REDUCED ULCEROGENECITY FROM *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~~s~~ white rats male strain Sprague Dawley (SD) age 2-3 months and weight 100-200 g~~rams~~ of 40 rats. Rats divided into 5 groups. Group I was a normal control group, ~~the test animals~~ which were given only water. Group II was a ~~suspending~~ vehicle control group which treated with a 1% polyvinylpyrrolidone (PVP) solution, group III ~~is~~was a carrier control group which treated with SNEDDS carrier ~~which is~~ (a mixture of tween 80, virgin coconut oil (VCO) and polyethylene glycol (PEG) 400), group IV was a group of piroxicam which reated with pyroxicam 1.08 mg/kg ~~drugs~~ suspended ~~with piroxicam 1.08 mg/kg in~~ 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 ANOVAand LSD test. The results showed that the normal control group, 1% PVP suspension and carrier groups had a ulcer index of 0.0, 0.0 and 0.0 respectively, while the piroxicam suspension ~~group~~ and the SNEDDS groups had an ulcer index of 0.88 and 0.0. These results were confirmed by histopathologic results of SNEDDS piroxicam to decrease the effect of pyroxicam ulcerogenic with results in the piroxicam suspension group has ulcer with necrosis by neutrophil infiltration, lymphocytes and mast cells in the mucosal tunica to submucosa. In the SNEDDS piroxicam group there was~~is~~ erosion with necrosis of the mucosal tunica epithelium ~~with~~ marked by infiltration of lymphocytes and mast cells in submucosal tunica. It can be concluded ~~the~~ that SNEDDS piroxicam can decrease the ulcerogenic effect.

Keyword : piroxicam, SNEDDS, ulcerogenic

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*

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al., 2011). WHO data showed that the deaths caused by peptic ulcers in Indonesia reached 0.99 percent, 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 hamperation 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 (Pol *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 METHODS

The tools used in this research consist of spectrophotometer (Shimadzu UV-1800), Ultrasonicator (Elmasonic), dissolution apparatus type 2, digital camera (C~~c~~annon), vortex, micropipette (Product?), microscope (Product?) and optilab (Product?).

The materials used in this study were piroxicam ((pharmaceutics); tween 80 (pharmaceutics); PEG 400 (pharmaceutics); VCO (pharmaceutics), polyvinylprolidone (PVP) (pharmaceutics); aquadest, chloroform, NaCl; technical formalin 0.5% and technical formalin 10%. The test animal used was~~is~~ white male rats of SD ~~strain~~ aged 2-3 months with body weight 150-200 g obtained from animal trader test from Solo.

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Research Procedure

1. Production of SNEDDS Piroxicam

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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 ~~are~~ were mixed in vials, then vortexed? 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 ~~is~~ was then homogenized with a vortex for 5 minutes, with a sonicator for 5 minutes, heated in a 45 °C waterbath for 5 minutes, repeated the cycle 2 times.

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2. Characteristics Test of SNEDDS piroxicam

a. Clarity Test

Clarity test wasis done through transmittance reading with spectrophotometer. One hundred microliter tThe piroxicam SNEDDS formula wasis taken 100 µL and then added up to 5 mL of aquadest, then vortex for 1 min. Furthermore, transmittance percent reading wasis done at 650 nm wavelength. The reading was conducted is replicated 3 times.

b. Emulsification Time Test

The emulsification time test wasis 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 ºC. One mililiter SNEDDS piroxicam of 1 mL is 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).

c. Ulserogenic Test

All test protocols have been approved by the Universitas Ahmad Dahlan University Ethics Committee numbered 011702020. The test animals rats of 40 male white rats SD were divided into 5 groups with 8 rats of groups each,-i.e divided into:

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

Provision of dosage is was done orally once daily for 28 days. Clinical symptoms occurring in mice rats? were observed during treatment. On the 29th day the rats mice were sacrificed by anesthetized with ether then performed surgery. The stomach was is removed then the gastric mucosa was is opened along the major curvatura, washed with physiological NaCl then spread on a flat surface and then photographed, macroscopically observed, then scored according to (Szabo *et al*., 1985) modified.

To strengthen macroscopic data, Mmicroscopic observation was performed by observing histopathology of gastric mucosa organs, The gastrics were organs cleaned with physiological NaCl, then stored in pots containing 10% technical formalin. Preparation was carried out is done by standard method in Pathology Laboratory, Faculty of Veterinary Medicine of UGM. Examination and interpretation Observation of histopathologic preparations were performed using the Optilab apparatus.

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RESULTS AND DISCUSSION

1. 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 ~~is~~ was done to prove that the emulsion droplet which has reached the nanometer size ~~of~~ (less than 100 nm). The measurements were performed using UV-Vis spectrophotometry at a wavelength of 650 nm with aquadest blanks (Lalwani *et al*., 2013). The results show that the average transmittance value of three replications are 99.38% ± 0.23. Because ~~T~~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 ~~it~~ which was taken ~~takes for the system~~ to form a homogeneous mixture in a medium with light stirring. This character describes the time it takes SNEDDS 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 peristalsis. SNEDDS is said to be good 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 ± 7.14. When the emulsion is formed there is interaction between tween 80 and PEG 400. PEG 400 as kosurfaktan 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

~~Macroscopic observations of gastric ulcers were performed on all test animals except for healthy control groups~~. The macroscopic gastric sighting differences in each treatment ~~can be seen~~is ~~-~~exhibited in Figure 1. The macroscopic observation of the gastric mucosa ~~stomach~~ shows that ~~28 days~~ of the giving of piroxicam with a dose of 1.08 mg/kg (group IV) for 28 days may lead to a peptic ulcer ~~on the stomach of the~~ in ~~mouse~~ rats? Rats were given by 1% PVP ~~group as suspending~~ and ~~healthy~~ normal control group indicated the presence of hemorrhage. 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).

In the group given SNEDDS piroxicam there was no hemorrhage 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.

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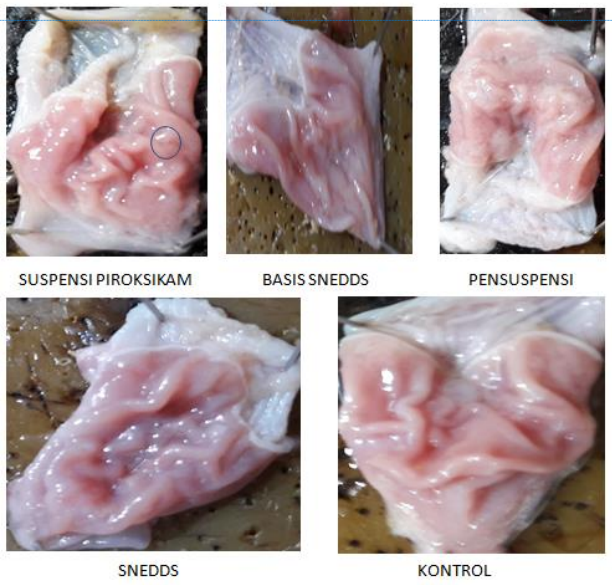

Figure 1. Macroscopic stomach: A. Normal control does not change, B. Suspension control does not change, C. SNEDDS base control does not change, D. Piroxicam suspension occurs hemorage, E. SNEDDS piroxicam no change.

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 susupension 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.

Table I. The ~~results of calculation of~~ mean of ulcer index ~~(x ± SD) (n = 8)~~ of ~~in the~~ the groups ~~given SNEDDS piroxicam.~~

Group	Ulcer Index ~~(x ± SD)~~
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
SNEDDS piroxicam	0.00 ± 0.00

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Microscopic Stomach Organs

Microscopic observations of the gastric mucosa ~~stomach organs~~ were performed to ~~at~~ observe ~~stomach~~ gastric conditions at a cellular level which not seen in macroscopic observations. ~~This observation is done by tissue histopathology. This microscopic observation is done to strengthen the observation macroscopically.~~ T~~in~~he ~~staining~~ organ ~~preparations are~~ was performed by ~~using~~ hematoxillyne and eosin (HE) dyes. The hematoxillyne dye is an alkaline dye that will give a blue or purple color to the acid component ~~used to dye~~ 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 ~~organs~~ mucosa is showed ~~can be seen~~ 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. 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 ulser (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, so it can be concluded that SNEDDS piroxicam formulas are able to protect the stomach from the ulcerogenic effects of piroxicam.

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Piroxicam is known to cause ulcerogenic effects in the stomach. Ulserogenic 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 (Pol *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.

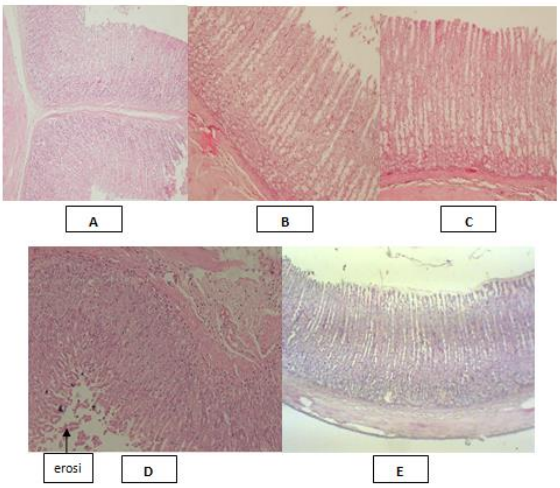

Figure 2. Section of Microscopic Stomach ~~Mouse~~ rats?Description: (a) Normal Control (water), (b) 1% PVP Suspension Control, (c) SNEDDS Control, (d) Piroxicam Suspension Group, (e) Piroxicam SNEDDS Group.

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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 piroxicam protects drug particles that are insoluble in gastric fluid, so that the particles do not come into direct contact with the gastric mucosa (Pol *et al*., 2013). This protection is thought to decrease the ulcerogenic effect of piroxicam.

CONCLUSION

The results showed that SNEDDS piroxicam decreased the ulcer score and improved histopathologic of gastric mucosa~~features~~. ~~SNEDDS piroxicam can decrease the effect of pyroxicam ulcogenic.~~

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ACKNOWLEDGMENT

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