The Potency of Ethanolic Extract of *Sauropus androgynus* (L.) Merr Leaves as Therapeutic herbal of Rats (*Rattus norvegicus*) Peptic Ulcer Model Induced by Aspirin

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Abstract. Peptic ulcer is an erosion of the mucosa gaster and duodenum. Aspirin can increase the activity of ROS in duodenum leading to be oxidative stress condition, followed by the increase expression of TNF-α and changes in the duodenum histopathology. Ethanolic extract from *Sauropus androgynus* (L.) Merr leaves contains flavonoid compound that act as antioxidants. The research aimed to study the therapeutic effect of *Sauropus androgynus* (L.) Merr leaves towards the expression of TNF-α and the duodenum histopathology. The study used five groups: the negative control group, the positive control group (peptic ulcer), and three therapeutic groups. Peptic ulcer induced by aspirin 200 mg/kg BW given orally once a day for five days. The therapeutic group (dose 16.2 mg/200 g BW, 32.4 mg/200 g BW, 48.6 mg/200 g BW) administrated orally once a day for fourteen days. Expression of TNF-α were analyzed by ANOVA followed by Tukey test (α=5%) and histopathology description of duodenum were analyzed descriptive qualitative. The result showed that treatment of the ethanolic extract from *Sauropus androgynus* (L.) Merr leaves at dose 48.6 mg/200 g BW was the effective dosage. The conclusion of this research indicate that ethanolic extract from *Sauropus androgynus* (L.) Merr leaves can be used as alternative therapy peptic ulcer towards decrease the expression of TNF-α and repair duodenum histopathology such as structure of epithelial duodenum, vile and decrease of inflammatory cell and vascular vasodilatation

1. Introduction

Peptic ulcer or ulcus pepticum are ulcer that generally occur in gastric and duodenum as a result of imbalance between aggressive and defensive factors. The aggressive factor includes acid, pepsin and *Helicobacter pylori*, while the defensive factor are gastric mucus and bicarbonate secretion, prostaglandin, nitric oxide and innate resistance of mucosal cells. The peptic ulcer is indicated by a gnawing or burning sensation shortly after meal for gastric ulcer and 2-3 hours afterward for duodenal ulcer. In a gastric ulcer, usually, the acid secretion is normal or low, whereas in duodenal ulcer, acid secretion is high in half of patients but normal in the rest. Gastric and duodenal ulcers can be induced by a variety of factors such as stress, smoking, nutritional deficiencies and noxious agents including non-steroidal anti-inflammatory drugs (NSAID)[1].

Aspirin is a drug of NSAID that affects both cyclooxygenase (COX -1 and COX-2 activity). Aspirin can inhibit COX-2 activity to reduce inflammation and pain while inhibition of COX -1 lowers the prostaglandin synthesis. The decrease of prostaglandin can reduce mucus secretion and vasoconstriction of capillary blood leading to disturbance of H+ diffusion from gastric. The excessive gastric acid can lower bicarbonate and prevent proliferation of mucus epithelia that can injure the mucosa [2].
The increase of HCl production in gastric can trigger the excessive production of ROS, consequently, the duodenal tissue undergo stress oxidative condition [3]. The oxidative stress enhance oxidation process in normal cells that can severe to the body. This condition is responded by immune system through activation of transcription factor NF-κB with TLR signal in transduction pathway. The increase of NF-κB activation will be encountered by macrophage to produce pro-inflammatory cytokine (TNF-α) as inflammation indicator [4]. The inflammation can damage the duodenal mucosa lead to the change of duodenal histopathology.

Currently available medication for peptic ulcers include antacids (systemic and nonsystemic) and drugs which reduce acid secretion such as H₂ anti-histaminics, proton pump inhibitors, anticholinergics, prostaglandin analogues, ulcer protectives, ulcer healing drugs and anti-H. pyloridrugs [5]. These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often expensive for the poor [6]. In light of the above, it is pertinent to study natural products from food/plants as potential anti-ulcer compounds. Due to less side effects compared to synthetic drugs, currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care [7]. The flavonoid can act as antioxidant to neutralize free radicals, inhibit hydrolysis, oxidation and anti inflammation. The flavonoid in Sauropus androgynus (L.) Merr is 831.70 mg/100 g DW that comprised of kaempherol 805.48 mg/100 g DW [8]. Sauropus androgynus (L.) Merr is believed hitherto improve the flow of breast milk of breastfeeding mothers, however, this plant has not been reported as pectic ulcer medicine. Therefore, this research aimed to elaborate the potency of ethanolic extract of Sauropus androgynus (L.) Merr leaves as alternative therapeutic herbal for peptic ulcer.

2. Materials and Methods

2.1 Chemicals and instrumentation
Aspirin (Sigma-Aldrich), ethanol (Merck), liquid paraffin, NaCl, Hematoxylene-Eosin, Xylol (merck) ,H₂O₂,rat anti TNF-α , Rabbit anti-rat IgG – biotin, strepavidin horse raddish peroxydase (SAHRP), Bovine serum albumin (BSA) (Sigma), DAB (Sigma). Phospho buffer saline. Light microscope Olympus DP71®, rotatory evaporator, rat cages, a set of surgery apparatus. Sauropus androgynus (L.) Merr was purchased from UPT Materia Media, Batu, the rats were purchased from Institute of BioScience, UB, Malang.

2.2 Experimental animals and Design
Male Wistar rats (180-200 g) were purchased from Institute of BioScience, UB, Malang. The rats were acclimatized under controlled room temperature, fed standard diet and water ad libitum for 7 days prior to commencing experiment. The experiments for care and use of animal were approved by Ethical Clearance committee of Brawijaya University (No:673-KEP-UB).

In the whole study, a total of 20 rats were randomly divided into five groups (Groups 1-5). Groups 1 was set as normal control. The 16 aspirin induced- peptic ulcer were divided into 4 groups (Groups 2 to 5) of 4 rats in each, namely peptic ulcer control groups (Groups 2), receiving ethanolic extract of Sauropus androgynus (L.) Merr leaves at dose of 16.2 mg/200 g BW; 32.4 mg/200 g BW; 48.6 mg/200 g BW (Groups 3,4,5).

The data of TNF-α were analyzed statistically using One Way Analysis of variance (ANOVA) followed by Tukey Test multiple comparation tests. The Level for statistical significane was set at a P value <0.05. The analysis used SPSS 23.0 for windows, while the duodenal histopathology was descriptively analyzed.

2.3 Induction of peptic ulcer in rats
The rats were administrated aspirin orally in dose 200mg/kg BW daily for 5 days. The administration of aspirin used sonde. Aspirin was dissolved in 1% CMC solution [9].
2.4. Preparation of ethanolic extract from *Sauropus androgynus* (L.) Merr leaves

Two hundreds g of *Sauropus androgynus* (L.) Merr leaves were macerated in 1.5 L of 80% ethanol, shake for 15 minutes at 50 rpm. Then, the mixture was left overnight. The liquid was separated from the *Sauropus androgynus* (L.) Merr dregs by using filter paper. The *Sauropus androgynus* (L.) Merr dregs were macerated 3 times. The liquid extract were concentrated by using rotary evaporator 60°C, the extract was further dried in vacuum oven, the result was thick liquid extract.

3. Results and Discussion

3.1 Therapeutic Effect of Ethanolic Extract of *Sauropus androgynus* (L.) Merr leaves towards TNF-α Levels of rats duodenum.

Naturally, TNF-α cytokine is produced in relative low level as immune component, which involved in apoptotic mechanism in body tissue such as duodenal organ. In the existence of antigen, macrophage, neutrophils, mast cell and lymphoid T will produce TNF-α cytokine in higher level to trigger or activate the nonspecific and specific immune system to responding inflammation [10].

| Groups                          | TNF-α Level (%area) | Difference of TNF-α Level to negative control |
|---------------------------------|---------------------|-----------------------------------------------|
| Negative Control (normal)       | 11.98±1.09a         | 0.00                                          |
| Positive control (peptic ulcer) | 46.75±1.58d         | 290.23                                        |
| Therapy 16.2 mg/200 g BW        | 32.12±1.83c         | 168.11                                        |
| Therapy 32.4 mg/200 g BW        | 20.70 ± 1.27b       | 72.79                                         |
| Therapy 48.6 mg/200 g BW        | 18.50±1.14ab        | 54.42                                         |

As shown in Table 1, The expression of TNF-α in duodenum at peptic ulcer were significantly higher compared to normal rats. The increase of TNF-α indicated the occurrence of inflammation in rats’ duodenum which caused by the aspirin induction of 200 mg/kg BW. Aspirin inhibits Cyclooxygenase isoenzyme (COX-1) through irreversible acetylation COX-1 leading to prevent the synthesis of prostaglandin. The decrease of prostaglandin lowered the mucus production in digestive tract. The mucus function as duodenal defensive barrier will decrease that lead to increase of Reactive oxygen species (ROS). The excessive ROS production in cell cause activation of NF-κB and phosphorylation of NF-κB inhibitor that bring to induce the transcription of cytokine pro inflammatory (TNF-α)[11].

Therapy with ethanolic extract of *Sauropus androgynus* (L.) Merr leaves may reduce the elevated level of TNF-α. The level of TNF-α decreased with the increasing dosage of ethanolic extract of *Sauropus androgynus* (L.) Merr leaves. Statistical test results showed there were significant difference (P<0.05) between TNF-α levels of pectic ulcer and therapeutic peptic ulcer mice. It suggests that the kaempherol of *Sauropus androgynus* (L.) Merr enable to act as an antioxidant, especially as a hydroxyl radical’s scavenger.

The kaempherol as an antioxidant function to neutralize ROS through releasing hydrogen atom of flavonoid hydroxyl group and produce unreactive stable radical with low energy. Therefore, the increase of ethanolic extract dosage declined ROS and oxidative stress in duodenum tissue. The decline of ROS can inhibit the activation of NF-κB to produce TNF-α. NF-κB remain bound to NF-κB inhibitor, and NF-κB cannot combine with response element which can trigger transcription and translation of pro inflammatory cytokine (TNF-α) as a result the lower pro inflammatory can reduce activity of inflammation cell [12].
3.2 Histopathology of duodenum tissue from control rats, peptic ulcer rats, and the therapeutic rats

Free radicals are the result of normal product of cell metabolism and phagocytes produce large amounts of ROS via the NADPH oxidase system as part of their defensive mechanism against pathogens [13], however, under circumstances may occur the imbalance between ROS production and endogenous antioxidants that lead to cell disfunction and cell damage. Epithelial and endothelial cell damage due to oxidative stress could cause damage to duodenum tissue and disfunction. The histology of duodenum tissue was observed to determine both the level of damage and organ repair.

![Image 1](histopathology.png)

**Figure 1.** Histopathology of Rats Duodenum magnified 400x and 600x. (A) Negative Control (normal), (B) Positive control (peptic ulcer), (C) Therapy 16.2 mg/200 g BW, (D) Therapy 32.4 mg/200 g BW, (E) Therapy 48.6 mg/200 g BW. epithelia cell ( ), vili epithelia cell erosion ( ), neutrophil cell ( ), epithelia cell regeneration ( ), Blood vessel vasodilatations ( ).

Epithelia vili structure in normal rat duodenum showed intact and compact, there are goblet cells and no inflammatory cell in lamina propia. Duodenum epithelial cell covered all mucosal layer by forming one cylindrical epithelial layer. In peptic ulcer rats showed erosion of cylindrical epithelial layer, inflammatory cell at lamina propia and increase blood vessel permeability. It indicates that aspirin induction has damaged to the epithelial cell of rats’ duodenum.

After receiving therapy of the ethanolic extract from *Sauropus androgynus* (L.) Merr leaves the duodenum vili showed better that less erosion of cylindrical epithelial layer and infiltration of inflammatory cell, and occur proliferation of epithelial cells. The higher dose of the ethanolic extract from *Sauropus androgynus* (L.) Merr leaves therapeutic bring out a better repair of histology of duodenum tissues and the therapeutic dose of ethanolic extract from *Sauropus androgynus* (L.) Merr leaves 48.6 mg/kg BW in peptic ulcer rats can restore duodenum vili structure almost like normal rats duodenum. *Sauropus androgynus* (L.) Merr leaves extract can maintain the integrity of cell membranes by inhibited lipid peroxidation reaction resulting in lower (TNF-α) expression and inflammation.
4. Conclusions
Ethanolic extract from *Sauropus androgynus* (L.) Merr leaves can be used as alternative therapy for peptic ulcer towards decreasing the expression of TNF-α and repair duodenum histopathology such as structure of epithelial duodenum, villos and decrease of inflammatory cell and vascular vasodilatation with the effective dosage of 48.6 mg/200 g BW.

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