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## **Reducing ulcerogenic effect of self-nano emulsifying drug delivery system of piroxicam**

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### **ABSTRACT**

Piroxicam is antiinflammatory non-steroidal (AINS) drug group that has anti-inflammatory, analgesic and antipyretic effects. Like most other AINS drugs, piroxicam has low solubility and has gastrointestinal (ulcerogenic) side effects on long-term use. The nano-emulsifying drug delivery system (SNEDDS) is one of the technologies that can be used to overcome it. This study aims to determine the effect of ulcerogenic SNEDDS piroxicam compared with piroxicam formulas instead of SNEDDS. This study used white rats male strain Sprague Dawley (SD) age 2-3 months and weight 100-200 g of 40 rats. Rats divided into 5 groups. Group I was a normal control group, which were given only water. Group II was a vehicle control group which treated with a 1% polyvinylpyrrolidone (PVP) solution, group III was a carrier control group which treated with SNEDDS base (a mixture of tween 80, virgin coconut oil (VCO) and polyethylene glycol (PEG) 400), group IV was a group of piroxicam which reated with piroxicam 1.08 mg/Kg suspended 1% PVP, group V was treated with SNEDDS piroxicam. Treatment was done for 28 days. After treatment, the gastric of rats were taken to be observed for ulcerogenic effects. Observations were made macroscopically by looking at ulcer scores followed by histopathological observations of tissue. The ulcer score data from each group were analyzed using one-way ANOVA and LSD test. The results showed that the normal control group, 1% PVP suspension and SNEDDS base groups had an ulcer index of 0.0, 0.0 and 0.0 respectively, while the piroxicam suspension and the SNEDDS groups had an ulcer index of 0.88 and 0.0. These results were confirmed by histopathologic results of SNEDDS piroxicam in order to decrease the effect of piroxicam ulcerogenic. It can be concluded that SNEDDS piroxicam can decrease the ulcerogenic effect.

**Keyword :** piroxicam, SNEDDS, ulcerogenic

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## INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs to reduce pain and swelling (Wallace and Vong, 2008). NSAID drugs have side effects of gastrointestinal disorders. Symptoms of gastrointestinal disorders such as dyspepsia occur in 15-60% of users of OAINS and peptic ulcers ranging from 0.1-0.19% of the total patients (Sung *et al.*, 2009). Hospital-based endoscopic data showed gastrointestinal complications resulting from the use of NSAID in Makassar 71%, Jakarta 67.7%, and Surabaya 61% (Margaretha *et al.*, 2011). WHO data showed that the deaths caused by peptic ulcers in Indonesia reached 0.99%, which is obtained from the death rate of 8.41 per 100,000 population. Peptic ulcer was ranked 10th in the category of causes of death in the age group of 45-54 years in men according to BPPK Depkes.

Piroxicam like other OAINS can cause side effects on the digestive tract, one of which is a peptic ulcer. It has been reported that OAINS crystals have poor solubility in gastric acid and contact with the gastric wall for prolonged periods resulting in dangerous local concentrations. This causes local irritation of the stomach wall followed by ulceration (Nagarsenker *et al.*, 2000). In addition, the systemic piroxicam inhibits COX so that prostaglandin synthesis is hampered (Laine *et al.*, 2008). The hampering of mucosal prostaglandin synthesis will lead to peptic ulcers (Kumar *et al.*, 2007).

Piroxicam is a second class drug in Biopharmaceutical Drug Classification System (BCS) which have a low dissolution and high permeability (Blagden *et al.*, 2007). The absorption rate and bioavailability rate for the hydrophobic drug are controlled by the dissolution rate in the digestive fluid. From the previous research we have obtained the optimum formula of self-nano emulsifying drug delivery system (SNEDDS) piroxicam (Dewi, 2016). SNEDDS is a mixture of isotropic oil phases, surfactants, cosurfactants and drugs that make up nanoemulsion of oil in water when added to the aqueous phase under slow stirring (Wang *et al.*, 2009). The nanoemulsion protects the drug particles that can not dissolve in the stomach fluid, so that the particles are not in direct contact with the gastric mucosa (Anuradha *et al.*, 2013). This research aimed to investigate the decrease of ulcerogenic effects of SNEDDS piroxicam with VCO as oil phase, tween 80 as surfactant and PEG 400 as cosurfactant compared with piroxicam formulas instead of SNEDDS.

## MATERIALS AND METHOD

### Materials

Piroxicam (pharmaceuticals) grade which used for study was found from PT Indofarma Tbk.

### Production of SNEDDS Piroxicam

SNEDDS piroxicam was made with a composition of 12% VCO; 64% tween 80; 24% PEG 400 v/v and 10 mg/mL piroxicam (Dewi, 2016). VCO, tween 80 and PEG 400 were mixed in vials, then vortexed (mix) for 5 minutes. Followed by sonication for 5 minutes and heated for 5 minutes with a temperature of 45 oC. The next step of piroxicam is mixed in the mixture. The piroxicam in the carrier was then homogenized with a vortex for 5 minutes, with a sonicator for 5 minutes, heated in a 45 °C waterbath for 5 minutes. Repeat the cycle procedure 2 times.

### Characteristics Test of SNEDDS piroxicam

#### Clarity test

Clarity test was done through transmittance reading with spectrophotometer. One hundred microliter the piroxicam SNEDDS formula was taken and then added up to 5 mL of aquadest, then vortex for 1 min to mix it into homogenous. Furthermore, transmittance percent reading was done at 650 nm wavelength with aquadest blanks (Lalwani *et al.*, 2013). The reading was conducted 3 times.

### Emulsification time test

The emulsification time test was performed using type 2 dissolution apparatus with aquadest as its medium. A total of 500 mL of aquadest was conditioned on the device with a temperature of 37°C. One milliliter SNEDDS piroxicam was included in the medium along with rotating the paddle at 100 rpm. The obtained time is calculated starting from SNEDDS entering to form a clear solution in aquadest medium. The time obtained is then recorded and replicated 3 times (Balakumar *et al.*, 2013).

### Ulcerogenic test

All test protocols have been approved by the Universitas Ahmad Dahlan Ethics Committee with Ref number 011702020. The test animal used was white male rats of SD aged 2-3 months with body weight 150-200 g obtained from animal trader test from Solo. The rats were divided into 5 groups with 8 rats of each, i.e.:

- a. Group I: given by water, as a normal control.
- b. Group II was given by a 1% PVP solution, as a vehicle control.
- c. Group III was given a mixture of tween 80, VCO, PEG 400, as a SNEDDS base control.
- d. Group IV was given a suspected piroxicam dose of 1.08 mg/Kg in 1% PVP.
- e. Group V was given SNEDDS piroxicam dose of 1.08 mg/Kg BW.

Treatment was done orally once daily for 28 days. On the 29th day the rats were sacrificed by anesthetized with ether then performed surgery. The stomach was removed, then the gastric mucosa was opened along the major curvatura, washed with physiological NaCl. After that, spread on a flat surface and then photographed, macroscopically observed, then scored according to (Szabo *et al.*, 1985) modified.

Microscopic observation was performed by observing histopathology of gastric mucosa. The gastrics were cleaned with physiological NaCl, then stored in pots containing 10% technical formalin. Preparation was carried out by standard method in Pathology Laboratory, Faculty of Veterinary Medicine of UGM. Observation of histopathologic preparations were performed using the Optilab apparatus.

### Data Analysis

The number of ulcers were counted and the ulcer index calculated using the method described by Szabo *et al.*, (1985) with equation 1.

The ulcer index = Mean (hyperemia score + hemorrhage score + erosion score) .....(1)

## RESULT AND DISCUSSION

### Characteristics of SNEDDS Piroxicam

The resulting SNEDDS piroxicam characters has met the clarity and emulsification time. Clarity is expressed in percent transmittance. Measurement of transmittance percent was done to prove that the emulsion droplet which has reached the nanometer size (less than 100 nm). The results show that the average transmittance value of three replications are 99.38% ± 0.23. Because this value is close to 100%, so it can be stated that the emulsion droplet in SNEDDS piroxicam formula has reached nanometer size (Bali *et al.*, 2010). The size of the dispersed phase greatly affects the appearance of the emulsion. When the nanoemulsion formed is passed through the light, the light beam is transmitted, resulting in a large transmittance value (Sahumena, 2014).

The emulsification time is the time which was taken to form a homogeneous mixture in a medium with light stirring. This character describes the time SNEDDS take to form an emulsion in the gastrointestinal tract. SNEDDS should be able to form spontaneous nanometer-sized emulsions

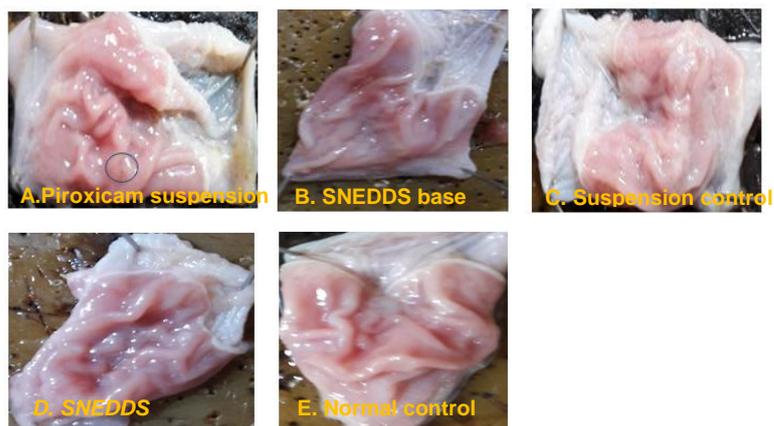
in the gastrointestinal tract with mild agitation such as peristaltic. SNEDDS should be good enough when the emulsification time is produced in less than a minute with a clear and transparent appearance (Balakumar *et al.*, 2013). The results showed that the emulsification time of SNEDDS piroxicam was able to fully emulsified to form nanoemulsion in less than one minute ie 38.97 seconds  $\pm$  7.14. When the emulsion is formed there is interaction between tween 80 and PEG 400. PEG 400 as cosurfactant can increase fluidity through penetration and form empty space between surfactant molecules, so PEG 400 plays a role in accelerating emulsification time (Belhadj *et al.*, 2013).

### The ulcerogenic effect of SNEDDS piroxicam on macroscopic observation

The gastric macroscopic differences on each treatment is exhibited in Figure 1. The macroscopic observation of the gastric mucosa shows that of the giving of piroxicam with a dose of 1.08 mg/Kg (group IV) for 28 days may lead to a peptic ulcer in rats. These results are consistent with the study (Nagarsenker *et al.*, 2000) who reported that OAINS crystals have poor solubility in gastric acid and contact with the gastric wall for prolonged periods to produce concentrations causing local irritation of the stomach wall followed by ulceration (Nagarsenker *et al.*, 2000). Otherwise, the systemic piroxicam inhibits COX so that prostaglandin synthesis is inhibited. Inhibition of prostaglandin synthesis will decrease mucosal resistance and trigger gastric mucosal damage (Laine *et al.*, 2008).

There was no hemorrhage found in the SNEDDS piroxicam treated groups as well as the group given SNEDDS carrier which was a mixture of 12% VCO; 64% tween 80; 24% PEG 400 v/v. This result shows that the formulation of SNEDDS piroxicam can protect the hemorrhagic occurrence of rat stomach.

Observation of gastric ulcer severity index was performed according to (Szabo *et al.*, 1985) modified. The results of the SNEDDS ulcer index can be seen in Table I. The results of the ulcer index are used to assess the state of the peptic ulcer formed. According to Table I, it was seen that in the groups given the piroxicam suspension had a severity index of 0.88 greater than the piroxicam SNEDDS group and the control group. The results of this ulcer indicated that the SNEDDS piroxicam formulation can protect the occurrence of ulcers compared to the piroxicam suspension group. Result and discussion should be combined in the manuscript. It is should be described concisely. Text, tables and figures must be internally consistent. Discussion should involve the significant findings presented with relevant and extensive discussion.



**Figure 1. Macroscopic of stomach: A. Piroxicam suspension occurs hemorrhage** ○ **B. SNEDDS base control does not change, C. Suspension control does not change, D. Piroxicam SNEDDS does not change, E. Normal control does not change**

**Table I. The mean of ulcer index (x ± SD) after treatment with SNEDDS piroxicam**

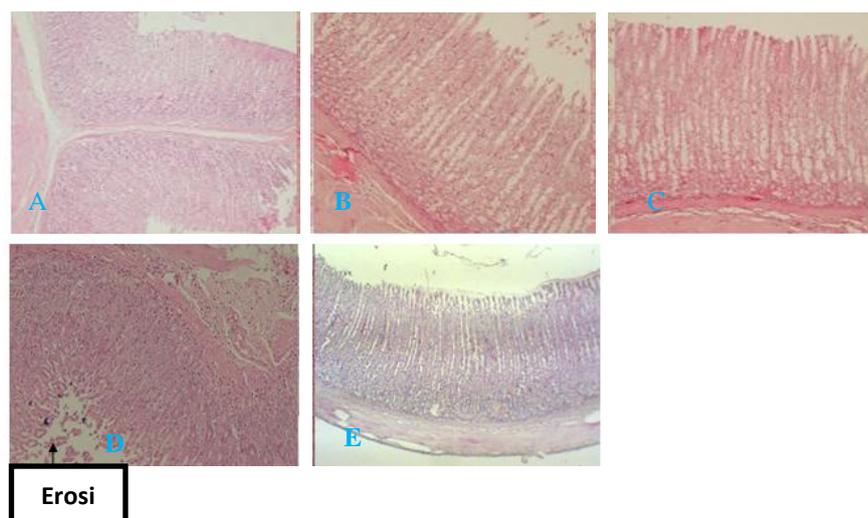
Group (n=6)	Ulcer Index
Normal control	0.00 ± 0.00
Suspension control	0.00 ± 0.00
SNEDDS base control	0.00 ± 0.00
Piroxicam suspension	0.88 ± 0.08
Piroxicam SNEDDS	0.00 ± 0.00

### Microscopic stomach organs

Microscopic observations of the gastric mucosa were performed to observe gastric conditions at a cellular level which not seen in macroscopic observations. The staining organ was performed by hematoxylyne and eosin (HE) dyes. The hematoxylyne dye is an alkaline dye that will give a blue or purple color to the acid component including the nucleus, while the eosin dye is an acid dye that gives the pink color in the cytoplasmic base component (Mescher, 2011). The histopathological results of the gastric mucosa is showed in Figure 2.

The result of histopathological observations on normal control (water), 1% PVP suspending control and carrier controls appear to be unchanged or tissue seen to be normal. In the piroxicam suspension group there is a change of erosion (Figure 1 A). In the piroxicam SNEDDS group there was no change. Mucosal damage can be said to erosion if the depth is less than 5 mm. If the mucosal damage reaches 5 mm or more until it reaches submucosa with necrosis it is called ulcer (Puspitasari, 2008). Cell damage (necrosis) will stimulate the release of inflammatory mediators. The task of this inflammatory mediator begins with acute inflammation and ends with healing (Reid *et al.*, 2011). This histopathological observation showed that the ulcerogenic effect was seen only in the piroxicam suspension group (Figure 2D), so it can be concluded that SNEDDS piroxicam formulas are able to protect the stomach from the ulcerogenic effects of piroxicam.

Piroxicam is known to cause ulcerogenic effects in the stomach. Ulcerogenic caused by topical effects as well as systemic effects. Topical effects occur because the piroxicam is acidic and lipophilic. Piroxicam has a weak acidic nature, so in the gastric fluid piroxicam is in unionized form and dissolves in lipids. Piroxicam diffuses through the gastric epithelial cell membrane to the cytoplasm, where the pH is neutral. At that pH, piroxicam is converted to ionized and lipophobic forms, so that piroxicam is trapped in the cell and causes cellular damage (Matsui *et al.*, 2011). In some studies and articles mentioned that piroxicam and other AINS drugs have low solubility in stomach acid and are in direct contact with the gastric wall for long periods of time, resulting in dangerous local concentrations. It causes local irritation of the stomach wall and ulcers (Anuradha *et al.*, 2013). Systemic effect of piroxicam occurs by inhibition of cyclooxygenase enzyme (COX) in arachidonic acid so that prostaglandin and prostacyclin production is reduced. Prostaglandins are found in the gastric mucosa. Prostaglandins are a cytoprotective substance for the gastric mucosa performed by maintaining mucosal blood flow, increasing mucus secretion and bicarbonate ions, reducing gastric acid secretion and enhancing epithelial defenses (Wallace, 2008). Through inhibition of this prostaglandin causes protection of the gastric mucosa of the insoluble piroxicam particles decreases.



**Figure 2. Microscopic profile of SNEDDS piroxicam: (A) Normal control, (B) Suspension control, (C) SNEDDS base control, (D) Piroxicam suspension group with erosi (▲), (E) Piroxicam SNEDDS group**

The results of this study are in line with the study (Putri, 2012) on the reduction of the piroxicam ulcerogenic effect through the formation of piroxicam-PVP solid dispersions in male white rats. Similar results are also obtained (Obitte *et al.*, 2013) on the decline in the ulcerogenic effects of piroxicam on solid lipid microparticle delivery. Nanoemulsion on SNEDDS protects drug particles that are insoluble in gastric fluid, so that the particles do not come into direct contact with the gastric mucosa (Anuradha *et al.*, 2013). This protection is thought to decrease the ulcerogenic effect of piroxicam.

## CONCLUSION

The results showed that SNEDDS piroxicam with VCO as oil phase, tween 80 as surfactant and PEG 400 as cosurfactant decreased the ulcer score compared with piroxicam suspension.

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