

The Histomorphometry of Liver and Kidney of Hyperglycemic Albino Rats after Treatment with *Tithonia diversifolia* Leaf Extract

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Abstract. *Tithonia diversifolia* can be used as an antidiabetic, so it is necessary to study the safety of its use, especially the side effects on the liver and kidneys. This study aimed to determine the effect of using *T. diversifolia* leaf extract through histomorphometry observations of the liver and kidneys of hyperglycemic albino rats. The study design used a completely randomized design (CRD). This study used 20 male albino rats which were divided into five treatment groups, there were T₀ (rat normal/control), T₁ (hyperglycemic rat without *T. diversifolia* leaf extract), T₂ (hyperglycemic rat administered with Glibenclamide 10 mg/kg BW), T₃ (hyperglycemic rat administered with *T. diversifolia* leaf extract 150 mg/kg BW), T₄ (hyperglycemic rat administered with *T. diversifolia* leaf extract 300 mg/kg BW). Every treatment was repeated four times. The damage of hepatocyte and the glomerular cell was observed through histological structure observation by histomorphometry method using a photomicrography microscope (Olympus BX51). The results indicate that there were significant differences ($P < 0.05$) in the variable of hepatocytes diameter and there was no significant different ($P > 0.05$) result on glomerular diameter, as well as kidney and liver weight. It was concluded that *T. diversifolia* leaf extract of 150 mg/kg BW and 300 mg/kg BW are safe to be used as antidiabetic. It does not cause any side effects on the liver and kidneys of hyperglycemic albino rats. Thereby *T. diversifolia* leaf extract can be further tested as preparation of biopharmaca which can be used as herbal medicines for diabetics.

Key words: glomerular; hepatocyte; histomorphometry; hyperglycemia; *Tithonia diversifolia*

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INTRODUCTION

Tithonia diversifolia is a herbal medicine native to Central America (Kato-Noguchi, 2020). In Indonesia, it is called *kembang bulan*, *paitan*, and insulin leaf (Ladeska et al., 2019). This plant can be used to treat diseases related to hyperglycemia (Chagas-Paula et al., 2012). Hyperglycemia is a condition of increasing blood glucose levels exceeding the normal limits (Mackey & Whitaker, 2015). Hyperglycemic conditions can damage body organs such as the liver and kidneys (Pecoits-Filho et al., 2016). Liver is an organ that plays an important role in detoxification to convert toxic compounds into non-toxic compounds (Chiang, 2014). The kidneys function for the disposal of metabolic waste products by the body such as urea and creatinine (Renda, 2017), and elimination of toxic substances (Genuis et al., 2011).

The liver and kidney can be damaged during hyperglycemic conditions. Hyperglycemic conditions in albino rats can be characterized by the presence of blood glucose of more than 140 mg/dL (Mackey & Whitaker, 2015). This condition causes glucose availability in cells to decrease, so the body will take the energy from other sources than carbohydrates

such as lipids (Michael et al., 2013). Over a long period, hyperglycemia will increase lipid oxidation that is able to increase reactive oxygen species (ROS) compounds that can bind to hepatocyte and glomerular cell organic matter, resulting in damage of hepatocyte and glomerular cell (Barrett et al., 2010).

The damage of liver and kidney cells can be observed through histomorphometry. That is the measurement method by calculating the average diameter of hepatocytes and glomeruli using a photomicrography microscope (El-Gohary et al., 2011). Cell damage due to hyperglycemia can be overcome with Glibenclamide and antidiabetic compounds obtained from plants (Naveen & Baskaran, 2018). Based on recommendations from the World Health Organization (WHO), many antidiabetic agents are obtained from plants (Kitukale & Chandewar, 2014). Plant extracts are widely and effectively used in lowering blood glucose and decrease the side effects (Baroni et al., 2016). One of the medicinal plants that can be consumed as an antidiabetic treatment is *T. diversifolia* (Lawal et al., 2012).

Khaing et al. (2019) reported that *T. diversifolia* contains many components of active compounds, but

only two main components have antidiabetic roles, namely flavonoids and sesquiterpenes. Flavonoids are antioxidants that can protect cells against damage caused by ROS. They can also inhibit pancreatic β cell damage, whereas sesquiterpenes can inhibit damage to the kidney in hyperglycemic conditions (Marianne et al., 2011; Wang et al., 2010). Winarsi et al. (2012) reported that flavonoids can stimulate peripheral glucose utilization, by increasing the glycolysis and glycogenesis pathways. Flavonoids can simultaneously suppress glycogenolysis, gluconeogenesis pathways, and controlling blood glucose so that blood glucose levels decrease. Antioxidants in flavonoids also can donate their hydrogen atoms to bind to the free radicals, so they become stable compounds. Flavonoids can stimulate the regeneration of hepatocytes, and glomeruli cells in the kidney due to damage caused by hyperglycemia (Abdelmoaty et al., 2010).

The research on *T. diversifolia* has been conducted by Sari et al. (2018), that showed that administration of 150 mg/kg BW and 300 mg/kg BW for *T. diversifolia* leaf extract 28 days can reduce blood glucose levels in hyperglycemic rats. According to Yuneldi et al. (2018), giving *T. diversifolia* 30 mg/BW/day and 60 mg/BW/day for 28 days can improve rat liver function. Fakunle and Abatan (2007), reported that *T. diversifolia* leaf extract at a dose of 400 mg/kg BW for 14 days caused degeneration to necrosis in the liver and kidneys. A lot of research on *T. diversifolia* has been done. However, there has not been an analysis of side effects and the safety of the doses of 150 and 300 mg/kg BW on the liver and kidneys. Furthermore, it was necessary to further investigate the effect of *T. diversifolia* leaf extract on the histomorphometry of liver and kidney of hyperglycemic albino rats. This study aimed to examine the effect of *T. diversifolia* leaf extract through histomorphometry observations of the liver and kidneys of hyperglycemic albino rats. This study was expected to provide scientific information about the effect of *T. diversifolia* leaf extract on the liver and kidneys of hyperglycemic albino rats.

METHODS

The preparation of *T. diversifolia* leaf extract

The fresh *T. diversifolia* leaves (1000 g) were collected and oven-dried at 50 – 60°C for 24 - 48 hours. The dried leaves then were mashed using a blender. The powder of 100 g was soaked in 1 L of distilled water for 24 hours. The filtrate was evaporated using a rotary evaporator at 80°C resulted in a viscous paste.

Animal study and treatment

The animals used in this study were 20 male albino rats (2-3 months old) consisting of 4 normal male albino rats and 16 hyperglycemic male albino rats weighing approximately 200 g. The albino rats were made hyperglycemic using alloxan at a dose of 150 mg/kg injecting by intraperitoneal for three days in a row (Yimam et al., 2014). Hyperglycemia in rats is a condition of rat blood glucose of more than 126 mg/dL (Zubaidah et al., 2017). The animals were randomly divided into 5 treatment groups, there were T₀ (rat normal/control), T₁ (hyperglycemic rat without *T. diversifolia* leaf extract), T₂ (hyperglycemic rat administered with 10 mg/kg BW of Glibenclamide), T₃ (hyperglycemic rat administered with 150 mg/kg BW of *T. diversifolia* leaf extract), T₄ (hyperglycemic rat administered with 300 mg/kg BW of *T. diversifolia* leaf extract) (Yuneldi et al., 2018; Sari et al., 2018). Each treatment was repeated 4 times. *Tithonia diversifolia* leaf extract with a dose of 150 and 300 mg/kg BW were made by dissolving 30 and 60 mg of the extract into each 0.2 mL distilled water. The other treatment was Glibenclamide 10 mg/kg BW. All treatment was done orally for 28 days (Yuneldi et al., 2018). The Ethics Committee of the Faculty of Medicine, Universitas Diponegoro approved all procedures.

The data collection and measurement

The research variables observed were hepatocyte and glomerular diameter as well as liver and kidney weight. The liver and kidney tissues were stained using hematoxylin-eosin (HE). Hepatocyte histomorphometry measurements were determined by calculating the average diameter as measured by the longest and widest part of the hepatocyte cell. Glomerular histomorphometry measurement was also done by calculating the average diameter as measured by the longest and widest part of the glomerular. Observations were carried out using a photomicrography microscope (Olympus BX51) equipped with a computer (El-Gohary et al., 2011; Kotyk et al., 2016).

Statistical analysis

The data that had been obtained were analyzed by using analysis of variance (ANOVA) at 95% confidence level by SPSS software version 15 (Yuneldi et al., 2021; Yuneldi et al., 2021). If there were significant different results, the analysis was continued using Duncan's test (Yuneldi et al., 2021; Yuneldi et al., 2021).

RESULTS AND DISCUSSION

The results on the average diameter of hepatocytes showed significantly different results ($P < 0.05$), but the glomerular diameter of hyperglycemic albino rats treated with *T. diversifolia* leaf extract after 28 days were not significantly different ($P < 0.05$) between treatments (Table 1). The effect of *T. diversifolia* leaf

extract on the average hepatocyte diameter of hyperglycemic albino rats showed significantly different results ($P < 0.05$). The results of the advanced test analysis using the Duncan’s test showed that there was a significant difference between T_1 and all treatments, but no significant difference between $T_0, T_2, T_3,$ and T_4 (Table 1).

Table 1. Average hepatocyte and glomerular diameter of hyperglycemic albino rats after treatment of *T. diversifolia* leaf extract.

Variable	Average \pm SD, Treatment				
	T_0	T_1	T_2	T_3	T_4
Hepatocyte Diameter (μm)	19.46 ^b \pm 1.00	22.75 ^a \pm 2.51	20.02 ^b \pm 0.54	19.31 ^b \pm 0.85	18.27 ^b \pm 0.38
Glomerular Diameter (μm)	92.00 ^a \pm 3.47	93.24 ^a \pm 2.77	93.30 ^a \pm 2.39	92.49 ^a \pm 8.87	94.94 ^a \pm 6.60

^{a-b} Different superscripts in the same row indicate significant differences ($P < 0.05$).

The significant different results of T_1 hepatocyte diameter (22.75 μm) was due to an increase in hepatocyte diameter. For the T_0 , the average diameter of the hepatocyte was situated in the normal range. Rosioru et al. (2012) reported that the normal hepatocyte cell diameter in albino rats ranges from 17.01-21.93 μm . The hepatocyte diameter of T_1 was increased by 3.29 μm as compared to T_0 . The increase in hepatocyte cell diameter was caused by

swelling of the cytoplasm in the form of hydropic degeneration (Figure 1b).

Histologically, there was a swelling of cells in the T_1 in the form of hydropic degeneration that was characterized by unevenly eosin-stained cytoplasm due to water entering the cytoplasm. Hastuti (2006) reported that swelled cells in the form of hydropic degeneration will make the cytoplasm cannot be evenly stained by eosin because it is accumulated by water.

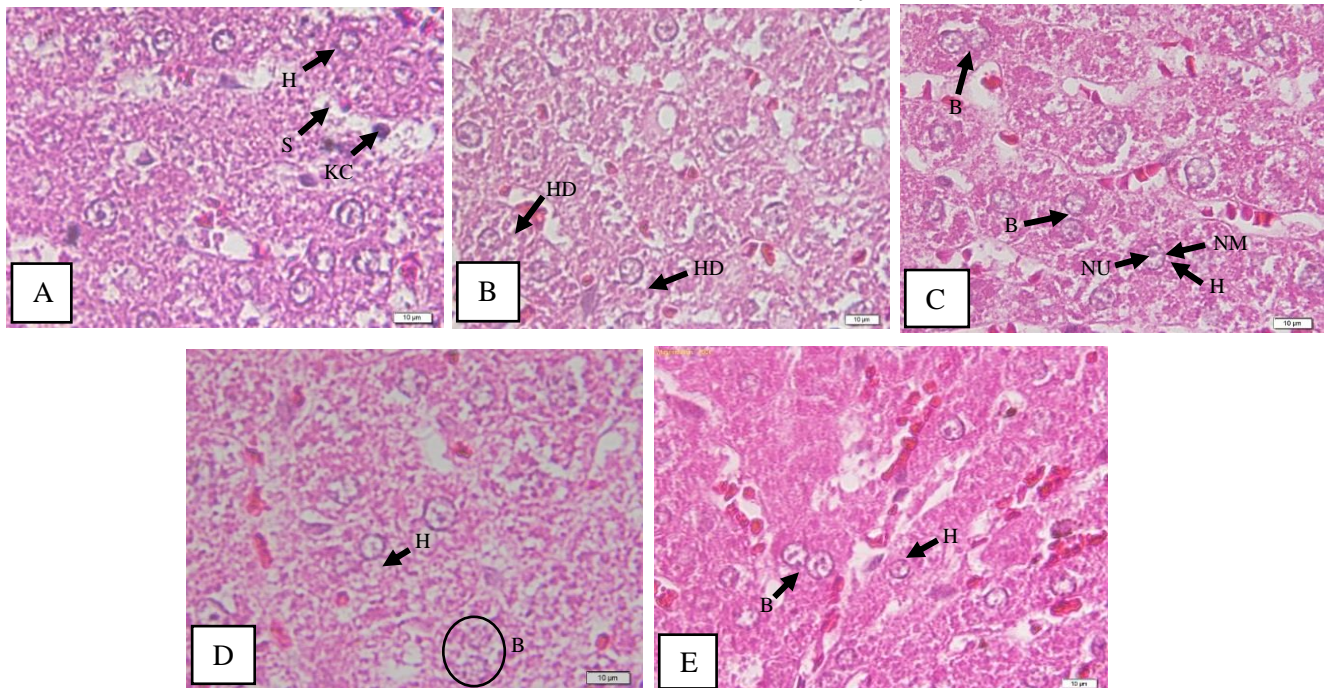


Figure 1. Liver histological structure of albino rats (*Rattus norvegicus*). A) T_0 , B) T_1 , C) T_2 , D) T_3 and E) T_4 . 1000x magnification. Description: hematoxylin-eosin. H: Hepatocytes, S: Sinusoid, KC: Kupffer Cell, HD: Hydropic Degeneration, B: Binucleated Cell, NM: Nucleus Membrane, and NU: Nucleolus.

Changes in hepatocyte structure in the form of hydropic degeneration are caused by ROS which was resulted from excessive lipid oxidation (Barrett et al., 2010). The mechanism of hepatocyte cells damaged by ROS starts from alloxan which damages pancreatic β cells, then inhibits insulin secretion into the blood, so that glucose in the blood increases beyond a normal level which results in hyperglycemic conditions (Rohilla & Ali, 2012). A hyperglycemic condition in albino rats causes glucose supply to the cells in a small amount so that the availability of glucose in cells decreases. The reduced availability of glucose in cells makes the body take the energy from other sources than carbohydrates, such as lipids (Michael et al., 2013). Hyperglycemic conditions over a long period will increase lipid oxidation and can produce excessive compounds of ROS that bind to hepatocyte cell organic matter, resulting in hepatocyte cell swelling (Barrett et al., 2010).

The average diameter of hepatocytes for T_1 was significantly different from T_3 and T_4 . The treatment of T_3 and T_4 can regenerate cells and the diameter can return to be normal (Table 1 and Figure 1). The structure of hepatocytes in hyperglycemic albino rats damaged can be repaired by giving *T. diversifolia* leaf extract which contained flavonoid. Flavonoids are antioxidants that can stimulate the regeneration of hepatocytes quickly (Biswas et al., 2011). The

content of flavonoids can also stimulate insulin secretion, regeneration of pancreatic β cells, and regeneration of hepatocytes (Dheer & Bhatnagar, 2010). Increased secretion of insulin will cause the increase of glucose oxidation that produces more energy so that it can be used for hepatocyte cell regeneration (Barrett et al., 2010).

The average diameter of glomeruli of hyperglycemic albino rats showed results that were not significantly different ($P > 0.05$) (Table 1). It happened because kidney glomerular diameter was still in the normal range and no glomerular damage occurred (Figure 2). These results are in accordance with Selmanoglu et al. (2012), that the normal glomerular diameter ranges from 76.0976 μm to 92.3549 μm . This statement is supported by Kotyk et al. (2016), that the normal glomerular diameter are 81.79 μm , 87.63 μm , and 97.40 μm . The administration of *T. diversifolia* leaf extract containing sesquiterpene compounds which can inhibit the damage to the kidney under hyperglycemic conditions and also inhibit glomerulonephritis. Wang et al. (2010) reported that sesquiterpene compounds in *T. diversifolia* can inhibit kidney damage in hyperglycemic conditions. Sesquiterpene compounds can also inhibit glomerulonephritis, as well as mesangial and podocyte cells damage (Sanchez-Niño et al., 2013).

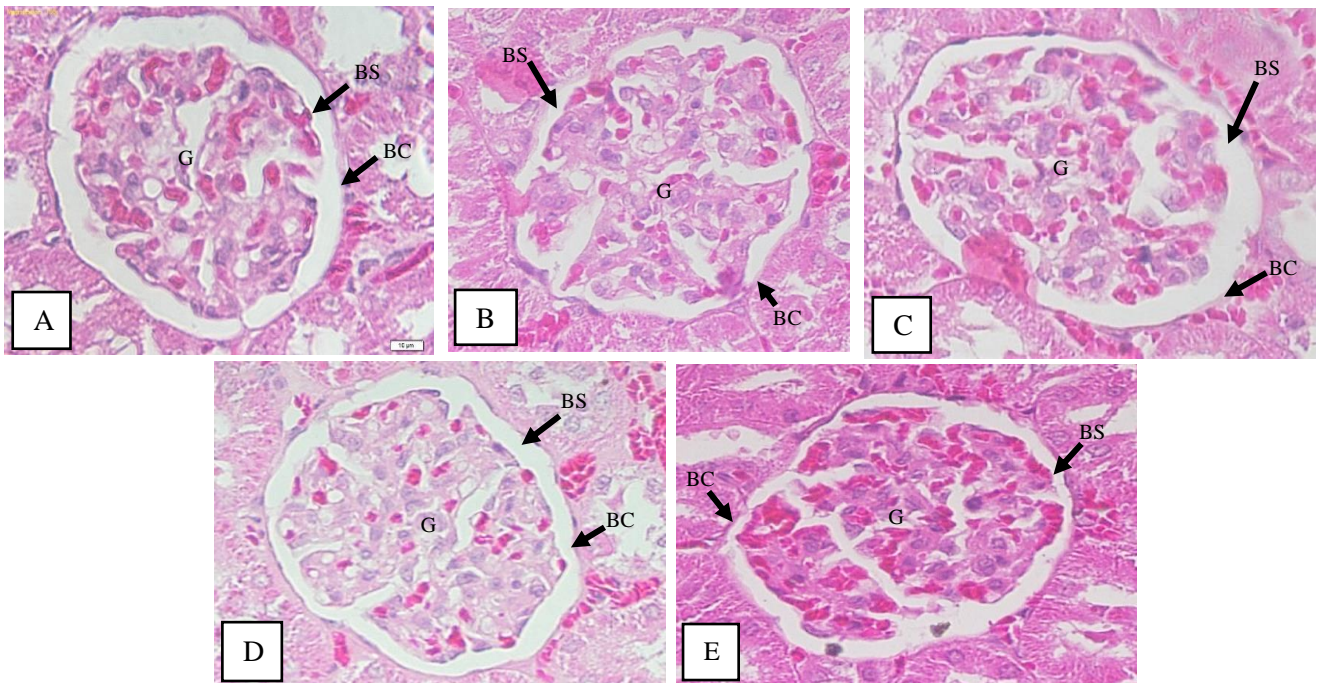


Figure 2. Kidney histological structure of albino rats (*Rattus norvegicus*). A) T_0 , B) T_1 , C) T_2 , D) T_3 , E) T_4 . 1000x magnification. Description: hematoxylin-eosin. G: Glomerular, BS: Bowman Space, and BC: Bowman Capsule.

The effect of the administration of *T. diversifolia* leaf extract to the liver weight of hyperglycemic albino rats using ANOVA showed that the results were not significantly different ($P > 0.05$) between treatments (Table 2). The results that were not significantly different showed that the liver structure was still in normal condition. Liver weight can be influenced by hepatocyte diameter. The increase in hepatocyte cell diameter has not been able to influence liver weight because it is in the form of hydropic degeneration which is still reversible, so that hepatocyte cells can regenerate (Kumar et al., 2018).

The increase in hepatocyte cell diameter was considered to have not affected overall hepatocyte cells so that there was no significant difference in liver weight. Increased liver weight is usually caused

by fatty liver, as a result of necrosis (Niendya et al., 2011). Giving alloxan in treatment T_1 has not caused fatty liver, so there was no significant difference in liver weight. Fatty liver is an excessive lipid accumulation in the liver exceeding 5% of liver weight (Green & Hodson, 2014). The mechanism for the fatty hepatocyte cells begins with a hyperglycemic condition. Over a long period, hyperglycemic will increase lipid oxidation. Increased lipid oxidation can produce more excessive ROS compounds, resulting in an imbalance between the antioxidants and free radicals produced. This imbalance causes ROS to bind to hepatocyte cell organic matter, resulting in damage characterized by the presence of fatty cells in hepatocytes (Pratiwi et al., 2016).

Table 2. Average liver and kidney weight of hyperglycemic albino rats after treatment *T. diversifolia* leaf extract

Variable	Average \pm SD, Treatment				
	T_0	T_1	T_2	T_3	T_4
Liver Weight (g)	9.76 ^a \pm 0.39	10.97 ^a \pm 0.60	9.48 ^a \pm 0.55	10.06 ^a \pm 1.06	9.68 ^a \pm 0.68
Kidney Weight (g)	1.71 ^a \pm 0.13	1.90 ^a \pm 0.14	1.55 ^a \pm 0.18	1.81 ^a \pm 0.19	1.83 ^a \pm 0.13

^{a-b} Different superscripts in the same row indicate significant differences ($P < 0.05$).

The effect of the administration of *T. diversifolia* leaf extract to the kidney weight of hyperglycemic albino rats showed results that were not significantly different ($P > 0.05$) (Table 2). The average result of kidney weight was not significantly different because the kidney's structure was still in normal condition. Kidney weight can be affected by glomerular diameter. According to Nyengaard & Bendtsen (1992), there is a correlation between the number and diameter of glomeruli and kidney weight. The results of glomerular diameter measurements were in the normal range and necrosis has not occurred, so the kidney weight in albino rats is also normal. Craig et al. (2015) reported kidney weight correlation with kidney histopathology. According to Rasch & Dørup (1997), diabetic rats can increase kidney weight by 53 - 93%. Another factor that is considered to cause changes in the kidney structure is the influence of toxic metabolic activities of chemical compounds (Craig et al., 2015).

Based on this study, *T. diversifolia* leaf extract of 150 mg/kg BW and 300 mg/kg BW are safe to be used as antidiabetic and does not cause side effects on the liver and kidneys of hyperglycemic albino rats. Therefore *T. diversifolia* leaf extract can be further tested as preparation of biopharmaca which can be expected as herbal medicines for diabetics.

CONCLUSION

Based on this research, it was concluded that *T. diversifolia* leaf extract of 150 mg/kg BW and 300 mg/kg BW are safe to be used as antidiabetic. It does not cause any side effects on the liver and kidneys of hyperglycemic albino rats.

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