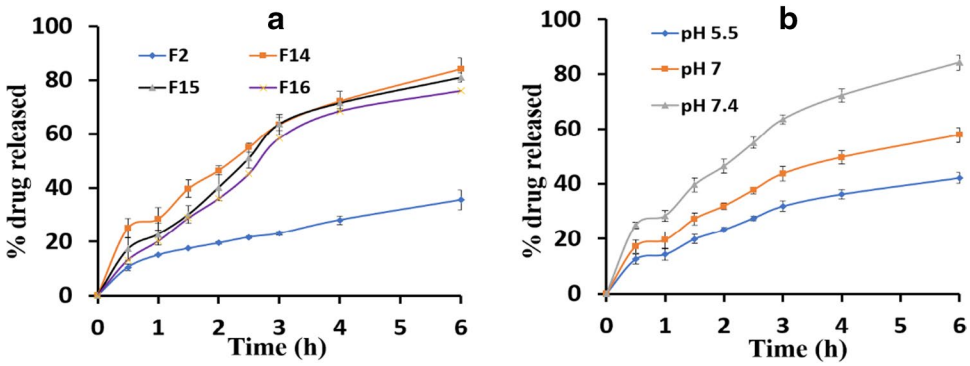

Fig. 6 **a** Effect of different concentrations of lecithin on the TX release from organogels base that contains 50/50 oil/aqueous phase ratio. The concentrations were (3, 5, 7, and 22.6)% w/v for F14, F15, F16, and F2, respectively. **b** Effect of pH of the release medium on the TX release

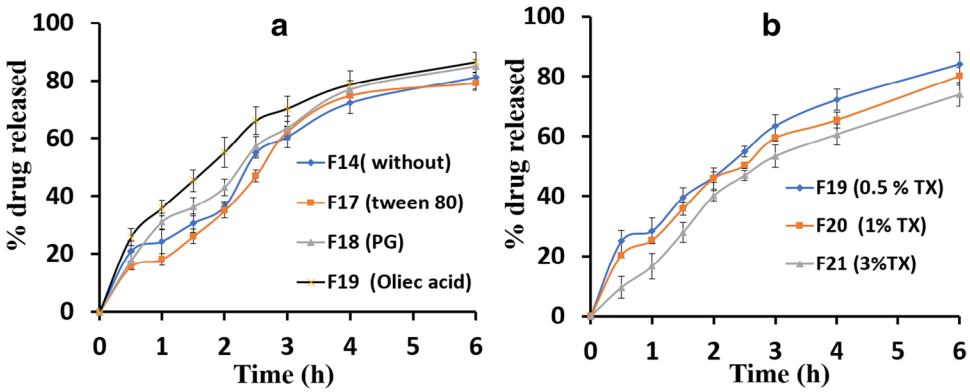

Fig. 7 **a** Effect of penetration enhancers (Tween 80, propylene glycol, and oleic acid) at a fixed concentration (5%w/w) on the TX release from PLO-F14. **b** Effect of drug concentration on its release profile via using three different TX concentrations (0.5, 1, and 3% w/v)

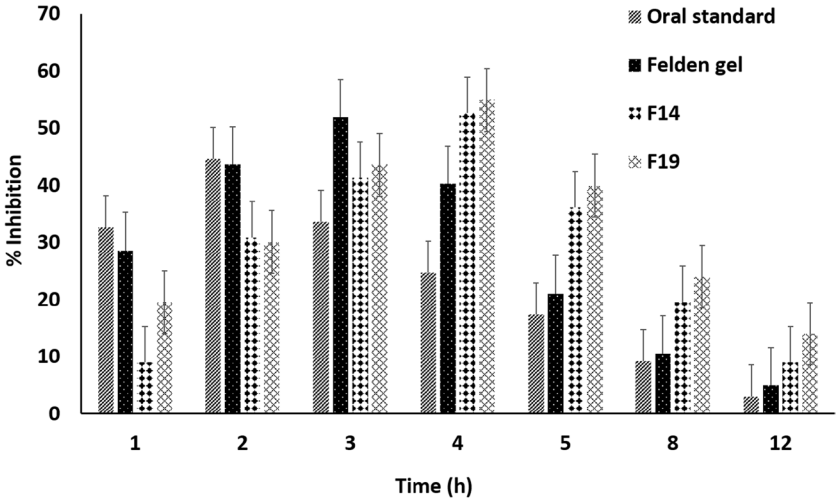

Fig. 8 The % inhibition of rat hind paw edema as a function of time (h) for the selected PLOs containing 0.5% w/w TX (F14, without percutaneous enhancers) and (F19, with enhancer) compared to either oral indomethacin® and topical marketed standard medication (Feldene® gel). The results are presented as mean ± SD ($n = 4$)

TX release from PLO containing 30% w/w oil phase. The release of TX from PLO was significantly reduced by the increase in lecithin concentration ($P > 0.05$). This finding might be attributed to a decrease in drug thermodynamic activity in the case of the formula of high lecithin concentrations. With greater lecithin concentrations, long cylindrical micelles get more entangled with each other, generating a network-like structure with extremely high viscosity. The drug's entrapment inside this network reduces the quantity of free drug available for release, resulting in a reduction of the drug release rate across the membrane. This finding and interpretation agree with the previously obtained data [52, 62]. Accordingly, TX's release rates from PLO were arranged in the following order:

$$F3 > F4 > F5 > F6 > F1 > F2.$$

On the other hand, the effect of varying lecithin concentrations on TX release from an equal oil/aqueous ratio (50% oil:50% aqueous) was also investigated. TX release was observed to be significantly ($P < 0.05$) influenced by the increase of lecithin concentration since the lower the lecithin concentration in the PLO formula the higher the drug release. Regarding the effect of pluronic concentration on the TX release, the results illustrated in Fig. 5c showed that there is an indirect relationship between the polymer concentration and the drug release since the release rate decreased with the increase of pluronic concentration. Accordingly, the release rate of TXs from different PLOs was arranged in the following order: F3 > F7 > F8. This result was attributed to the difference in viscosity behavior of all formulae since the viscosity values of F3, F7, and F8 were 2840 Poise, 3920 Poise, and 4400 Poise, respectively.

Figure 6a shows the release profiles of F14 (3% lec), F15 (5% lec), F16 (7% lec), and F2 (22.65% lec). The results showed that TX release from the respected PLO formulae was arranged in the following order: F14 > F15 > F16 > F2. This finding mainly accounted for the viscosity values since they were 760 Poise, 810 Poise, 840 Poise, and 4500 Poise for F14, F15, F16, and F2, respectively. Noteworthy, PEG300 plays an important role in the formation of organogels. At a low concentration of PEG300 (4%), lecithin concentration should be high enough (not less than 22%) to formulate PLO, as in the case of F2 which is composed of 22.6% lecithin concentration. Otherwise, no gel can be formed, as in the cases of F9, F10, F11, F12, and F13. The other option to formulate stable PLO, at low lecithin concentration, is the increase of pluronic phase ratio (e.g., 70% v/v) against oil phase (30% v/v). In this case, the organogel will be formed even at a very low concentration of lecithin (3% w/v).

Considering the medium pH, Fig. 6b depicts its influence on TX release, taking PLO-F14 as a selected formula due to it showed the highest release rate. The results showed that the release of TX was shown to be significantly quicker ($P < 0.05$) in the case of phosphate buffer pH 7.4, followed by water and phosphate buffer pH 5.5. This result might be related to enhanced TX ionization at pH 7.4, which increased TX solubility and release rate. This finding is consistent with the previously reported findings of Chawla *et al.*, who noted that the rise in pH value was accompanied with a considerable increase in the release patterns of naproxen sodium from tablet [63].

Figure 7a depicts the impact of penetration enhancers on TX release from the chosen PLO formula (F14). The results showed that the use of penetration enhancers was accompanied by a non-significant increase in the drug release compared with the F14 (without enhancers). Regarding the difference between the utilized enhancers, the results indicated that there are no significant differences ($P > 0.05$) between them. However, their effect on the release rate was arranged as follows: F19 > F18 > F17 > F14. This finding might be attributed to the varying viscosities of the investigated PLOs as a

function of different penetration enhancers. Therefore, F19 with the lowest viscosity showed the highest drug release. As a result of the predicted and desirable *in vivo* penetration enhancer effect, F19 was chosen to finish the investigation. Also, the influence of drug concentration on TX release from the selected PLO formula (F14) was studied. Practically, there were three different TX concentrations investigated (0.5, 1, and 3% w/v), and the data is illustrated in Fig. 7b. The results showed that the increase in drug concentration was accompanied by the delay in the release rate profile.

Kinetic Analysis

The *in vitro* release data are theoretically described by four kinetic models: zero-order model, first-order model, Peppas model, and Higuchi model. From the *in vitro* drug release kinetic study, as presented in Table VI. TX release from PLO organogel formulations was shown to follow zero-order in formulas from F1 to F8 as indicated by the values of (R), while, in the cases of formulae from F14 to F19, the release mechanism follows the Higuchi diffusion model. Korsmeyer and Peppas models were used to calculate the Fickian constant "*n*" that distinguishes distinct release processes. For all cases, the obtained *n* values were less than 0.5 ($n < 0.5$) suggesting that the release mechanism was Fickian diffusion. This result indicated that the release of TX is controlled by diffusion through the matrix [64]. As a conclusion, the composition of PLO plays an important role in the mechanism of drug release from topical PLO.

The release of the drug from the formula of low viscosity follows the diffusion mechanism as indicated by *R* and *n* values, whiles the highly viscous formulae follow zero-order models, indicating the controlled release behaviors with low values of *n* ($n < 0.5$) indicating the involvement of diffusion mechanism together with zero. Noteworthy, the formula that follows zero-order release mechanism is suitable for controlled release applications. Similar results and conclusions were reported by other groups [41, 65–67].

In Vivo Release Study

Acute Anti-inflammatory Activity

The anti-inflammatory effect of the TX-loaded PLO formulations was investigated and compared with a control group by utilizing Winter technique [52]. Figure 8 depicts the inhibitions of edema thickness following the application of PLOs containing 0.5% w/w TX with (F14) or without (F19) penetration enhancers. The production of acute inflammation in the control group resulted in a significant rise in paw thickness since it was observed that the percentage swelling ranged from 158 to 213% within 12 h of carrageenan injection. Furthermore, it was observed that the formula F19, which contains oleic acid as a penetration enhancer, has a

Table VI Mechanism of TX release from different PLO formulations

Code	Zero order		First order		Higuchi-diffusion		Korsmeyer-Peppas		The best model fitted
	R	K_0	*R*	K_1	*R*	K_H	*R*	*N*	
F1	0.998	8.62	0.991	0.133	0.991	27.57	0.998	0.136	
F2	0.994	4.35	0.993	0.057	0.989	13.94	0.991	0.087	
F3	0.991	11.96	0.941	0.239	0.983	39.44	0.982	0.138	
F4	0.996	10.46	0.982	0.189	0.981	33.21	0.995	0.133	Zero order
F5	0.994	9.95	0.988	0.168	0.983	31.66	0.991	0.139	
F6	0.997	9.45	0.986	0.154	0.978	29.94	0.995	0.138	
F7	0.998	9.04	0.980	0.140	0.961	28.75	0.965	0.137	
F8	0.993	8.177	0.986	0.119	0.970	26.43	0.983	0.142	
F14	0.945	11.39	0.994	0.295	0.998	37.13	0.969	0.098	
F15	0.915	12.62	0.973	0.289	0.989	41.27	0.966	0.124	
F16	0.920	12.31	0.967	0.255	0.995	40.32	0.980	0.135	Higuchi-diffusion
F17	0.904	13.73	0.963	0.292	0.987	45.18	0.974	0.162	
F18	0.882	12.34	0.992	0.339	0.994	41.37	0.969	0.110	Zero order
F19	0.835	12.24	0.984	0.354	0.993	41.63	0.929	0.109	
F20	0.998	11.31	0.969	0.294	0.977	37.13	0.946	0.093	
F21	0.994	14.11	0.982	0.325	0.987	45.81	0.983	0.16	

R correlation coefficient; *n* Peppas exponent which indicates Fickian and non-Fickian mechanism; K_0, K_1, and K_H are the kinetic release constants for zero, first, and Higuchi models

larger percentage suppression of edema volume than F14 (with no enhancer), Feldene® gel, and oral indomethacin® capsule. This finding might be explained by the introduction of a penetration enhancer, which enhances drug permeability by disturbing the highly organized structure of the stratum corneum lipid and hence promotes drug partitioning into the stratum corneum. It was also noticed that there is no significant difference in the % inhibition of edema thickness between the indomethacin® capsule, Feldene® gel, and the produced TX PLO formulas (F14 and F19). Moreover, the obtained data revealed that all the investigated formulae inhibited significantly ($P < 0.05$) the edema size when compared with the control group (untreated group), as indicated in Fig. 8. Noteworthy, F19 showed the highest effect compared with other investigated groups.

Similar results were obtained with Jhawat *et al.*, who study the anti-inflammatory activity of optimized mefenamic acid organogels (F2) against standard marketed preparation (Volini gel), and the results showed that the test F2 formulation showed a non-significant effect when compared with marketed formulation but showed a significant effect when compared with the control group (dose 1) [68]. Also, similar results were obtained with Abu-Elyazid *et al.* [69] who studied the anti-inflammatory activity of TX nano-emulsion gel since the results showed that 1% TX nano-emulsion in HPMC gel exhibited a non-significant higher anti-inflammatory effect than 0.5% TX nano-emulsion in MC gel base and Feldene gel (R) [69].

Noteworthy, a factorial design was constructed to measure the effect of two factor on the % of edema inhibition: factor 1, dosage forms (four levels) F14, F19, Feldene gel, and oral standard; factor 2, time (seven levels) 1, 2, 3, 4, 5, 7, 8, and 12 h as illustrated in Fig. 9. The result showed a statistically significant difference in the % of edema inhibition because of dosage forms by time. PLO F19 showed the maximum inhibitory effect by time ($P = 0.04$), compared with other investigated formula which arranged as follows:

$$F19 > F14 > Feldene\ Gel > Oral\ standard$$

Dose–Response Relationship

Figure 10 illustrates the effect of TX concentration (0.5, 1%, and 3 g% w/w) on the extent of edema inhibition. F19, composed of PLO with oleic acid as a penetration enhancer, was selected for this experiment. The results indicated that there are significant differences in the degree of edema inhibition with the increase in drug concentration. The difference between the group was clearer after a long duration (8-h duration) since the formula of higher concentration still had the activity compared with the formula of lower concentration. Furthermore, the findings showed that F20 and F21 had

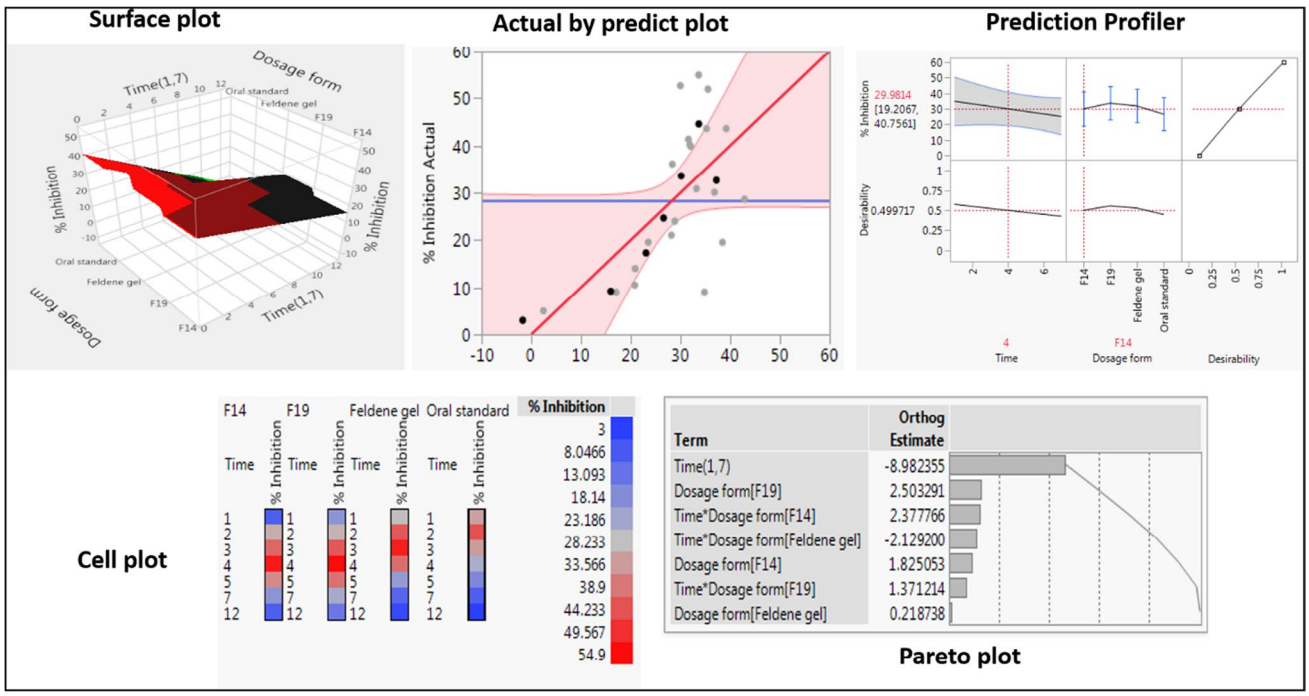

Fig. 9 Different statistical analysis plots of the *in vivo* anti-inflammatory profiles for the modified TX-PLO showed that the model was fit with a *P* value of 0.04; i.e., both time (7) and dosage form have a significant effect on the anti-inflammatory effect of edema thickness by TX, a significant effect of the dosage form (F20 and F21) by time, compared to oral indomethacin® and topical marketed standard (Feldene® gel)

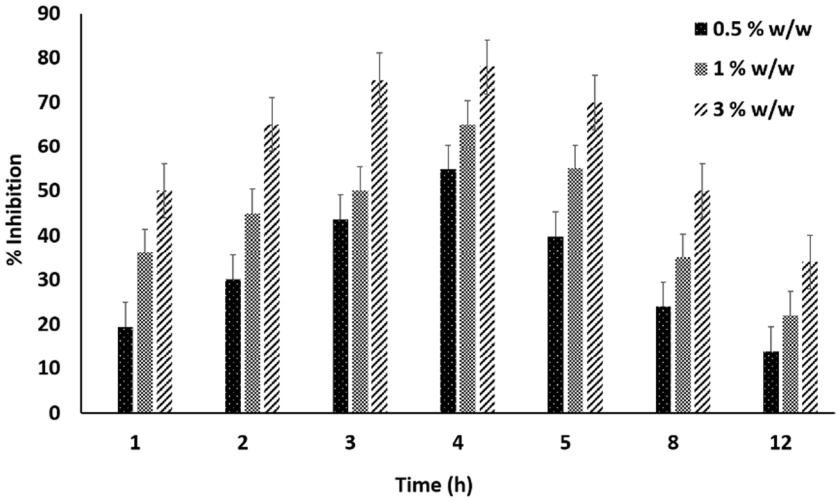

Fig. 10 Effect of the loaded TX concentration (0.5%, 1%, and 3% w/w), represented by formulae F19, F20, and F21, respectively, on the % inhibition of rat hind paw edema. The results are presented as mean ± SD ($n = 4$)

substantial edema inhibition ($P < 0.05$) when compared to oral indomethacin® suspension and control group, but not when compared to Feldene® gel and F19. The sustained effect of F20 and F21 confirm the results of kinetic release studies since they follow zero-order mechanisms.

Statistically, factorial design was done and illustrated in Fig. 11 to measure the effect of different dosage forms (factor 1) and time in hours (factor 2) on the % of edema inhibition (response): factor 1, dosage forms (four levels) F19, F20, F21, and Feldene gel; factor 2, time (seven levels) 1, 2, 3, 4, 5, 7, 8, and 12 h. The result showed a statistically significant difference in the % of edema inhibition due to the effect of dosage forms by time. PLO F21 showed the maximum inhibitory effect by time ($P = 0.004$), compared with other groups which were arranged in the following manner:

$$F21 > F20 > F19 > Feldene\ Gel$$

Analgesic Effect of the Modified PLO

Table VII presents the impact of TX PLO formulas on glacial acetic acid–induced abdominal constriction in

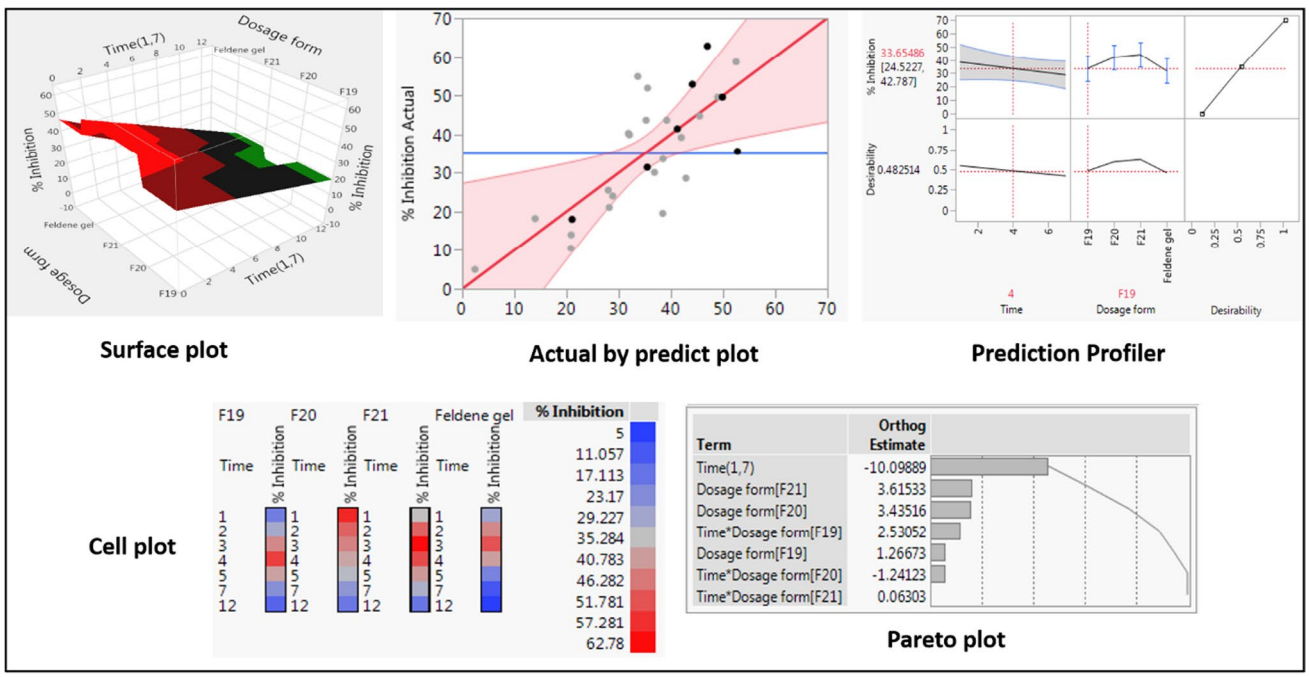

Fig. 11 Different statistical analysis plots of the *in vivo* anti-inflammatory profiles for the modified TX-PLO showed that the model was fit with a *P* value of 0.004; i.e., both time (7) and dosage form have a highly significant effect on the anti-inflammatory effect as a function of TX concentration, a significant response of the dosage form (F20 and F21) when compared with the marketed Feldene gel by time

Table VII Effect of TX organogel formulations on the glacial acetic acid–induced abdominal constrictions in mice compared to Feldene gel

Group	Treatment	Mean number of writhing in mice	The percentage protection against abdominal writhing
Group 1	Nothing	92	0
Group 2	Feldene gel	0	100%
Group 3	F19	6	93.47%
Group 4	F20	0	100%
Group 5	F21	0	100%

mice. As compared to the control group, both the tenoxicam-loaded PLOs (F19, F20, and F21) and the reference Feldene® gel markedly reduced the abdominal writhing in mice, lowering the mean number of writhing from 92 to zero. There were no significant differences ($P > 0.5$) between the modified PLOs and Feldene ® gel. In contrast, it was observed that there is a significant difference ($P < 0.5$) between the treated groups and the control group.

In Vitro and *In Vivo* Correlations

Regarding the association between *in vitro* and *in vivo* outcomes, pluronic lecithin organogel base (F19) with oleic acid as a penetration enhancer demonstrated a modest percent increase in TX release rate *in vitro* compared to F14 and provided better edema inhibition (*in vivo*). This finding might be explained by the fact that oleic acid increases drug diffusion through the skin by changing the intercellular lipoprotein barriers on the skin and, as a result, boosting drug partitioning into subcutaneous layers [31, 42]. In addition, the inclusion of a high concentration of TX into an organogel base resulted in the greatest percentage of edema inhibition (*in vivo*) and the greatest quantity of drug release in the *in vitro* release assays. This might be because the high dose of tenoxicam increases thermodynamic activity and thus increase drug diffusion [66].

Skin Irritation Study

The results of skin irritation studies showed no signs of erythema score even after 24 h (showed zero of erythema score) which indicated the safety of prepared medicated hydrogels and advising for the next marketed topical administration.

Conclusion

Different formulations of PLO were modified and examined *in vitro* and *in vivo*. According to the results, all the modified organogels have acceptable physical properties in terms of drug content, homogeneity, consistency, appearance, pH value, and spreadability. Also, the results revealed that TX was released more quicker upon using phosphate buffer pH 7.4. Also, the release rate of TX increased by increasing the concentration of either lecithin or pluronic in the same PLO formula. Formulation F19, which incorporates oleic acid as a penetration enhancer, had the highest percentage of drug release of any organogel formulation. The anti-inflammatory results revealed a considerably significant difference ($P < 0.05$) between the control group and the groups treated with the specified topical formulations. Furthermore, the anti-inflammatory impact of the selected topical organogel formulations on the generated paw edema was shown to be greater than that of either indomethacin (orally administered standard medicine) or Feldene (R) gel (topical marketed drug). Moreover, as a dose–response relation, the incorporation of a high concentration of TX into an organogel base resulted in the highest percentage of edema inhibition with a longer duration of action up to 12 h.

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Data Availability All data are contained within the article.

Declarations

Ethics Approval The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Faculty of Pharmacy, Al-Azhar University, with approval number AZ-AS/PH/3/C/2021, for studies involving animals.

Informed Consent Not applicable.

Conflicts of Interest The authors declare no competing interests.

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