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Self-nanoemulsifying drug delivery system (SNEDDS) of piroxicam: evaluation on anti-inflammatory activity in wistar rats

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ABSTRACT

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) that is commonly used for arthritis, gout, and other musculoskeletal disorders. Piroxicam is poorly soluble in water and according to the biopharmaceutical drug classification system (BCS) is classified as a Class II drug with good permeability but poor dissolution. The self-nanoemulsifying drug delivery system (SNEDDS) has been extensively employed to improve the dissolution and absorption of water-insoluble drugs within the gastrointestinal tract, leading to enhanced oral bioavailability and increased therapeutic effect of the loaded drugs. Therefore, the present study aims to evaluate the anti-inflammatory activity of piroxicam-loaded SNEDDS as compared to conventional piroxicam suspension that was observed in male Wistar strain rats. The SNEDDS was tailored from a mixture of oleic acid, tween 80, and propylene glycol. Twenty male Wistar strain rats (aged 2-3 months, weighed 150-250g) were selected and were divided equally into 4 different groups receiving 1% PVP, SNEDDS carrier, piroxicam suspension (1.8 mg/kg BW), and SNEDDS piroxicam (1.8 mg/kg BW). Acute inflammation was induced by a carrageenan-induced paw edema model where the carrageenan was injected sub plantar in the hind paw of the rats to induce edema. Several parameters including paw edema volume, AUC_{0-6}, and percent anti-inflammatory effect, were measured to evaluate the anti-inflammatory activity experienced in each group. At the end of this study, the piroxicam SNEDDS group significantly demonstrated better protection from paw edema compared to the piroxicam suspension group ($\rho<0.05$), suggesting that SNEDDS may enhance the anti-inflammatory activity of piroxicam.

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Keywords: SNEDDS, piroxicam, anti-inflammatory

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INTRODUCTION

Even though various novel routes of administrations of drugs have been recently developed, yet oral administration is still predominantly preferred by most patients when taking drugs. About 40% of new drugs, however, suffer from poor water solubility that leads to poor drug absorption and ultimately poor oral bioavailability. This issue is observed mainly in the lipophilic drugs group including piroxicam (Obitte et al., 2013; Yang et al., 2016).

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam group that has been widely used for various musculoskeletal disorders such as osteoarthritis, rheumatoid arthritis, and gout, thanks to its anti-inflammatory effect by blocking cyclooxygenase. According to the Biopharmaceutical Drug Classification System (BCS), piroxicam is classified as a Class II drug which attributes to low solubility and high permeability (Motawea et al., 2017). This condition is not ideal for orally administered drugs as poor solubility will ultimately affect the therapeutic effect exerted by the drug. Since dissolution is a rate-limiting step, designing a proper oral drug formulation that focuses on enhancing absorption and bioavailability is demanded (Yang et al., 2016). Many attempts have been employed to solve this problem including microemulsion (Abd-Allah et al., 2010), nanostructured lipid carrier (Otarola et al., 2015), solid dispersion (Barzegar-Jalali et al., 2014; Hussain et al., 2019), and lipid formulation (Jannin et al., 2015), yet further investigations are still needed for these approaches before clinical application.

A self-nanoemulsifying drug delivery system (SNEDDS) is an advanced technology of lipid-based drug delivery system to overcome drugs with inherent problems of low solubility and poor oral absorption (Buya et al., 2020). SNEDDS comprises oil, surfactant, and co-surfactant that form a translucent, anhydrous isotropic mixture. SNEDDS is a thermodynamically stable formulation that can spontaneously emulsify into fine oil-in-water nanoemulsions when exposed to the aqueous environment of the gastrointestinal tract (GIT) facilitated by gentle agitation of the digestive motility (Cherniakov et al., 2015; Rehman et al., 2017).

Recent studies suggest SNEDDS may enhance solubility (Ke et al., 2016) and oral bioavailability (Baloch et al., 2019; Mohsin et al., 2016) of drug targets as well as improve the therapeutic effect (Hapsari et al., 2016) of drugs of interest. Such findings and, not to mention, its relatively high solubilization capacity for lipophilic drugs (Cherniakov et al., 2015) and its feature to produce nanometric-sized emulsion droplets (usually below 200 nm) (Rehman et al., 2017), SNEDDS is likely an ideal approach for improving solubility, bioavailability, and pharmacological effect of piroxicam. Therefore, this study aims to evaluate the anti-inflammatory activity of piroxicam-loaded SNEDDS compared to conventional piroxicam suspension. The composition of SNEDDS employed in this study is per with the previous research (Zaerosa, 2016). The differences of anti-inflammatory activities between groups are assessed based on the measurements of paw edema volume, AUC_{0-6}, and % anti-inflammatory effect.

MATERIALS AND METHOD

Materials

Materials and tools used in this study include piroxicam (Indofarma), carrageenan (Lansida Group), oleic acid, tween 80, propylene glycol (Brataco), PVP (Cipta Kimia), NaCl 0.9% (Widatra Bhakti), HCl 37%, MgCl2 p.a, CaCl2 p.a, KCl p.a (Merck), male Wistar rats aged 2-3 months weighed 150-250 gram, ultrasonicator (Elmasonic), dissolution test apparatus EDT 08Lx (Electrolab), vortex maxi mix II (Thermo Scientific), and spectrophotometer UV-1800 (Shimadzu).

Methods

Preparation of 1% PVP and 1% carrageenan

One percent of PVP was prepared by adding 1 gram of PVP into aquadest up to 100 mL while 1% carrageenan was made by diluting 50 mg of carrageenan into 0.9% NaCl up to 5 mL.

Preparation of piroxicam suspension

Piroxicam suspension was made by diluting 200 mg of piroxicam into 1% PVP up to 100 mL with a final concentration of 2 mg/mL. The mixture was then stirred thoroughly to ensure homogenization.

Preparation of SNEDDS piroxicam

The SNEDDS piroxicam was prepared by loading the piroxicam into the SNEDDS carrier. The carrier was made from a mixture of oleic acid; tween 80; propylene glycol with a ratio of 4:33:13, respectively (Zaerosa, 2016). A 3.3 mL of tween 80 and 1.3 mL of propylene glycol were initially mixed and vortexed for 5 minutes. Afterward, 0.4 mL of oleic acid was added gradually and underwent an additional vortex for 10 minutes. The mixture was then sonicated at 45°C for 5 minutes before 70 mg of piroxicam was finally added into it. The final mixture was then ultrasonicated to ensure homogenization with a concentration equal to 70 mg/5mL.

Orientation assay of carrageenan

A 1% carrageenan was used as an irritant to induce paw edema in rats as described by (Mansouri et al., 2015). Eight rats were equally divided into two groups to undergo an orientation assay to ensure the carrageenan can induce paw edema in rats. The first group received 1.0 mL of 1% carrageenan whereas the second group received 1.0 mL of 0.9% NaCl only, a solvent of carrageenan. The treatment for both groups was induced by sub-plantar injection in hind paws. The progress of paw edema was then monitored every 30 minutes for 6 hours. All procedures as to animal handling described herein follow the ethical clearance issued by the Animal Ethics Committee for Research of Universitas Ahmad Dahlan (registered number: 01170209).

SNEDDS characterizations

Percent transmittance (%)

A 100 µl of the prepared SNEDDS was mixed with aquadest up to 5.0 mL and vortexed for 30 seconds. The % transmittance was measured using an ultraviolet spectrophotometer at a wavelength of 650 nm with aquadest as blank (Patel et al., 2011).

Emulsification time

The emulsification time was done using dissolution apparatus 2. As much as 500.0 mL of aquadest was initially conditioned as a medium in the dissolution apparatus 2 at 37℃. A 1.0 mL of the resulting SNEDDS was added into the medium while being constantly stirred at 100 rpm using the paddle. The emulsification time measurement was performed by observing the time needed from the moment SNEDDS was firstly introduced until the emulsion was completely formed in the medium.

Anti-inflammatory activities assay

All procedures described herein were approved by the Animal Ethics Committee for Research of Universitas Ahmad Dahlan with registration number 01170209. The dose of piroxicam for the animals was calculated according to the dose conversion factor from human to rats as described by the Laurence-Bacharach Method (Wilmana and Gunawan, 2007). The dose employed in this study was 0.36 mg/200 g BW or 1.8 mg/kg BW.

The anti-inflammatory effect was evaluated using paw edema rat assay by measuring the increase of mercury volume in rats that were previously induced by 1% carrageenan sub-plantar. Twenty male Wistar rats were equally divided into 4 groups and received treatments orally as follow:

Negative Control I	: received piroxicam suspension carrier orally
Negative Control II	: received SNEDDS carrier orally
Positive Control	: received piroxicam suspension 1.8 mg/kg BW orally
Treated Group	: received SNEDDS piroxicam 1.8 mg/kg BW orally

All groups were given treatment with the same volume and after 5 minutes they were injected with 1% carrageenan sub-plantar in the hind paw to induce edema. Evaluation on edema volume alteration in the paw was done every 30 minutes for 6 hours using a plethysmometer. The edema volume was calculated using Equation 1 with baseline is defined as observation at 0 min.

$$Edema\ volume = volume\ after\ treated - volume\ at\ baseline \quad \ldots\ldots\ldots\ldots(1)$$

The Anti-inflammatory activity was assessed by measuring the Area under Curve (AUC) that was derived from the edema volume data. The AUC at 0 hour to 6 hours was calculated using the trapezoid method by Equation 2:

$$AUC_{0\text{-}6} = \left(\frac{X0.5 + X0}{2} \times t0.5 - t0\right) + \left(\frac{X1 + X0.5}{2} \times t1 - t0.5\right) \ldots\ldots + \left(\frac{X6 + X5.5}{2} \times t6 - t5.5\right) \ldots\ldots(2)$$

Note:
AUC 0-6 = area under curve at 0-6 hour
x = volume at hour
t = time

Besides the above equation, the anti-inflammatory effect (referred to as %AE) was assessed by measuring the percentage of inflammation inhibition using Equation 3:

$$\%\ inflammation\ inhibition = \frac{(AUC_{0\text{-}6})0 - (AUC_{0\text{-}6})n}{(AUC_{0\text{-}6})0} \times 100\% \quad \ldots\ldots\ldots(3)$$

Note:
$(AUC_{0\text{-}6})0$ = AUC $_{0\text{-}6}$ average of negative control group (mm.hour)
$(AUC_{0\text{-}6})n$ = AUC $_{0\text{-}6}$ of each rat that received treatment (mm.hour)

Data Analysis

Data presented herein were statistically analyzed by the Kruskal-Wallis test, Kolmogorov-Smirnov test, and the Mann-Whitney test ($p<0.05$).

RESULT AND DISCUSSION

The SNEDDS carrier used for loading the piroxicam was a composition of oleic acid (8%), tween 80 (66%), and PG (26%) as described by (Zaerosa, 2016). Oleic acid serves as the oil phase while tween 80 and PG serve as the surfactant and co-surfactant, respectively.

Before the main test, the freshly-prepared SNEDDS piroxicam was firstly characterized for percent transmittance and emulsification time. The percent transmittance defines the amount of light that can pass through material in percentage. This parameter allows to see the clarity of the prepared mixture that later can indicate whether the dispersed droplets are in the nano range (<100 nm) or not. A percent transmittance close to 100% is preferable for nanosized as the higher the value is, the smaller the size of dispersed droplets. In this study, the formed nanoemulsion was clear and transparent with the transmittance average of 99.16% ± 0.06 (n=3), suggesting that the droplets of resulting SNEDDS piroxicam are already nanosized. This result is in line with (Ke et al., 2016) and (Zaerosa, 2016).

The emulsification time enables the evaluation in which the droplets quickly forming a homogenous mixture with mild stirring. Since the SNEDDS is a self-nanoemulsified carrier, this assay is of importance to take place. A good SNEDDS must feature immediate emulsification within 1 minute as soon as it reaches GIT (Makadia et al., 2013). In the GIT, the spontaneous emulsification is further facilitated by a relatively low-energy agitation generated by peristaltic movement. Data obtained in this study exhibit an average time of emulsification at 44.82 ± 0.53 seconds (< 1 minute)

(n=3). This suggests that the prepared SNEDDS, once reaching the GIT, may be able to be emulsified spontaneously and meets the criteria of a good SNEDDS. This phenomenon has also been described by (Balakumar et al., 2013). Further, it is suggested that, regardless of tween as a surfactant, the accelerated emulsification time is also activated thanks to the presence of propylene glycol as a co-surfactant by increasing the fluidity through penetration (Belhadj et al., 2013).

Male Wistar strain rats aged 2-3 months with weights ranging from 150 to 250 grams were selected in this study. Males were preferred because it is relatively stable from hormonal imbalance. All animals used were acclimated by being kept in a standard laboratory condition for 7 days before the test to avoid unwanted variations caused by stress factors. All procedures as to animal handling were based on the ethical clearance issued by the Animal Ethics Committee for Research of Universitas Ahmad Dahlan (registered number: 01170209).

After the characterization of SNEDDS had been established, a preliminary study was then performed before the anti-inflammatory assay by exposing the animals with carrageenan, an edema-induced agent, in the hind paw to ensure that this irritant is indeed able to cause edema at the injection site. This preliminary was done by grouping 8 male Wistar rats into 2 groups equally. The first group received 0.1 mL of 1% carrageenan while the second group received 0.1 ml of the carrier (0.9% NaCl). The paw edema was observed every 30 minutes for 6 hours. The results demonstrated that only the group exposed with carrageenan experienced escalated paw edema volume while the second group which only received 0.9% NaCl had no edema at all (no edema volume escalation was observed as compared to the baseline). The difference between the groups is depicted in Figure 1. This confirms that carrageenan could induce edema.

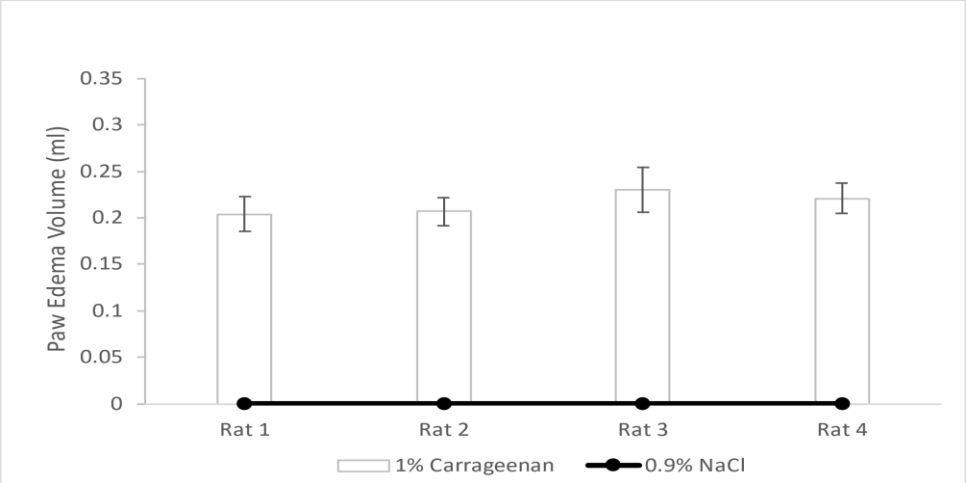

Figure 1. Paw edema volume alteration between groups from the preliminary test. All rats that received 0.1 mL of 1% carrageenan (white bar) show excessive paw swollen marked by increasing edema volume (in ml) after injection, while rats with 0.1 mL of 0.9% NaCl (black line), a solvent of carrageenan, had no edema throughout 6-hour observation ($\rho<0.05$)

The anti-inflammatory activity was assessed by the carrageenan-induced paw edema method where the animals were injected using carrageenan sub-plantar in the hind paw to allow edema. As soon as the carrageenan was given, redness and swollen paw were immediately observed in the rats in each group. The edema was then measured for the volume of swelling produced in the paw as compared to the baseline. The paw edema volume was observed every 30 minutes for 6 hours using the plethysmometer. The lower the edema volume is, the greater the rats are protected from paw edema (Cong et al., 2015). The results in each group can be seen in Figure 2.

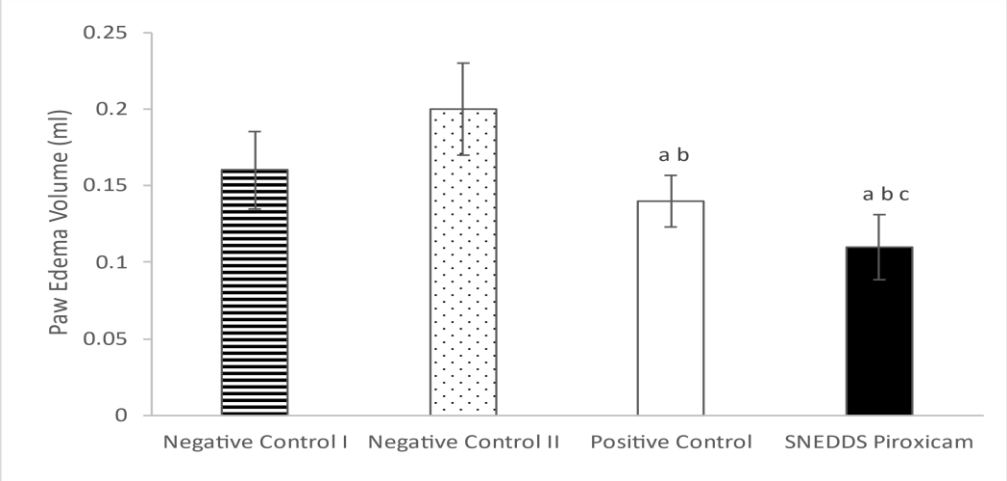

Figure 2. SNEDDS piroxicam group (solid black bar) exhibits the least paw edema volume while other groups were at a higher level. This indicates that the rats injected by SNEDDS piroxicam experienced the smallest swollen paw, suggesting that this group also had better protection from edema than the group injected with piroxicam suspension (white bar). a. significantly different from negative control I ($\rho<0.05$); b. significantly different from negative control II ($\rho<0.05$); c. significantly different from positive control ($\rho<0.05$)

As depicted in Figure 2, negative control I and negative control II which received 1% PVP and SNEDDS carrier, respectively, showed a relatively high edema volume. This suggests that 1% PVP and SNEDDS carrier is least likely to have an anti-inflammatory effect which results in no protection from edema on the rats at all (it was observed during the study that the edema on the rats of these groups kept increasing even after a 6-hours observation was done). On the contrary, positive control which received piroxicam suspension and SNEDDS piroxicam group, showed lower edema volume as compared to negative control I and II with SNEDDS piroxicam group had the lowest paw edema volume ($\rho<0.05$). From the study, it was observed that the paw swelling on the rats in positive control started decreasing 4 hours after injection while in the SNEDDS piroxicam group was even quicker, the paw swelling started reducing 3 hours after injection ($\rho<0.05$). This result demonstrates that piroxicam-loaded SNEDDS has the greatest protection from paw edema on the rats between groups that may also suggest that SNEDDS piroxicam has better anti-inflammatory activity than the conventional piroxicam suspension.

To further assess the anti-inflammatory activities of the SNEDDS piroxicam, the AUC_{0-6} value was calculated using the trapezoid method. The AUC_{0-6} was derived from the edema volume data and reflects the AUC value obtained from 0 to 6 hours data. This parameter is employed to further evaluate to what extent the anti-inflammatory activity in each group is. The smaller the value of $AUC_{0-6,}$ the greater the anti-inflammatory activity exerted by the compound (Sahlan et al., 2019). In other words, if the AUC_{0-6} value was relatively low, it indicates that the rats were prominently protected from severe edema as the swelling paw was gradually reduced over time thanks to the compound given. The result of the calculated AUC_{0-6} can be seen in Figure 3.

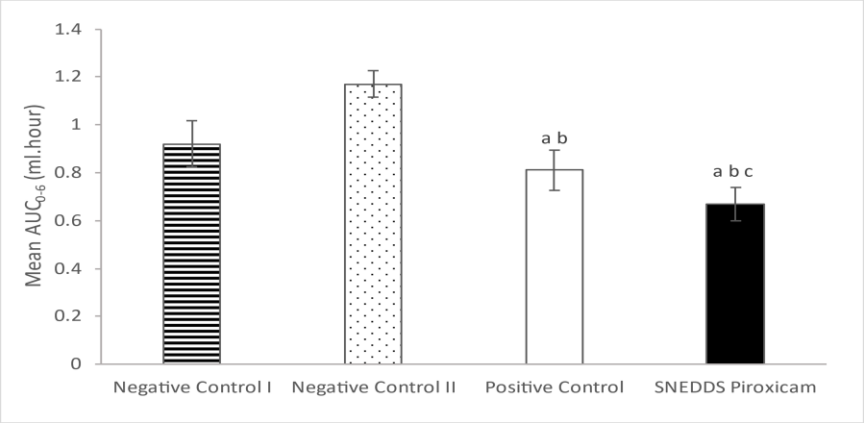

Figure 3.The average of AUC_{0-6} between the groups. The negative control I (striped bar) and negative control II (dotted bar) present a relatively high AUC_{0-6} value while positive control (white bar) and SNEDDS piroxicam group (solid black bar) show a markedly smaller value with the latter had the smallest. a. significantly different from negative control I (ρ<0.05); b. significantly different from negative control II (ρ<0.05); c. significantly different from positive control (ρ<0.05)

As shown in Figure 3, the SNEDDS piroxicam group had the smallest value of AUC_{0-6} and is followed by positive control, negative control I, and negative control II in the second, third, and fourth place, respectively. This result suggests that the rats which received SNEDDS piroxicam were least affected by the paw edema (ρ<0.05). In addition, as the positive control had a higher value of AUC_{0-6} than the SNEDDS piroxicam (ρ<0.05), this demonstrates that SNEDDS is likely to augment the anti-inflammatory activity of the loaded piroxicam, thus leading to a better effect on the rats.

To measure the anti-inflammatory activity, the AUC_{0-6} data can also be used to evaluate the percentage of anti-inflammatory effect (%AE) between groups using Equation 3. The %AE of the positive control group and SNEDDS piroxicam group were compared to further assess the anti-inflammatory effect between these two groups. The %AE is displayed in Figure 4.

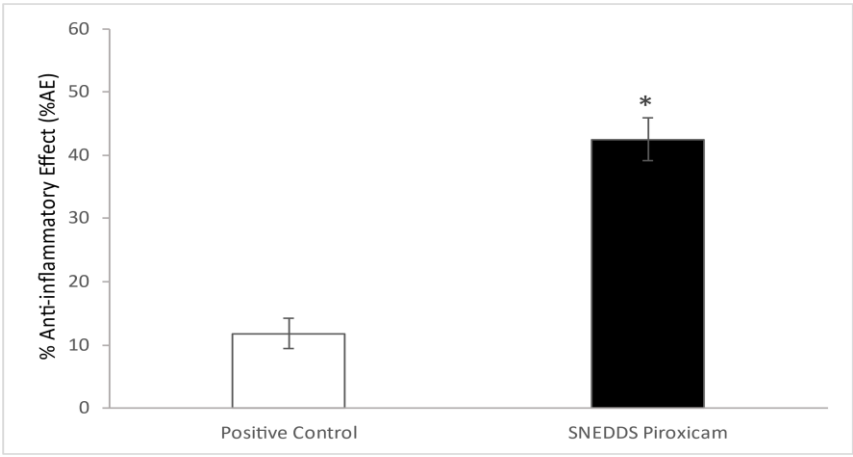

Figure 4. A comparison of %AE between positive control group (white bar) and SNEDDS piroxicam group (black bar). This graph reveals that the rats given with SNEDDS piroxicam received over 3 times higher anti-inflammatory effects than the piroxicam suspension group did. *Significantly different from the positive control group (ρ<0.05)

Based on Figure 4, the SNEDDS piroxicam group exhibits a considerably higher %AE than that of the positive control with 42.5±3.4 % and 11.8±2.6%, respectively. The gap between these groups is significantly huge (over 30%) ($\rho<0.05$), meaning that the SNEDDS piroxicam managed to alleviate the swollen paw on the rats to a greater extent compared to those that received piroxicam suspension only. Based on this result, the rats with SNEDDS piroxicam were most likely to experience an enhanced anti-inflammatory effect; therefore, the %AE is higher than the ones with piroxicam without SNEDDS.

A better anti-inflammatory activity of SNEDDS piroxicam over piroxicam suspension as demonstrated in this study may be explained by several factors: first, the SNEDDS may improve piroxicam solubility via spontaneous emulsification in the GI fluid. Within the GIT, the SNEDDS may promote immediate nanoemulsion formation, where the piroxicam is presented in the solubilized state that leads to dissolution rate enhancement (Belhadj et al., 2013). Second, nanosized emulsion droplets produced by SNEDDS may significantly increase the surface area needed for enhanced drug absorption within the intestine, thereby allowing better bioavailability (Kanwal et al., 2019). Third, the use of lipid in the SNEDDS is thought to also promote the absorption rate via the intrinsic lipid pathway (Mirza et al., 2010). Fourth, by loading into SNEDDS, the piroxicam tends to be well-protected from liver-induced first-pass-elimination preventing the early drug degradation. In such conditions, the ratio of active compound that can be presented/ available in systemic circulation may be raised, thus optimum therapeutic concentration can be achieved, therefore enabling high systemic bioavailability (Motawea et al., 2017). These combined factors are likely to cause an improvement of anti-inflammatory effect on SNEDDS piroxicam as compared to piroxicam in conventional suspension form. However, since this study only describes the anti-inflammatory effect as evaluated via paw edema volume, $AUC_{0\text{-}6}$ and %AE, further studies such as in vivo and pharmacokinetic studies are still needed for improvement and establishing the findings in more depth.

CONCLUSION

SNEDDS significantly enhances the anti-inflammatory activity of loaded piroxicam compared to that of piroxicam suspension. The rats treated with SNEDDS piroxicam demonstrated better protection from paw edema than other groups, suggesting SNEDDS is a promising approach for the delivery of water-insoluble drugs.

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