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Ruellia tuberosa L. Leaf Extract Improves Histopathological Damages in Kidneys of Alloxan-Induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is a Non-Communicable Disease (NCD) characterized by hyperglycemia and insulin dysfunction, leading to redox imbalance and increased Reactive Oxygen Species (ROS). This oxidative stress condition can lead to kidney damage. While drugs like metformin are commonly used to treat diabetes, they can also cause damage to the kidney. Therefore, exploring natural alternatives, such as *Ruellia tuberosa* L. leaves, is important to minimize side effects and protect kidney function.

Methods: This study used a Completely Randomized Design (CRD) with five treatments and five replications. The negative control group received distilled water, while the positive control group was injected with alloxan (150 mg/kgBW) and treated with metformin (50 mg/kg BW). Treatment groups (P1, P2, and P3) received alloxan (150 mg/kg BW) and *Ruellia tuberosa* L. extract at doses of 200, 400, and 800 mg/kg BW, respectively. Data were analyzed using the Kruskal-Wallis test, and if the extract showed an effect on the kidney condition, post hoc analysis with the Mann-Whitney test was performed.

Results: The results of the study showed that there was kidney damage characterized by glomerular necrosis, degeneration of tubular cells, and necrosis of renal tubular cells due to alloxan induction. However, giving *Ruellia tuberosa* L. extract at doses of 200, 400, and 800 mg/kgBW can prevent kidney damage.

Conclusion: The data analysis results indicate that the *Ruellia tuberosa* L. leaf extract can prevent kidney damage in alloxan-induced diabetic white rats, with the recommended effective dose of 800 mg/kgBW.

Introduction

Diabetes mellitus is a disease related to metabolic disorders involving carbohydrates, proteins, and fats [1, 2]. This condition arises because the body is unable to produce or respond to insulin effectively, thereby failing to maintain normal blood glucose levels [3]. Diabetes mellitus is classified as a Non-Communicable Disease (NCD). A key characteristic of this condition is hyperglycemia, which results from disruptions in insulin secretion, insulin action, or both [1]. This dysfunction is associated with a redox imbalance caused by uncontrolled cellular levels of Reactive Oxygen Species (ROS) [4, 5].

Every year, the number of people with diabetes mellitus has increased. Indonesia is ranked fifth globally in terms of the number of cases of diabetes mellitus, according to a 2021 survey by the International Diabetes Federation (IDF). Type 1 and type 2 diabetes are the two forms of the disease that are most prevalent in Indonesia [6].

Diabetes mellitus is a substantial risk factor for heart disease, kidney failure, and blindness in addition to being a global contributor to mortality [7]. The kidneys play a crucial role in maintaining fluid balance in the body. They also secrete the enzymes renin, angiotensin, and aldosterone to regulate blood pressure, as well as erythropoietin, which is essential for red blood cell synthesis [8]. Diabetes mellitus is related to kidney disease due to complications arising from vascular and renal dysfunction, a condition known as diabetic nephropathy [9]. It is characterized by glucosuria and can be detected by the presence of glucose in the urine. Histopathological damage in diabetes mellitus patients can affect all types of renal cells, impacting the kidneys ability to function properly [10]. Damage to the glomerulus, a component of the nephron, involves the destruction of glomerular capillary cells, the basal membrane, and the glomerular capsule, often resulting in necrosis [11]. Damage to the renal tubules can include deposits or degeneration (hyaline, fatty, hydropic), glycogen accumulation, atrophy, or hypertrophy in the proximal or distal convoluted tubules [12].

Currently, metformin is commonly used in the treatment of diabetes mellitus. However, metformin has the potential to cause lactic acidosis, a condition where elevated levels of lactic acid in the body, which is able to lead to an imbalance in pH that affecting both blood and urine. This imbalance will put a strain on the kidneys and potentially cause kidney damage [13].

Herbal plants are now being utilized as alternative antidiabetic treatments to chemical drugs [14]. The extract of *Ruellia tuberosa* L. leaves contains four active chemical compounds (Hexadecanamide, 9-Octadecenamide, (Z)-Octadecenamide, and 1,2-Benzenedicarboxylic acid), which are polyphenols that promote the repair of pancreatic beta cells and enhance insulin production [15]. Analysis of the aqueous extract of *Ruellia tuberosa* L. leaves reveals the presence of flavonoids, saponins, tannins, alkaloids, and steroids [16]. These phytochemicals induce autophagy in kidney podocyte cells. It inhibits the overactive renin-angiotensin-aldosterone system, also reduces oxidative stress and inflammatory pathways in renal cells. Besides, it alleviates diabetes mellitus by inhibiting the synthesis of TGF-β and ROS [17]. Based on the above discussion, research has been conducted on the effects of *Ruellia tuberosa* L. leaf extract on the histopathological pictures of diabetic white rats induced by alloxan [18].

Methods

Tools and Materials

The tools and materials used in this study were *Ruellia tuberosa* L. leaves from Banyuwangi District, Banyuwangi Regency, East Java Province. Sampling of *Ruellia tuberosa* L. leaves was performed randomly without comparing to those from other regions. Other materials used in the study were male white rats (Rattus norvegicus), feed (CP511), sawdust, metformin, 10% formalin, 96% ethanol, 80%, 90%, and 95% alcohol, xylene, liquid paraffin, 0.9% NaCl solution, alloxan (PT. Nitra Kimia), alcohol spray, and Carboxymethyl Cellulose (CMC) (Medical and Laboratory Supplier).

Experimental Design

This study used an experimental design by using a Completely Randomized Design (CRD). The research was conducted from August to September 2023, including seven days for preparation of equipment and materials, seven days for adaptation, one day for alloxan injection, diabetes checking in five days after alloxan injection, 21 days for extract administration, seven days for histopathological preparation, and seven days for histopathological examination of the left kidneys of the white rats. Ethical test is carried out to ensure that all actions and treatments given to experimental animals are in accordance with the Standard Operating Procedures implemented at the Faculty of Veterinary Medicine, Universitas Airlangga, Campus C, Surabaya with the number 2.KEH.098.06.2023. The study population consisted of 25 male white rats which aged approximately two months. The rats were healthy, without physical defects, and weighed between 150-180 grams [19].

The treatment groups were as follows:

P1: Diabetic rats administered *Ruellia tuberosa* L. leaf extract at dose of 200 mg/kg BW.

P2: Diabetic rats administered *Ruellia tuberosa* L. leaf extract at dose of 400 mg/kg BW.
P3: Diabetic rats administered *Ruellia tuberosa* L. leaf extract at dose of 800 mg/kg BW.
K-: Control group of rats given only distilled water without treatment.
K+: Diabetic rats administered metformin at a dose of 50 mg/kg BW.

After a seven-day adaptation period and confirming the rats weight within the range of 150-180 grams, and measuring initial blood glucose levels, diabetes induction was carried out in all groups of white rats except the normal control group. The rats were induced with a single dose of alloxan (150 mg/kg BW) administered intraperitoneally [20–22].

Kidney samples were collected on the 22nd day following the administration of the medication and extracts. Euthanasia was performed with intraperitoneal injections of xylazine and ketamine at doses of 10 mg/kg BW and 100 mg/kg BW, respectively [23]. After taking organ samples, histopathology preparations are made. Then, histopathological observations and assessments were carried out for each treatment group using the following parameters:

Score	Glomerular Necrosis
0 (zero)	If glomerular necrosis is <10% of all glomeruli
3 (three)	If glomerular necrosis is 11 - 25% of all glomeruli
5 (five)	If glomerular necrosis is 26 - 50% of all glomeruli
7 (seven)	If glomerular necrosis is 51 - 75% of all glomeruli
8 (eight)	If glomerular necrosis is >76% of all glomeruli
Score	**Tubular Cell Degeneration**
0 (zero)	If the number of degenerative cells is <10% of the field of view
1 (one)	If the number of degenerative cells is 11 - 25% of the field of view
2 (two)	If the number of degenerative cells is 26 - 50% of the field of view
3 (three)	If the number of degenerative cells is 51 - 75% of the field of view
4 (four)	If the number of degenerative cells is >76% of the field of view
Score	**Tubular Cell Necrosis**
0 (zero)	If the number of necrotic cells is <10% of the field of view
2 (two)	If the number of necrotic cells is 11 - 25% of the field of view
4 (four)	If the number of necrotic cells is 26 - 50% of the field of view
6 (six)	If the number of necrotic cells is 51 - 75% of the field of view
8 (eight)	If the number of necrotic cells is >76% of the field of view

Table 1: Histopathological Scoring Parameters for Kidney [1].

Table 1 presents the histopathological scoring parameters used for assessing kidney damage. The scoring system quantifies the extent of glomerular necrosis, tubular cell degeneration and tubular cell necrosis, based on the percentage of affected structures observed under microscopic examination. Scores range from 0 to 8 for glomerular necrosis and tubular cell necrosis, and 0 to 4 for tubular degeneration. The higher scores indicating greater severity of kidney damage.

Data Analysis

The collected data were analyzed using the non-parametric Kruskal-Wallis test followed by the Mann-Whitney test [25]

Results

Histopathological examinations

The results of the assessment observed with a trinocular microscope showed glomerular and tubular necrosis, as well as tubular degeneration in the kidneys. Analysis of glomerular and tubular cell necrosis, as well as tubular degeneration in white rat, showed significant differences ($p<0.05$) between treatment groups based on the results of the Kruskal-Wallis test. Based on the results, it suggests that *Ruellia tuberosa* L. leaf extract has an impact on the healing of kidney cells in white rats. Further analysis using the Mann-Whitney test was conducted to determine differences among groups.

Mann-Whitney test

Based on the Mann-Whitney test in this study, it showed that there was no significant difference ($p>0.05$) between the dose treatment group (P3) and the negative control group (K-). However, a significant difference ($p<0.05$) can be seen between this treatment group and other treatment groups. The positive control group (K+) did not show a significant difference ($p>0.05$) from the dose treatment group (P1), but there was a significant difference ($p<0.05$) compared to the dose (P2) and dose (P3) treatment groups. Significant differences ($p<0.05$) were also observed between dose (P1) and dose (P2) treatments compared to dose (P3) treatments.

Treatment	Mean Rank		
	Glomerular Necrosis	Tubular Degeneration	Tubular Necrosis
K- (Negative Control, distilled water)	4.20^{a}	4.60^{a}	4.90^{a}
K+ (Positive Control, Metformin)	22.30^{c}	22.60^{c}	21.70^{c}
P1 (200 mg/kg BW *Ruellia tuberosa* L. extract)	17.30^{bc}	15.70^{bc}	17.70^{bc}
P2 (400 mg/kg BW *Ruellia tuberosa* L. extract)	14.30^{b}	14.90^{b}	14.60^{b}
P3 (800 mg/kg BW *Ruellia tuberosa* L. extract)	6.90^{a}	7.20^{a}	6.10^{a}

Table 2: Mean rank values of glomerular necrosis, tubular degeneration, and tubular necrosis in diabetic white rats induced by alloxan under different treatments.

Table 2 presents the mean rank values for histopathological scoring of glomerular necrosis, tubular degeneration, and tubular necrosis in alloxan-induced diabetic rats under different treatments. The treatments include a negative control (distilled water), a positive control (metformin), and *Ruellia tuberosa L.* extract at three different dosages (200, 400, 800 mg/kg

BW). Significant differences are indicated by different superscripts ($p<0.05$).

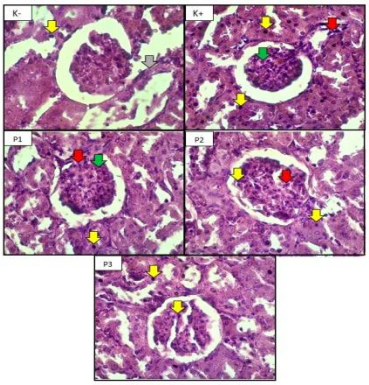

Figure 1: Results of histopathological scoring for glomerular necrosis in white rats K(-), K(+), P1, P2, and P3, using H.E. staining at 400X magnification. Red arrows = Karyolysis, Yellow arrows = Pyknosis, Green Arrows = Karyorrhexis, Grey arrows = Hyaline Degeneration.

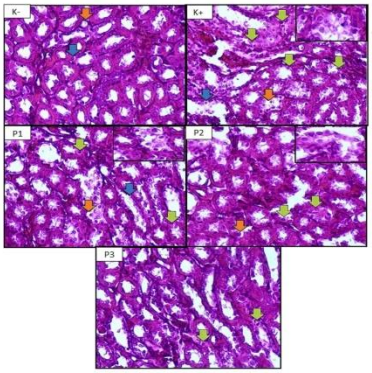

Figure 2: Results of histopathological scoring for tubular degeneration in white rats K(-), K(+), P1, P2, and P3, using H.E. staining at 400X magnification. Blue arrows = hydropic degeneration. Green arrows = hyaline degeneration. Orange arrows = fatty degeneration.

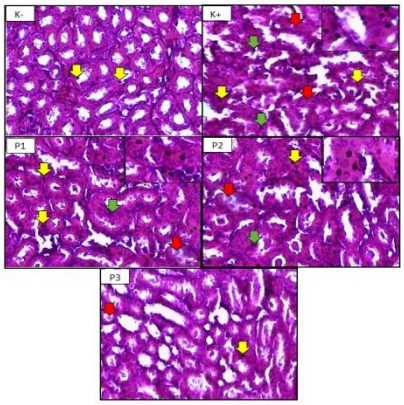

Figure 3: Results of histopathological scoring for tubular necrosis in white rats K(-), K(+), P1, P2, and P3, using H.E. staining at 400X magnification. Red arrows = Karyolysis. Yellow arrows = Pyknosis. Green arrows = Karyorrhexis.

Discussion

This study aims to determine the effect of *Ruellia tuberosa* L. leaf extract on the histopathological picture of the kidneys of diabetic white rats (Rattus norvegicus), which was induced by alloxan. Microscopic observations and examinations were carried out on five treatment groups with five different fields of view. The results were histopathological changes in the form of glomerular necrosis, degeneration of tubular cells, and necrosis of tubular cells which were quite significant. These histopathological changes occurred because the kidney cells were damaged due to the administration of alloxan.

Based on the research data, alloxan induction at a dose of 150 mg/kg BW, in the positive control group (K+), as well as in the treatment groups P1, P2, and P3, resulted in diabetes with fasting blood glucose levels reaching ⩾200 mg/dl. Increased blood glucose levels in white rat occur due to alloxan induction that attacks pancreatic cells, causing anomalous function of pancreatic β cells. The process of alloxan to enter pancreatic β cells is at the GLUT-2 receptor, which is the same receptor on pancreatic glucose receptor cells, which carries alloxan to the cytosol or fluid from the cells [26].

Alloxan undergoes a redox reaction after reaching the cytosol. This reaction forms free radicals (superoxide) and produces dialuric acid. This triggers hydroxyl radicals to activate various enzymes, thereby causing protein fragmentation, lipid peroxidation, DNA fragmentation, and pancreatic cell death [27], [28]. Disruption of this function causes uncontrolled glucose levels resulting in hyperglycemia or diabetes. [29].

Hyperglycemia affects the body's regulatory system due to abnormal increases in blood glucose levels. The increased number of free radicals in the body caused by high blood pressure can be toxic as they damage cells through oxidative stress [30]. Blood glucose regulation is not only performed by the pancreas but also involves (1) nerves, (2) kidneys, and (3) other hormones in the body. The kidneys are a crucial parameter in the mechanism of regulating hyperglycemia, releasing hormones and regulating blood glucose levels by filtering blood, excreting excesses, and reabsorbing blood [31].

The kidneys have two mechanisms to maintain normal blood glucose levels which are hormonal and hemodynamic. The hemodynamic mechanism regulates blood pressure in the renal artery. If there is excess blood passing through the artery, the kidneys filter large amounts of blood, then leading to the excretion of water, salt, and a reduction in blood volume. The kidneys also stimulate hormones such as renin, angiotensin, and aldosterone, which collectively regulate excess blood glucose levels. Blood pressure

and fluid balance are important parameters that are regulated by the renin-angiotensin system (RAS) [32]. The kidneys produce the hormone renin in response to low sodium, high potassium, or decreased blood volume. The liver produces angiotensinogen, which is converted into angiotensin I by the enzyme renin. It is produced in the juxtaglomerular cells and macula densa. Then, angiotensin I is changed into angiotensin II by the angiotensin-converting enzyme (ACE), which is mostly present in lung endothelial cells. Vasoconstriction brought on by angiotensin II stimulates the pituitary gland to release antidiuretic hormone (ADH), which lowers fluid loss and increases the adrenal glands' production of aldosterone. Aldosterone acts on the renal tubules to retain sodium and excrete potassium, ultimately increasing blood pressure. RAS functions to maintain blood pressure and regulate sodium, potassium, and fluid levels [33].

The administration of *Ruellia tuberosa* L. leaf extract in treatments (P1), (P2), and (P3) for 21 days resulted in a decrease in blood glucose levels and improvement in the histopathological structure of the kidneys in white rats which damaged by alloxan exposure. Degenerations such as hyaline, hydropic, and fatty degenerations were repaired, and necrosis such as karyolysis, karyorrhexis, and pyknosis were avoided. Based on the data and results of the study, the negative control group (K-) showed the least amount of kidney damage. This was due to the white rats in the negative control group (K-) not receiving any treatment other than distilled water, making them a normal group. The low level of damage in this group could be attributed to external factors, such as uncontrolled variables during the study. For example, the rats' condition might not have been optimal or they could have been stressed.

The positive control group (K+) that was given alloxan at a dose of 150 mg/kg BW and metformin as a comparison to the *Ruellia tuberosa* L. extract showed severe kidney damage. This was due to cell damage resulting from hyperglycemia caused by alloxan induction and metformin, which could exacerbate necrosis conditions due to excessive drug accumulation and cause kidney failure or worsen kidney function, particularly in those with pre-existing kidney disease or patients with high blood pressure or hyperglycemia. The use of metformin in patients with kidney disease can lead to lactic acidosis, affecting the pH or acid-base balance of the blood and urine, causing kidney damage and chemical accumulation in the tubules, which makes the renal tubule cells work too hard, eventually leading to cell death if prolonged [34]. Additionally, hyperglycemia can cause oxidative stress, ultimately leading to cell death. Alloxan is known as a diabetogenic agent that damages pancreatic beta cells, worsening hyperglycemia, and accelerating kidney damage. Excessive oxidative processes also lead to inflammation and further dysfunction in kidney tissues, exacerbating the degenerative condition of tubular kidney cells [35].

Treatment groups P(1) and P(2), which received *Ruellia tuberosa* L. extract at doses of 200 mg/kg and 400 mg/kg BW respectively, showed mild microscopic damage compared to white rats treated with alloxan and metformin. However, there was no significant difference between treatment groups P1 and P2, indicating that the doses of *Ruellia tuberosa* L. in both treatment groups were not optimal, as the antioxidant content was insufficient to neutralize the increase in ROS and its toxicity. The antioxidant levels in *Ruellia tuberosa* L. at doses of 200 and 400 mg/kg BW were not effective in preventing cell damage. Meanwhile, treatment group P3 showed a pattern similar to the negative control group (K-) and had a mean rank value not significantly different from the negative control group (K-). This suggests that a dose of 800 mg/kg BW of *Ruellia tuberosa* L. leaf extract is the most effective in preventing kidney damage. The extract's activity can prevent and repair damage to renal tubular cells due to its phytochemical content, including saponins, carotenoids, flavonoids, alkaloids, and polyphenols such as Hexadecanamide, 9-Octadecenamide (Z), Octadecenamide, and 1,2-Benzenedicarboxylic acid, which can induce autophagy in necrotic renal tubular cells, and also acts as a diuretic, antidiabetic, antipyretic, analgesic, and antihypertensive agent [15].

The data analysis results indicate that the administration of *Ruellia tuberosa* L. leaf extract can improve kidney damage in diabetic white rats induced by alloxan. The recommended effective dose of *Ruellia tuberosa* L. leaf extract for preventing histopathological kidney damage is 800 mg/kg BW.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

All authors were responsible for the study design, data gathering, data analysis, manuscript preparation, and editing of the manuscript. All authors have approved the submission of the manuscript.

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