Efek Terapeutik Ekstrak *Spirulina platensis* pada Gambaran Histopatologi Kerusakan Hati Tikus (*Rattus norvegicus*) yang diinduksi Ethanol

**Therapeutic Effect of Spirulina Platensis Extract on Histopathological Appearance of Ethanol Induced Liver Injury in Rat (*Rattus Norvegicus*)**

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**Abstract**

Penelitian ini bertujuan untuk membuktikan potensi ekstrak *Spirulina platensis* dalam mengurangi kerusakan hati yang disebabkan oleh etanol di Tikus (*Rattus norvegicus*). Dua puluh tikus jantan dibagi menjadi lima kelompok yang berisi masing-masing empat tikus. Terdapat dua kelompok kontrol dan tiga kelompok perlakuan, yang diberikan 200, 400, dan 800 mg/kg BB ekstrak *Spirulina platensis* secara peroral. Selama tujuh hari pertama masing-masing kelompok diberi etanol 50% 10 ml/kg BB kecuali untuk kelompok kontrol. Pada hari ke-8, pemberian etanol dihentikan dan dilanjutkan dengan pemberian ekstrak *Spirulina platensis* untuk kelompok perlakuan dan solusi CMC Na 0,5% untuk kelompok kontrol selama total 14 hari. Pada hari ke-28, 24 jam setelah pemberian terakhir, evaluasi histopatologi dilakukan untuk menghitung nilai kerusakan hati berdasarkan degenerasi dan nekrosis hepatosit menggunakan pewarnaan HE dengan pembesaran 400x. Data skor dianalisis menggunakan Kruskal Wallis dan Mann-Whitney. Hasil penelitian menunjukkan dosis ekstrak 200 mg/kg BB *Spirulina platensis* dapat mengurangi kerusakan hati yang diinsuksi ethanol pada Tikus (*Rattus norvegicus*) dan memberi perbedaan yang signifikan (p <0,05) antara kelompok perlakuan. Akan tetapi, ekstrak Spirulina dosis 400 dan 800 mg/kg BB menunjukkan hasil negatif dimana gambaran histopatologi menunjukkan banyak degenerasi dan nekrosis di beberapa daerah.

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

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**Background of Research**

Liver diseases are some of the fatal disease in the world today as they pose a serious challenge to international public health (Giri et al., 2011). Due to its detoxification role, liver is prone to injury as it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, alcohol and prescribed over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease (Sharma et al., 1991).

According to Jaeschke (2010), there are many factors that can cause liver cell death by stress but the central to this inflammatory response is the promotion of reactive oxygen species (ROS)
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formation. Byrnes (2003) mentioned in his article that ROS may be triggered by many things such as poor diet, detoxification of drugs, food preservative, cigarette smoke, pesticides, radiation, heavy metals, and alcohol consumption. Oxidative stress plays an important role in the pathogenesis of ethanol-induced liver injury (Lindros, 1995 and Zima et al., 2001) because ethanol leads to disturbances in the balance between pro-oxidant and antioxidant mechanism (Saravanan et al., 2006). In the research of Hall (1995), ethanol consumption implies several stages of liver injury: steatosis, alcoholic steatohepatitis, alcoholic hepatitis, and cirrhosis.

Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant-based preparations, which are employed for their treatment of liver disorders, but there are not much drug available for the treatment of liver disorders (Torres-Duranet al., 1999; Chatterjee, 2000). Therefore, the potential of the health promoting and disease preventing properties of plant-derived compounds has received increased attention from researchers in recent years (Amar and Maysa, 2010).

*Spirulina platensis* (SP) now named Arthrospira, is a filamentous cyanobacterium (blue green alga) that has a long history for use as food. Spirulina has a lot of nutrients that are very beneficial for the body, such as the C-phycocyanin, β-Carotenoids, vitamin E, zinc, and many trace elements and other natural phytochemicals (Ismet, 2009). Particularly, C-phycocyanin and β-carotene in Spirulina have strong antioxidant and anti-inflammatory activity (Cherng S, 2007).

The objective of present study was to evaluate *Spirulina platensis* extract effects in treating acute ethanol-induced liver injury of *Rattus norvegicus*. Hopefully, this research can contribute and benefit the development of the veterinary and medicine world in the future, mainly regarding alcohol intoxication or other ROS influenced injury.

**Material of the Research**

This research had been conducted at the Laboratory Animals Model of Biochemical department at the Faculty of Medicine, Universitas Airlangga for the treatment of experimental animals. The making of histopathology slide, observation and scoring of rat’s liver was done in Laboratory of Veterinary Pathology, Universitas Airlangga. Implementation of this research was carried out from January to February 2016. The population used in this study were healthy male white rats (*Rattus norvegicus*) strain Wistar with an average weight of 150-250 grams, 3 months old, maintained at the same place and were given the same feed.

The equipments used in this study were include spuit, oral gavages tube, places to eat and drink, enclosure plastic box 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, ethanol 50%, 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, and aquadest.

**Methods of the Research**

Male rats were with a number of 20 head reared in Laboratory Animals Model at Faculty of Medicine Universitas Airlangga, randomized by means of a lottery and was divided into five groups, and then adapted to the environment for one week. In the second week of experiment, animals was treated
respectively for twenty-one days, seven days of ethanol treatment followed by fourteen days of *Spirulina platensis* treatment. Animals was fasted for 3 hours before treated with ethanol. Feeding and drinking was ad libitum.

**Ethanol Extraction Using Maceration Method**

In this process, 3 kg of plant material (*Spirulina platensis* powder) was placed in a stopper container with the solvent (ethanol) and allowed to stand at room temperature for seven days with frequent agitation until the soluble matter has dissolved. The mixture then strained, the marc (the damp solid material) was pressed, and the combined liquids was clarified by subsidence or filtration. The extracts was evaporated and concentrated using rotary evaporator after filtration. To evaporate the ethanol residue, the extract was oven in 60°C for 24 hours.

**Spirulina platensis** Extract 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 CMC Na 0.5%, then adding aquadest gradually while stirring until homogenized up to a suspension was formed.

**Liver Damage Scoring**

Light microscope was used for the scoring of the liver damage in 400 (40x) magnification. The grading technique used in this research was a modification of Knodell (1981). Forms of lesions observed and the score of each lesions are for degeneration: 0: none, 1: degeneration change in <1/3 of the field of view, 3: degeneration change in 1/3-2/3 of the field of view, 4: degeneration change in >2/3 of the field of view; for necrosis: 0: none, 1: necrosis in <25% of the field of view, 3: necrosis in 25-50% of the field of view, 4: necrosis on >50% of the field of view, 5: necrosis in 25-50% of the field of view with bridging necrosis, 6: necrosis in >50% of the field of view with bridging necrosis, 10: multilobular necrosis.

**Processing and Data Analysis**

Data obtained in the form of scores overview of histopathological changes in rat (*Rattus norvegicus*) liver, organized in table form. Further statistical analyses was done using *kruskalwallis* test. The degree of change was processed by ranking method and if noticeable change were observed, data analyses would be continued using *Mann-Whitney* test. The entire analysis was done using computer statistic program SPSS 23.0 for Windows (Kusriningrum, 2012)

**Research Result and Discussion**

Histopathology examination of white rat (*Rattus norvegicus*) liver given *Spirulina platensis* extract post ethanol induction was done microscopically by *Hematoxylin Eosin* (HE) staining and 400 (40x) magnification. The variables observed in this observation were hepatocytes that undergo degeneration and necrosis.

**Degeneration**

Analytic result of hepatocyte degeneration observation is shown in the table 2.

Table 2. Hepatocyte degeneration value after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (C-)</td>
<td>4.125b ±3.1983</td>
</tr>
<tr>
<td>Positive control (C+)</td>
<td>15.750a ±2.5981</td>
</tr>
<tr>
<td>Spirulina 200 mg/kg BW (T1)</td>
<td>4.875b ±2.5981</td>
</tr>
<tr>
<td>Spirulina 400 mg/kg BW (T2)</td>
<td>13.750a ±3.2787</td>
</tr>
</tbody>
</table>
Spirulina 800 mg/kg BW  14.000±
(T3)  4.6188

*The different superscript show there is significant difference between treatment groups (p<0.05).

Statistical analysis of hepatocyte degeneration in Negative control (C-) and Spirulina extract 200 mg/kg BW (T1) treatment groups show no significant difference. Similarly shown in Positive control (C+), Spirulina extract 400 mg/kg BW (T2), and Spirulina extract 800 mg/kg BW (T3) treatment groups also show no significant difference. However, treatment groups Negative control (C-) and Spirulina extract 200 mg/kg BW (T1) show significant difference among Positive control (C+), Spirulina extract 400 mg/kg BW (T2), and Spirulina extract 800 mg/kg BW (T3) treatment groups shown by the difference of superscript.

Both cellular swelling and fatty change are reversible cell damage, meaning it is reversible when the cause is eliminated (Hayes, 2001). According to Desai (2004) and Bhat and Madyastha (2000), Spirulina contains very high amounts of beta-carotene and tocopherol; combined form of these antioxidants make Spirulina a very good source of natural antioxidant along with high protein. These antioxidants can scavenge free radicals, thus leads to rebalance between prooxidants and antioxidants.

Between Positive control (C+), Spirulina extract 400 mg/kg BW (T2) and Spirulina extract 800 mg/kg BW (T3) there was no significant difference. This could happen when Spirulina is consumed in high dose, which lead to stomach upset and diarrhea (Grunert, 2014). As stated by Lee (2016), Laxatives and diarrhea, which speed up the passage of substances through the digestive tract, may reduce drug absorption. Such happened in the research animals at 400 (T2) and 800 (T3) mg/kg BW dose of Spirulina administration which undergo diarrhea, as evidenced in the feces’ pungent smell and dirty and wet bedding, thus decreasing absorption of Spirulina platensis extract.

Another possibility is that since Spirulina platensis contain C-Phycocyanin and Beta Carotene, it increased GST, thus increasing doses of the extract will increase the GST enzyme accordingly (Thomas, 2010; Abdoloh et al., 2006; El-Baky, 2003). GST (glutathione S-transferase) is a group of multifunctional cytosolic enzyme that produced in the liver and plays an important role in detoxifying xenobiotic compounds electrolyte through conjugation with glutathione (GSH). However, if the amount of GST production is too high, it will cause the conjugation of the extract itself by the GST within a fairly short time. Then GST become incapable to do the feedback mechanism or undergo homeostatic levels of the enzyme, so that the mechanism of the extract in the body does not last long (Kolaez et al., 2007).

Necrosis

Analytic result of hepatocyte necrosis observation is shown in the Table 3

Table 3. Hepatocyte necrosis value after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (C-)</td>
<td>3.875b ± 2.3936</td>
</tr>
<tr>
<td>Positive control (C+)</td>
<td>15.750a ± 1.4434</td>
</tr>
<tr>
<td>Spirulina 200 mg/kg BW</td>
<td>5.125b ± 2.3936 (T1)</td>
</tr>
<tr>
<td>Spirulina 400 mg/kg BW</td>
<td>13.000a ± 2.9155 (T2)</td>
</tr>
<tr>
<td>Spirulina 800 mg/kg BW</td>
<td>14.750a ± 5.4848 (T3)</td>
</tr>
</tbody>
</table>

*The different superscript show there is significant difference between treatment groups (p<0.05).
Statistical analysis of hepatocyte degeneration in Negative control (C-) and Spirulina extract 200 mg/kg BW (T1) treatment groups show no significant difference. Similarly shown in Positive control (C+), Spirulina extract 400 mg/kg BW (T2), and Spirulina extract 800 mg/kg BW (T3) treatment groups also show no significant difference. However, treatment groups Negative control (C-) and Spirulina extract 200 mg/kg BW (T1) show significant difference between Positive control (C+), Spirulina extract 400 mg/kg BW (T2), and Spirulina extract 800 mg/kg BW (T3) treatment groups shown in the superscript.

King (2005) stated that ethanol metabolism could cause cell hypoxia. Based on Castilla et al (2004) study, the effect of ethanol on cell necrosis was related to lipid peroxidation in hepatocytes, explaining the damage caused in positive control (C+). Although, this experiment was conducted under short amount of time and could be grouped as acute damage, which explain why necrosis results were considered minimum.

A potential treatment for liver fibrosis is to inhibit activated hepatic stellate cell (HSC) proliferation and, subsequently, to induce HSC apoptosis. It has been reported that antioxidants are able to inhibit the proliferation of HSCs. The aqueous extract of Spirulina was observed to trigger HSC cell cycle arrest at the G2/M phase which resulted in apoptosis. In conclusion, this finding reveals the potential antifibrotic action of blue-green algae, confirming their potential as therapeutic agent for the treatment of liver fibrosis (Wu, 2005). Based on the theory of Desai (2004), Bhat and Madyastha (2000), and (Wu, 2005), Spirulina acted as strong antioxidant, scavenging the free radicals out from degenerative cell and prevent the forming of fibrosis, leading to normal proliferation of hepatocyte thus restoring the liver histology and functions, which explain why Spirulina extract of 200 mg/kg BW dose succeeded in restoring hepatocyte morphology.

The reason why Spirulina extract 400 mg/kg BW (T2) and Spirulina extract 800 mg/kg BW (T3) having the opposite effect as in Spirulina extract 200 mg/kg BW (T1) is probably because the experimental animals were having a diarrhea as it was exposed to high doses in one apply. According to Diver (2015), it is not advised to take more than 20 gram of Spirulina (360 mg in Rat) daily and beginners should start with low dose for the course of first week so that the body could adjust. Other than the possibility of higher stress resulted from being gavaged with higher amount of Spirulina platensis extract in one go, intoxication of ingesting a high amount of dietary supplement at once might be considered. Heller (2015) stated that any ingredient in a multiple vitamin supplement can be toxic in large amounts, but the most serious risk comes from iron or calcium. According to a food report from United States Department of Agriculture (2016), Spirulina is rich in iron (28.5 mg/100 gram). The most common side effects of iron supplements involve the gastrointestinal system such as diarrhea, nausea, heartburn, abdominal pain and constipation (Tourney, 2015). Other assumption is because Spirulina 400 and 800 mg/kg BB animals group was added too much fiber too fast. Michigan Bowel Control Program (2011) advised to add fiber slowly to diet, because the bacteria in stomach and small intestines need time to adapt. Adding too much fiber or adding fiber too quickly may cause gas, bloating, cramps and diarrhea since fiber draws in fluid from body to add bulk to stools and can make bowel movements soft or firm.

Histopathological observation and examination of hepatocyte degeneration and necrosis in rat’s liver (Rattus norvegicus) is shown in figure 1.
Figure 1. Comparison of hepatocyte degeneration and necrosis among treatment groups. (C+: positive control group, C-: negative control group, T1: Spirulina extract 200 mg/kg BW group, T2: Spirulina extract 400 mg/kg BW group, T3: Spirulina extract 800 mg/kg BW group). Showing: (1) Normal hepatocyte (2) Hepatocyte undergoing hydropic degeneration. (3) Clear fat vacuole inside hepatocyte cytoplasm (microsteatosis) (4) Hepatocyte nucleus undergoing pyknotic. (5) Hepatocyte nucleus undergoing karyolysis, leaving the cytoplasm appeared homogenous. (6) Complete loss of hepatocyte. HE staining, 400x magnification.

Conclusion
In this experiment, *Spirulina platensis* extract of 200 mg/kg BW can reduce liver injury caused by ethanol in white rat (*Rattus norvegicus*). However, this dose cannot be said to be the effective dose in this study because the experimental animals of 400 and 800 mg/kg BW dosage were having minimum absorption of the extract from having diarrhea.

References


Thomas SS. 2010. The Role of Parry Organic Spirulina in Health Management.


