Effects of Onion Extract on Hepar Histopatology in Alloxan-Induced Diabetic Rattus Novergicus

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Abstract

This study was aimed to know the effect of giving shallot extract (*Allium ascalonicum L*) to histopathological feature of rat (*Rattus norvegicus*) liver induced by alloxan. Alloxan was intraperitoneally injected 120 mg/kg BW (single dose). A total of twenty male Wistar rats of three months old were used in the study. The rats were divided into five groups. 1) negative control group (K-) treated by CMC Na 0.5 %, 2) positive control group (K+) treated by metformin 45 mg/kg BW as a standard drug, 3) extract of *Allium ascalonicum* 250 mg/kg BW (P1), 4) extract of *Allium ascalonicum* 500 mg/kg BW (P2), 5) extract of *Allium ascalonicum* 750 mg/kg BW (P3). Rats were treated for 14 days. The data of this study were analyzed by Kruskal Wallis Test, then it continued by Mann-Whitney Test. The result of this study is *Allium ascalonicum* can improve the histopathological feature of rats liver induced by alloxan.

Keyword: *Allium ascalonicum, alloxan, liver, Rattus norvegicus.*

Introduction

Diabetes mellitus (DM) is a degenerative disease that continues to grow in Indonesia. The increasing prevalence of this disease is caused by people's lifestyles and eating patterns. Diabetes mellitus (DM) occurs due to decreased function of the pancreas to produce insulin or insulin receptors do not occur due to complications, whereas diabetes does not turn into glycogen and cannot enter cells[(1)]. Clinical manifestations include disorders of lipid metabolism, carbohydrates, and proteins which will then stimulate the condition of hyperglycemia. Then the condition of hyperglycemia will develop into diabetes mellitus[(2)].

In the treatment of diabetes, oral antidiabetic drugs may be useful for sufferers who are allergic to insulin or who do not use insulin injections. While its use must be understood, so there is conformity with the indications without causing hypoglycemia conditions. Therefore, experts develop a traditional treatment system for diabetes mellitus that is relatively safe by using ingredients from herbal plants[(3)].

One of the plants that can be used as an alternative is onion (Allium ascalonicum L). Onion are very beneficial for health because they are rich in antioxidants to inhibit free radicals[(4)]. The dominant flavonoid content in the onion tubers, especially quercetin, is thought to have a hypoglycemic effect and is beneficial for people with diabetes mellitus[(5)].

This study uses alloxan to induce the condition of diabetes mellitus. Giving alloxan can increase blood glucose levels, causing disruption of insulin production due to damage to pancreatic β cells. Disruption of glucose entry into cells results in high blood glucose levels[(6)]. This hyperglycemia condition can cause oxidative stress from several organs, including the liver, heart, brain, and skeletal muscles[(7)]. Oxidative stress can cause lipid metabolic reactions, proteins including enzymes, which can cause oxidative damage if it continues it can cause liver cell damage and death. This study aims to determine

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the effect of the onion extract (Allium ascalonicum L) on the histopathological picture of the liver of white rats (Rattus norvegicus) induced by alloxan.

Material and Method

This research was conducted in February - March 2015 at four locations, namely the Experimental Animal Laboratory, Department of Basic Medicine, Faculty of Veterinary Medicine, making histopathological preparations of white rat liver (Rattus norvegicus) in the Diagnostic Center Building Dr.Soetomo General Hospital Surabaya, as well as observation and scoring of preparations histopathology in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Airlangga University.

This study used 20 white male Wistar rats divided into 5 treatment groups, each group containing 4 white rats. The treatments consisted of K-, K +, P1, P2, P3. On the first day, all white rats measured their blood glucose levels and then induced an alloxan dose of 18 mg / 150 grams BW intraperitoneally. Giving alloxan is done once on the first day of treatment. After four days of the alloxan induction process (to get a constant rise in blood glucose levels) then the blood glucose level was measured again. After an increase in blood glucose levels, in the K-group given 0.5% CMC Na, the K + group was given a dose of 45 mg/kg BW of metformin, while groups of P1, P2, P3 were given onion extract at a dose of 250 mg/kg BW, 500 mg/kg body weight, 750 mg/kg bodyweight for 14 days.

After the next stage of treatment is carried out euthanasia using chloroform, then performed surgery on white mice for liver organs. The liver is then fixed to a place containing a 10% BNF solution. Histopathological preparation using Haematoxylin Eosin (HE) staining. Examination of liver histopathology preparations using a 400 times magnification microscope on five different fields of view for each slide. Assessment criteria using the scoring method[8].

Table 1 Scoring assessment of the degree of liver cell histopathology [8]

	Degeneration	Necrosis
None	0	0
Minimal (0-25%)	1	1
Mild (25-50%)	2	2
Moderate (50-75%)	3	3
Severe (75-100%)	4	4

Result

This study used 20 white rats which were divided into five treatment groups with four replications. Before being divided into treatment groups, all-white rats were induced by alloxan at a dose of 18 mg / 150 grams BW intraperitoneally. The results of the examination of blood glucose levels of alloxan-induced white mice 18 mg / 150 grams BB intraperitoneally showed an increase in blood glucose levels that exceeded the normal range. Examination of blood glucose levels of rats using the EasyTouch brand glucometer.

After the condition of hyperglycemia, the K-group was treated with 0.5% CMC Na 0.5 ml by giving once a day for 14 days orally. For the K + group treated with metformin at a dose of 45 mg/kg body weight by giving once a day for 14 days orally, while in the groups P1, P2 and P3 treated with shallots extract at a dose of 250 mg/kg body weight (P1), 500 mg/kg BW (P2), and 750 mg/kg BW (P3) by giving once a day for 14 days orally. Then data were analyzed with the SPSS using the Kruskal-Wallis test and if there were significant differences between treatment groups ($p < 0.05$), then continued with the Mann-Whitney test.

1. Liver cell degeneration

Microscopic observations of white rat liver cell degeneration showed no significant difference between treatment groups ($p > 0.05$). The statistical analysis of observations of liver cell degeneration can be seen in table 1.

Table 2 Median values of liver cell degeneration

Treatment	Median
K-	2.8000[a]
K+	2.0000[a]
P1	2.4000[a]
P2	2.3000[a]
P3	2.1000[a]

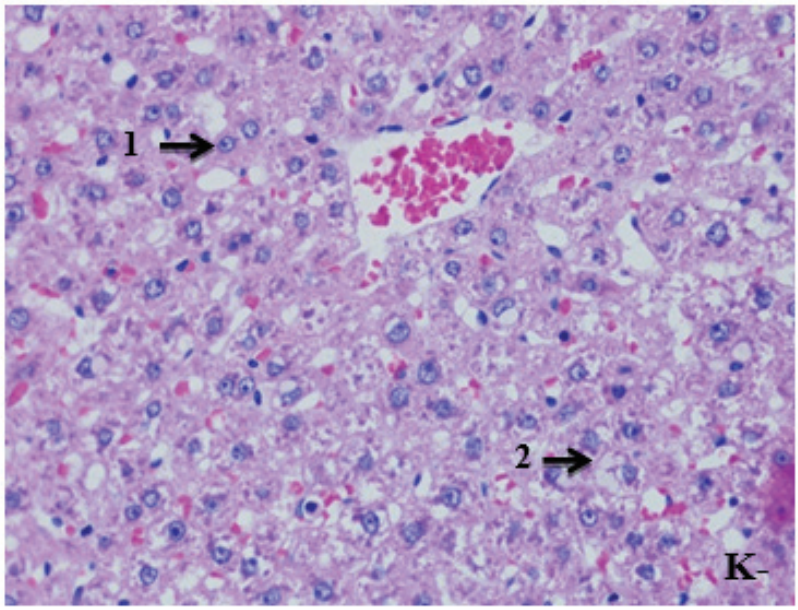

Figure 1 Histopathology of liver cell degeneration in rat K-treated. (1) Normal liver cells. (2) The degenerated liver cells are seen to have swollen so that the cavity looks wider (HE staining; 400x magnification).

In the K-group treated with alloxan and CMC Na 0.5%, the most severe degeneration occurred (2.8000), when compared to the K + group who were treated with alloxan and metformin 45 mg/kg BW degeneration occurred with a value (2.0000). Groups P1, P2 and P3 treated with alloxan and onion extract 250 mg / kg BW, 500 mg / kg BW and 750 mg / kg BW showed degeneration statistics with median values (2.4000), (2.3000) and (2.1000). The results show different figures for each treatment, but the Kruskal-Wallis statistical test did not show any significant difference ($p > 0.05$). Histopathological picture of liver cells in degenerated mice can be seen in the following figure.

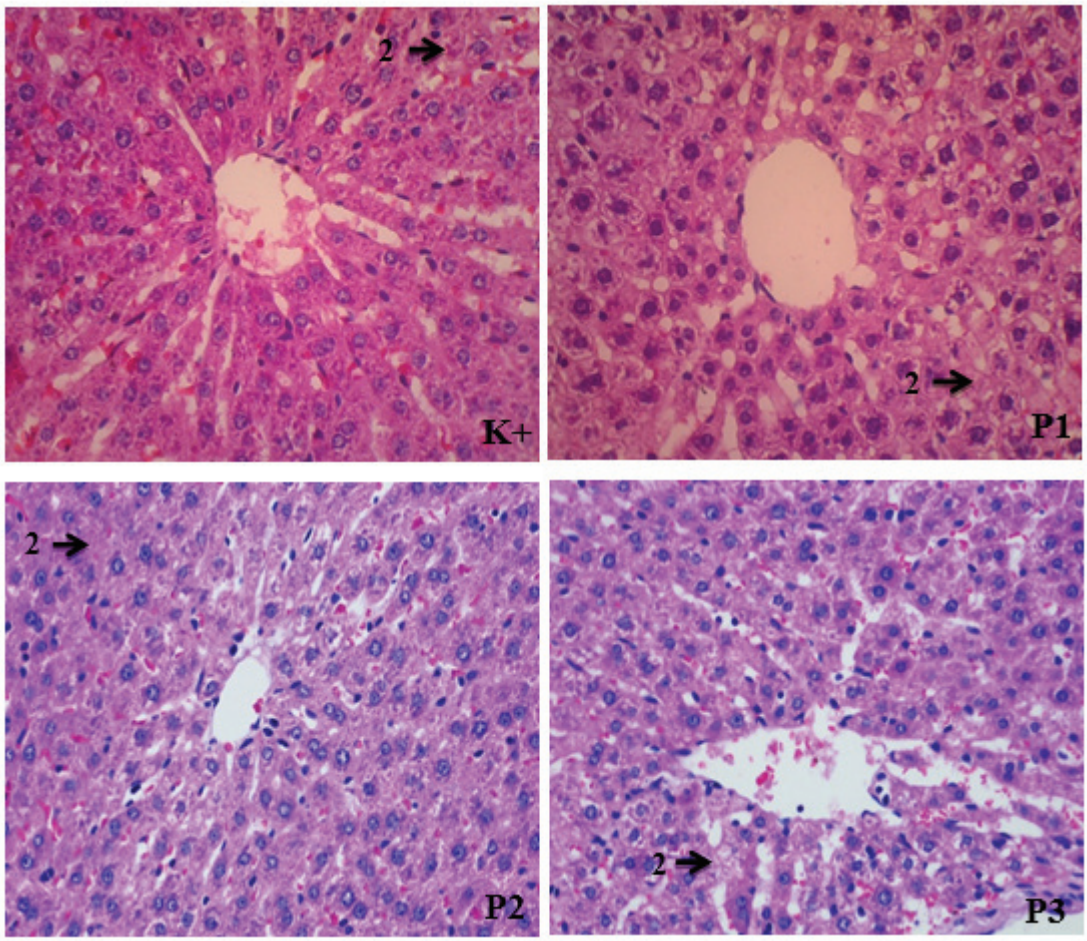

Figure 2 Histopathology of rat liver cell degeneration treated with K +, P1, P2, P3. (2) The degenerated liver cells are seen to have swollen so that the cavity looks wider (HE staining; 400x magnification).

2. Liver cell necrosis

Microscopic observations of white rat liver cell necrosis showed significant differences between treatment groups ($p < 0.05$). The statistical analysis of observations of liver cell necrosis can be seen in table 2.

Table 2 Median values of liver cell necrosis

Perlakuan	Median
K-	1.6000[a]
K+	1.4000[a]
P1	1.3000[a]
P2	1.0000[b]
P3	1.0000[b]

Statistical analysis of the occurrence of liver cell necrosis in the K-, K +, and P1 groups were not significant differences in the three treatments. In the P2 and P3 groups, there were also no significant differences. However, the K-, K +, and P1 treatment groups showed significantly different results when compared to the P2 and P3 treatments seen from the superscript listed. Histopathological picture of liver cells of mice undergoing necrosis can be seen in the following figure.

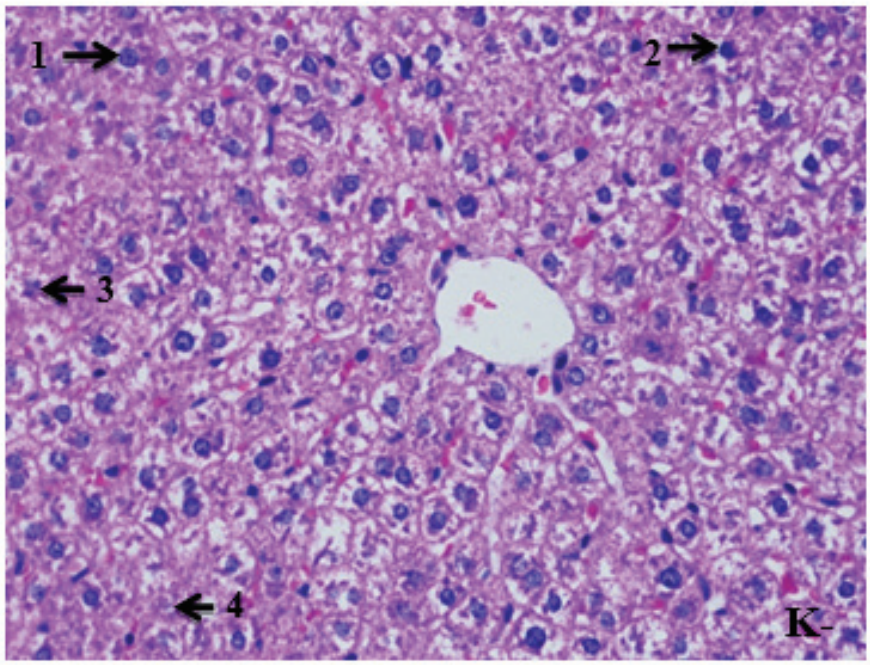

Figure 3 Histopathology of liver cell necrosis in rat K-treated. (1) Normal liver cells. (2) Liver cells experience necrosis in the form of picnotics. (3) The nucleus of the liver cell experiences karyoreksis. (4) The nucleus of the liver undergoes cariolysis. (HE coloring) 400x magnification.

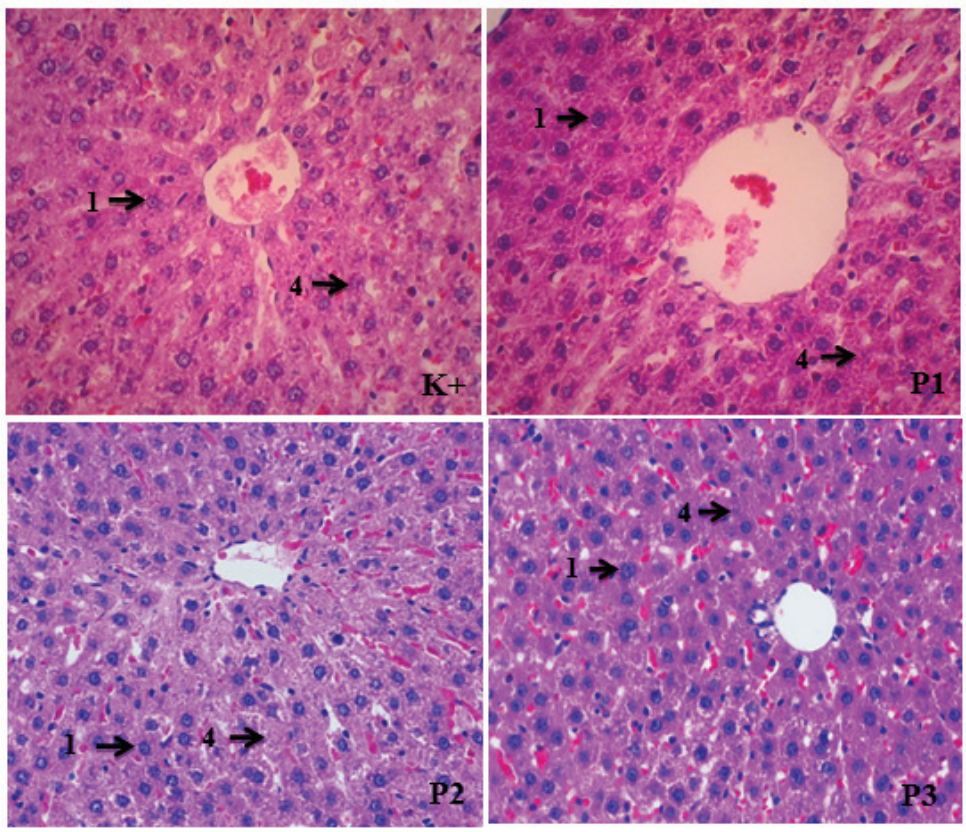

Figure 4 Histopathology of rat liver cell necrosis with treatment K +, P1, P2, P3. (1) Normal liver cell nucleus. (4) The nucleus of the liver cell undergoes karyolysis. (HE coloring) 400x magnification.

Discussion

This research was conducted to see the effect of the onion extract (Allium ascalonicum L) on the histopathological picture of the liver of the white rat (Rattus norvegicus) induced by alloxan. Statistical results of the occurrence of liver cell degeneration showed no significant difference, while the occurrence of liver cell necrosis there were significant differences in each treatment. This is enough to prove that the onion extract plays a fairly good role in repairing liver cell damage due to alloxan induction.

Giving alloxan can increase the blood glucose levels of white mice that cause disruption of insulin production due to damage to pancreatic β cells. This compound can enter rapidly into pancreatic β cells and is reduced to dialuric acid which will then be oxidized back into alloxan which results in a redox cycle with the end result of radical peroxide compounds. This radical peroxide compound will undergo a process of dismutase to hydrogen peroxide. Hydrogen peroxide together with Fe2 + will form a reactive hydroxyl (OH^-) radical compound so that it can cause damage to pancreatic β cells. Disruption of glucose entry into cells results in high blood glucose levels[6].

This hyperglycemia condition can cause oxidative stress from several organs, including the liver, heart, brain, and skeletal muscles[7]. Oxidative stress can cause lipid metabolic reactions, proteins including enzymes, which can cause oxidative damage, if this continues it can cause liver damage and death. The liver functions in the process of biotransformation and detoxification of endogenous and exogenous substances that enter the body, and functions to filter blood from various organs that contain food, medicine, toxins, and bacteria. The state of diabetes will affect the occurrence of cell morphological changes from the liver[9].

1. Liver cell degeneration

Degeneration is a state of decreased intracellular biochemical changes accompanied by morphological changes due to nonfatal lesions in cells or as a cell reaction to lesions that are still reversible storage process or accumulation of fluids or other substances in cell organelles[10].

Based on the results of statistical analysis with the Kruskal Wallis test, there were no significant differences between the treatment groups (p> 0.05). In the K- (2.8000) treatment showed the most severe degeneration when compared to the K + (2.0000) treatment, this clearly happened because in the K (-) treatment no therapy was given and only 0.5% CMC Na was given which did not provide a therapeutic effect. in the liver, whereas in the K + treatment therapy is given with the drug metformin dose 45 mg/kg BW. Metformin is an oral anti-hyperglycemia biguanide class. Metformin works to reduce blood glucose levels by inhibiting hepatic glucose production (by reducing glycogenolysis and gluconeogenesis) and reducing insulin resistance especially in the liver and skeletal muscles[11].

In groups P1, P2, and P3 treated with onion extract doses of 250 mg / kg BW, 500 mg / kg BW, 750 mg / kg BW showed statistical results of median values (2.4000), (2.3000) and (2.1000). These data indicate that there was a decrease in the mean value of liver cell degeneration when compared to the K-treatment group which was only given CMC Na 0.5%. This means that onion extract can be an alternative therapeutic reference to improve the histopathological picture of the alloxan-induced liver. Onion has high phenolic phytochemicals. Recent research has reported that phenolic phytochemicals from onion have a blood glucose-lowering effect and high antioxidant activity in the alloxan-induced diabetic rat[12].

Shallots are believed to contain chemical components that have anti-inflammatory, anti-cholesterol effects, anticancer, and antioxidants such as quersetin[4]. There are studies that report that shallots contain high levels of quercetin, saponins, isorhamnetin and glycosides[13]. Most plants that contain bioactive compounds such as glycosides, alkaloids, terpenoids, flavonoids, and ceratenoid have antidiabetic activity. [14].

2. Liver cell necrosis

Necrosis is the death of liver cells. Dead cell nuclei can appear smaller, chromatin and reticular fibers multiply. The nucleus appears more dense and dark (picnotic) which can be broken or broken into several segments (karyoreksis) and then the cell nucleus is not visible because it has undergone perfect lysis (karyolysis) [15]. The mechanism of the occurrence of necrosis occurs

when tissue experiences hypoxia or the entry of a foreign body that is considered poisonous, the mitochondria will be injured, resulting in ATP down and Na + and K + pomp disturbed. Na + enters the cell which causes the lysosome to burst, releasing the hydrolytic enzyme so that it dissolves the cell[16].

Based on the results of statistical analysis with the Kruskal Wallis test showed that there were significant differences in each treatment group ($p < 0.05$) and continued with the Mann-Whitney test. In the group K- (CMC Na 0.5%), K + (metformin 45 mg / kg BW), and P1 (onion extract 250 mg / kg BW) there were no significant differences in the three treatments. In the P2 and P3 groups, there were also no significant differences. However, the treatment groups K-, K +, and P1 showed significantly different results when compared with treatments P2 and P3. The effect of decreasing the occurrence of different liver cell necrosis in each treatment may be influenced by different amounts of concentration at each dose of therapy.

Hepatocyte damage begins with changes in membrane permeability followed by cell death (necrosis). Increased enzymes in the blood caused by severe liver damage and accompanied by necrosis, so that enzymes from the mitochondria also come out of the cell[17].

Conclusion

Based on the results of this study it can be concluded that the administration of onion extract (Allium ascalonicum L) can improve the histopathological picture of the liver of white rats (Rattus norvegicus) induced by alloxan.

Conflict of Interest: There is no conflict of interest to be declared**.**

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Ethical Clearance: This study was approved by the Faculty of Veterinary, Universitas Airlangga

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