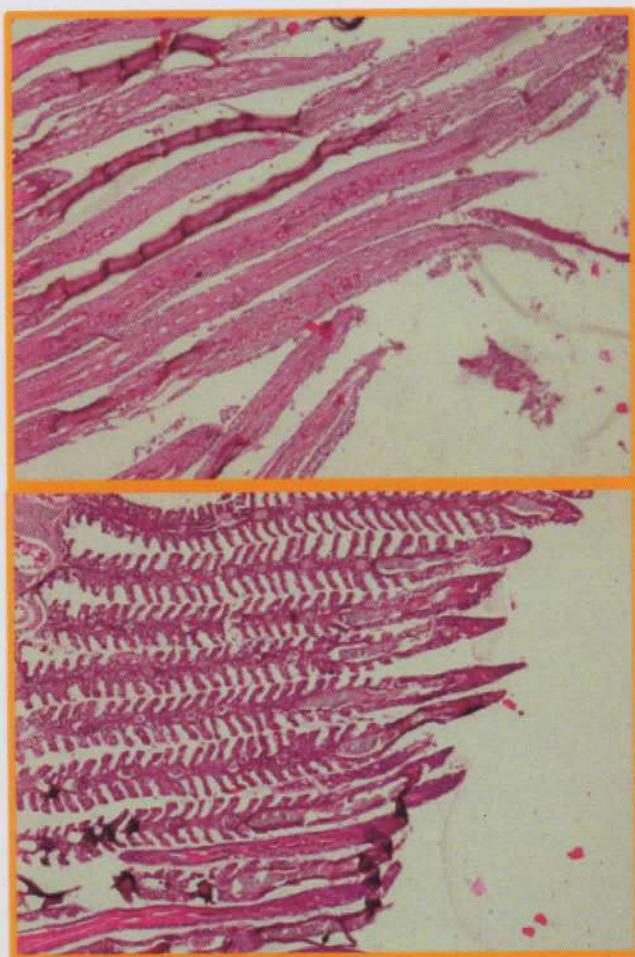


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**Efek Terapi Ekstrak *Spirulina platensis* Terhadap Kerusakan Lambung yang Diinduksi dengan Ethanol pada Tikus (*Rattus norvegicus*)**

**Therapeutic Effect Of *Spirulina platensis* Extract Against Gastric Damage Induced By Ethanol In Rats (*Rattus Norvegicus*)**

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**ABSTRACT**

The aim of this study is to prove the potential of *Spirulina platensis* extract in reducing gastric damage induced by ethanol in Rats (*Rattus Norvegicus*). This experimental search used twenty male rats which were divided into five groups, each group consisted of four rats. They were two control group, negative (C-) and positive (C+) control and three treatment groups which were given 200mg/kg BW (T1), 400mg/kg BW (T2) and 800mg/kg BW (T3) of *Spirulina platensis* extract orally. For the first seven days each group (C+,T1,T2 and T3) were given with 50% ethanol 10 ml/kg BW except for negative control (C-), then continued for 14 days with *Spirulina platensis* extract administration for the treatment groups and CMC Na 0.5% solution for negative control groups. 24 hours after the last treatment, histopathological evaluation was done to score gastric damage based on epithelial damage and submucosal edema. The data was analyzed using Kruskal Wallis test . If there was a significant result, then it was continued using Mann-Whitney test. The result showed 200 mg/kg BW dosage of *Spirulina* extract can reduce gastric damage induced by ethanol in Rat (*Rattus norvegicus*) and gave significant difference result ( $p < 0.05$ ) among the treatment groups.

**Keywords :** *Spirulina platensis*, ethanol, gastric damage

**ABSTRAK**

Penelitian ini bertujuan untuk membuktikan pengaruh ekstrak *Spirulina platensis* dalam mengurangi kerusakan lambung yang diinduksi dengan ethanol pada tikus (*Rattus norvegicus*). Percobaan penelitian ini menggunakan 20 tikus jantan yang dibagi menjadi 5 kelompok, setiap kelompok terdiri dari 4 tikus. Ada 2 kelompok kontrol yaitu kontrol negatif (C-) dan kontrol positif (C+) dan 3 kelompok perlakuan yang diterapi dengan ekstrak *Spirulina platensis* dengan dosis 200mg/kg BW (T1), 400mg/kg BW (T2) dan 800mg/kg BW (T3). Selama 7 hari pertama setelah adaptasi, kelompok C+,T1,T2 dan T3 diberi ethanol 50% 10ml/kg BW kecuali C- yang diberi CMC Na 0.5% 10ml/kg BW, kemudian dilanjutkan dengan pemberian terapi ekstrak *Spirulina platensis* untuk setiap kelompok perlakuan dan CMC Na 0.5% untuk kelompok kontrol negatif. 24 jam setelah terakhir pemberian terapi, evaluasi histopatologi dilakukan untuk menilai kerusakan lambung berdasarkan kerusakan epitel dan edema submukosa. Data yang didapatkan dianalisa dengan Kruskall wallis. Jika ada hasil yang signifikan kemudian dilanjutkan dengan uji Mann-Whitney. Hasil menunjukkan bahwa dosis ekstrak *Spirulina platensis* 200mg/kg BW bisa mengurangi kerusakan lambung yang diinduksi dengan ethanol dan memberikan hasil yang signifikan ( $p < 0.05$ ) diantara kelompok perlakuan.

**Keywords :** *Spirulina platensis*, ethanol, kerusakan lambung

## Background of Research

Gastric has one of important parts, with a complete function and structure due to pathological process that take place on, that is gastric mucosa (Laine et al., 2008). According to Brzozowski et al., (2005), gastric mucosa can be damaged or injured caused by imbalance between aggressive chemical agents with protective substances, such as superoxide dismutase, catalase , prostaglandin and glutathione peroxidase. In another side, Loguercio et al., (1993) stated that one of factors that can risk the gastric mucosal damage and gastric ulcer is ethanol.

Ethanol is a two-carbon alcohol also known as *Ethyl alcohol*, *grain alcohol*, or simply as spirits. Ethanol is found in variety of products, such as solvents, fuels, paints, medications and alcoholic beverages. Ethanol toxicosis in animals most commonly results from accidental or intentional ingestion of alcoholic beverages. There are reports of alcohol poisoning in dogs after the ingestion of unbaked bread dough or rotten apples ( Means, 2003 ; Kammere et al., 2001). The oral administration of absolute ethanol produced significant ulcers in glandular part of gastric mucosa of rats. Gastric mucosa showed a significant loss of glandular cells, disruption of epithelium, sub mucosal edema, and infiltration of neutrophils (Al asmari et al., 2013).

Polymorphonuclear (PMN) cell is inflammatory cells that have an important function in protective response to eliminate cause of the injury, by destroying or neutralizing the harmful agents, and dispose original cause of injury so that healing process can be carried out. One of those is Neutrophil which acts as tissue debridement and phagocytocytes infectious agents (Kim et al., 2004). On the other hand, the existence of a prolonged neutrophil is the main cause of the conversion of acute wounds

become chronic wounds that will never be healed (Gurtner, 2007).

Consequently, it is needed to find new agents with safe effective mechanism that are possessing potential property and counteracting different etiological factors (Al Batran et al., 2013). Especially, numerous marine algae have been found to be effective for anti-inflammatory and anti-allergic therapeutics for prevention or treatment (Thanh-Sang et al., 2012). One of popular marine algae nowadays is *Spirulina platensis*.

*Spirulina* is a group of cyanobacteria which has a blue pigment (phycocyanin) the highest compared with other microalgae (Ismet, 2009). The main biological and pharmacological effects of *Spirulina* are played by calcium (Ca-SP) and C-phycocyanin (C-PC). *Spirulina* contains a variety of compounds that potentially play a role in accelerating the inflammatory phase in which the blood vessels and inflammatory cells such as PMN, macrophages and lymphocytes have an important role in killing and destroying antigens and develop an antibody. (Coockbill, 2002).

This study aims to evaluate the biological activities of compounds extracted from medicinal plants and are currently commercially available, the author attempts to investigate further the potency of *Spirulina platensis* extract against gastric damage induced by ethanol in rat (*Rattus norvegicus*).

## Material of the Research

The equipments used in this study were include sput, oral gavages tube, places to eat and drink, enclosure plastic box (36 x 28 x 12) cm with wire cover, objects glass, cover glass, microscopes, rotary microtome, water bath, mortar and pestle, scales, watch glass, Erlenmeyer, measuring cylinder,

spatula, hot plate, and rotary evaporator.

Materials used in this study were pure *Spirulina platensis* extract powder, rats, 50% ethanol, CMC Na 0.5%, pellet feed, water, Hematoxyline Eosin staining, paraffin, 30 %, 50 %, 70 %, 80 %, 90 %, 100 % concentration of alcohol, Xylol absolut, 10 % of Formalin solution for tissue fixation, CH<sub>3</sub>COOH, H<sub>2</sub>SO<sub>4</sub> and aquadest.

## Methods of the Research

This research was conducted in Biochemistry Laboratory of Medicine Faculty Universitas Airlangga for treatment of experimental animals. Histopathology slide of rat's gastric, observation and scoring were conducted in Pathology Laboratory of Veterinary Medicine Universitas Airlangga. Implementation of this research was carried out from January to February 2016.

Male rats were 3 months old, weighing 150-250 grams with a number of 20 head reared in Laboratory Animals Model at Biochemistry laboratory of Medicine Faculty Universitas Airlangga, randomized by means of a lottery and was divided into five groups. Each group consisted 4 rats, then adapted to environment for 7 days. The administration of 50% ethanol was given for 7 days. The treatment of *Spirulina platensis* extract with a dosage 200mg/kg BW, 400mg/kg BW and 800mg/kg BW were given for 14 days. Experimental animals were fasted for 3 hours before being treated with 50% ethanol. Feeding and drinking was ad libitum.

## Spirulina Extraction Using Maceration Method

In this process, plant material (*Spirulina platensis* powder) was placed in a stoppered container with the solvent (ethanol) and allowed to stand

at room temperature for seven days with frequent agitation until the soluble matter had been dissolved. The mixture was strained, marc (damp solid material) was pressed, and combined liquid was clarified by subsidence or filtration. The extract was evaporated and concentrated by using rotary evaporator after filtration (Singh, 2008). To evaporate the ethanol residue, the extract was oven in 60°C for 24 hours.

## Spirulina Suspension

The suspension made was *Spirulina platensis* extract suspension in 200, 400, and 800 mg dosage W/V. The method used to make the suspension of *Spirulina platensis* was to weight it then suspended it with 0.5% CMC Na then adding aquadest gradually while stirring until homogenized up to a suspension was formed.

## Grading Techniques

For the histological assessment, specimens were assessed according to the criteria of Modified Laine and Winstein (1988) and Barthel (2003). The histological scoring for epithelial damage was assessed: 0: none, 1: < 1/3 field of view, 2: 1/3-2/3 field of view, 3: > 2/3 field of view. The histological scoring for submucosal edema was scored as follows : 0 : none, 1: <50% of gastric mucosa's diameter, 2: 50%-80% of gasric mucosa's diameter, 3: >80% of gastric mucosa's diameter. The edema was determined by space (empty room) between tunica muscularis and lamina propria.

## Research Result and Discussion

Histopathology examination of white rat (*Rattus norvegicus*) gastric given *Spirulina platensis* extract after ethanol induction was done microscopically by Hematoxylin Eosin (HE) staining and 100x magnification

for epithelial damage and 40x magnification for submucosal edema.

### **Epithelial Damage**

Analytical result of epithelial damage observation is shown in the table 1.

**Table 1.** Epithelial Damage Value After Treatment

Treatment	Mean±SD
C- (Negative control)	3.250 <sup>b</sup> ±1.5000
C+ (Positive control)	15.500 <sup>a</sup> ±3.3166
T1 (200mg/kg <i>Spirulina platensis</i> )	5.750 <sup>b</sup> ±2.3629
T2 (400mg/kg <i>Spirulina platensis</i> )	11.750 <sup>a</sup> ±1.5000
T3 (800mg/kg <i>Spirulina platensis</i> )	16.250 <sup>a</sup> ±3.7749

\*The difference a and b superscript show a significant difference between treatment groups ( $p<0.05$ ).

Statistical analysis of epithelial damage in C- and T1 treatment groups showed no significant difference. Similarly shown in C+, T2, and T3 treatment groups also showed no significant difference. However, treatment groups C- and T1 showed significant difference among C+, T2, and T3 treatment groups shown in the superscript.

Statistical analysis showed negative control (C-) and *Spirulina platensis* extract 200mg/kg BW (T1) no significant difference, while showing significant difference with positive control (C+), *Spirulina platensis* 400mg/kg BW (T2) and *Spirulina platensis* 800mg/kg BW (T3). This data showed *Spirulina platensis* 200mg/kg BW (T1) was able to reduce gastric

epithelial. It could happen possibly caused by rat groups treated with *Spirulina platensis* 200 mg/kg BW did not undergo diarrhea so the drug metabolism and absorption was good and caused by existence of trepenoids and polysaccharides in *Spirulina platensis* have been reported to strengthen the mucosal lining of gastric, making it more resistant to ulcer development and polysaccharides have been used to treat ulcers (Cheng and Koo, 2000).

Between positive control (C+), *Spirulina platensis* 400mg/kg BW (T2) and *Spirulina platensis* 800mg/kg BW (T3) were no significant difference. This could happen when *Spirulina platensis* is consumed in high dose can lead to stomach upset and diarrhea (Grunert, 2014). As stated by Le (2016), Laxatives and diarrhea, which speed up the passage of substances through the digestive tract, may reduce drug absorption. As it happened to this research animals that were given with *Spirulina platensis* 400mg/kg BW (T2) and *Spirulina platensis* 800mg/kg BW (T3) which caused diarrhea, as evidenced in the feces' pungent smell, dirt and wet bedding, thus it decreased the absorption of *Spirulina platensis* extract when it was given to the research animals.

On the other hand, as it was stated by Quader et al. (2013), the high consumption of *Spirulina platensis* extract can reduce the existence of prostaglandin on gastric mucosa while prostaglandin in the gastric mucosa plays role as a protective mechanisms. Phycocyanin and beta carotene existence in the *Spirulina platensis* contents as the main factor of reducing in prostaglandin when it is consumed in a high dosage (Cherng et al., 2007).

### **Submucosal Edema**

The result of submucosal edema histopathology examination was ana-

lyzed using *Kruskal Wallis* and showing significant difference ( $p<0.05$ ) between treatment groups, the analysis was continued using *Mann whitney* test. Analytical result of submucosal edema observation shown in the table 2

Table 2. Submucosal Edema Value After Treatment

Treatment	Mean±Standard Deviation
C- (Negative control)	3.250 <sup>b</sup> ±2.3629
C+ (Positive control)	15.625 <sup>a</sup> ±4.1908
T1 (200mg/kg <i>Spirulina platensis</i> )	5.750 <sup>b</sup> ±1.5000
T2 (400mg/kg <i>Spirulina platensis</i> )	11.625 <sup>a</sup> ±1.9738
T3 (800mg/kg <i>Spirulina platensis</i> )	16.250 <sup>a</sup> ±2.8723

\*The difference a and b superscript showed a significant difference between treatment groups ( $p<0.05$ ).

Statistical analysis of submucosal edema in C- and T1 treatment groups showed no significant difference. Similarly shown in C+, T2, and T3 treatment groups also showed no significant difference. However, treatment groups C- and T1 showed significant difference between C+, T2, and T3 treatment groups shown in the superscript.

Statistical analysis showed that negative control (C-) and *Spirulina platensis* 200mg/kg BW (T1) no significant difference, while they showed significant difference with positive control (C+) ,*Spirulina platensis* 400mg/kg BW (T2) and *Spirulina platensis* 800mg/kg BW (T3). Negative control (C-) and *Spirulina platensis* 200mg/kg BW (T1) showed lower

occurrence of submucosal edema. This result accorded to the statement of Yonathan et al. (2006) that the higher concentration of *Spirulina platensis* given might suppress the release of the pro-inflammatory mediators (serotonin, histamine, bradykinin) as well as the production and release of prostaglandin. Prostaglandins have a function as gastric mucosal barrier so that when production is inhibited, can result in gastric damage and inflammation.

According to Baharuddin et al., (2013) stated that the injury can cause inflammatory reactions and hyperemia. Hyperemia occurs because of an injury that causes vasodilatation of blood vessels so that blood flow and increase hydrostatic pressure, then the liquid plasma and protein will come out. Fluid and proteins resulting in interstitial edema out. Tissue edema is seen as the rooms were expanded like an empty room (space) and filled with fluid (Widyastuti et al., 2012). It was also proven with the existence of congestion in *Spirulina platensis* extract 400mg/kg BW (T2). Congestion is one of the patogenesis of edema appearance because congestion cause vasodilatation of blood vessel by increasing of microvascular permeability due to vasodilatation of blood vessel. Then it results in increasing hydrostatic pressure and decreasing of osmotic pressure causing protein containing plasma leaking to extravascular site as transudate. This process is called as edema (Arimbi et al., 2013).

According to Diver (2015) stated that it was not advised to take more than 20g of spirulina (360mg in rat) daily. Beginners should start with low dosage the course of first week so that the body could adjust. Diarrhea can lead to minimum body absorb the extract, so it can cause the decrease of spirulina potential virtue.

## Conclusion

A dosage of 200mg/kg BW *Spirulina platensis* could reduce gastric damage caused by ethanol.

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