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# The Effect of Torch Ginger (*Etlingera elatior*) Flower Extract on Creatinine Levels and Kidney Histophatology in Alloxan-Induced White Rats (*Rattus norvegicus*)

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**Abstract**

Kidney function impairment is a common complication of hyperglycemia, characterized by increased creatinine levels and structural damage to renal tissue. Alloxan is frequently used to induce kidney injury through oxidative stress mechanisms. Torch ginger (Etlingera elatior) flower contains antioxidant compounds with potential nephroprotective effects. This study aims to determine the effect of torch ginger (Etlingera elatior) flower extract on creatinine levels and kidney histopathology in alloxan-induced white rats (Rattus norvegicus). The study used a Completely Randomized Design (CRD) with an experimental approach. The negative control group received destilled water, while the positive control group was induced with alloxan (120 mg/kgBW) intraperitoneally and treated with glibenclamide (0.09 mg/kgBW). The treatments groups (P1, P2, and P3) were given alloxan (120 mg/kgBW) and torch ginger (Etlingera elatior) flower extract orally at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, respectively. This study showed that torch ginger (Etlingera elatior) flower extract significantly reduced creatinine levels and improved kidney histopathology (renal tubular necrosis). The 400 mg/kgBW dose was the most effective in lowering creatinine levels and repairing kidney tissue damage.

**Keywords:** *Etlingera elatior* flower; creatinine levels; kidney histopathology; alloxan; *Rattus norvegicus*.

## INTRODUCTION

The kidneys are essential organs responsible for maintaining internal physiological balance. They filter the blood to regulate fluid homeostasis, acid–base equilibrium, and electrolyte levels within the body (Jannah & Budijastuti, 2022). Blood flow to the kidneys accounts for nearly 25% (Rafe et al., 2020). This relatively large amount increases the likelihood that the kidneys will be exposed to toxic substances. The presence of toxins in the circulatory system poses a high risk as they can damage the structure and function of kidney tissue. Functional and structural impairment of the kidneys can lead to various diseases such as chronic kidney failure or even death. Hyperglycemic conditions are one of the causes of kidney damage. An imbalance between the production of ROS (Reactive Oxygen Species) and the antioxidant defense system is the main trigger for kidney damage, as indicated by increased creatinine levels in the blood. Elevated creatinine levels indicate a decrease in glomerular filtration rate (Perdanawati et al., 2021). This can be observed in rat models (*Rattus norvegicus*) induced with alloxan.

Alloxan, as one of the toxic compounds, affects kidney histology, causing tubular necrosis, loose tubular structure, and fatty degeneration (Sholihah & Qomariyah, 2021). The mechanism by which alloxan causes kidney damage involves oxidative stress, leading to non-enzymatic glycation of amino acids and protein kinase C. This process results in the irreversible formation of Advanced Glycation End Products (AGEs). These AGEs accumulate and trigger ROS to react with polyunsaturated fatty acids (PUFA) in kidney cell membranes, increasing the production of malondialdehyde (MDA) in kidney cells as a result of lipid peroxidation in the cell membrane (Ariani et al., 2024). The decline in kidney function caused by this condition can be treated using either conventional medicine or traditional remedies. Medical treatments are often more expensive and may cause side effects. Therefore, the use of locally available medicinal plants needs to be enhanced. Many plant species have the potential to lower blood glucose levels, including torch ginger *(Etlingera elatior*).

Torch ginger flowers contain numerous secondary metabolites, including flavonoids, saponins, alkaloids, steroids, phenols, and tannins (Wardani et al., 2022).

Phytochemical screening by Salman & Indriana (2021) showed that fresh flowers, torch ginger simplicia, and ethanolic extracts contain chemical compounds such as alkaloids, flavonoids, steroids, glycosides, and essential oils. The extract can inhibit amylase and glucosidase enzymes, neutralize free radicals, and protect pancreatic beta cells from damage (Putri, 2021).

These properties highlight its potential as a nephroprotective agent against alloxan-induced kidney damage. Therefore, researched are interested in evaluating the effectiveness of antioxidant compounds contained in *Etlingera elatior* flower extract at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW in lowering creatinine levels and repairing kidney tissue structure resulting from alloxan induction.

## MATERIALS AND METHODS

### Tools and Materials

The tools used included a blender, rotary evapotaror, water bath, whatman paper No.1, dark bottles, feeding and dinking tools, cages, injection syringes, oral gavage, weighing scales, surgical scissors and forceps, paraffin bath, 1 cc syringes, blood tubes, spectrophotometer, sample bottles, microtome, cassettes, tissue processor, incubator, microtome, glass slides, and microscope.

The materials used in this study included torch ginger (*Etlingera elatior*) flowers, alloxan, male white rats (Rattus norvegicus), glibencalmide, 96% ethanol, 0.9% NaCl, *Carboxy Methyl Cellulose* (CMC) 0.5%, feed, formalin, hematoxylin eosin (HE), alcohol, xylol and distilled water.

**Figure 1.** *Etlingera elatior was* found by author on Bingkawan Villages.

### Plant collection and extraction

Torch ginger flowers (*Etlingera elatior*) were obtained from Bingkawan Village, Sibolangit Subdistrict, Deli Serdang Regency, North Sumatra Province. Sampling was done manually by selecting 4 kg of fresh, young flower buds (unbloomed) that were undamaged and free from defects. The freshly harvested young flowers were then wet-sorted and thinly sliced. The sliced flowers were dried using an oven at 40°C, followed by dry sorting to ensure the samples were free from foreign materials. 250 grams of simplicia powder was weighed and added with 2500 ml of 96% ethanol solvent in a 1:10 ratio (Nasir et al., 2023). Maceration was carried out for 3×24 hours with stirring three times a day. After 3 days, the mixture was filtered using Whatman No.1 filter paper and concentrated using a rotary evaporator at 50°C. The concentrate was then dried using a water bath 60°C to obtain a thick extract of Etlingera elatior flowers.

### Experimental animals

The experimental animals used in this study were 25 healthy male white rats (*Rattus norvegicus*), aged between 2 to 3 months and weighing approximately 150–200 grams. These animals were obtained from the Pharmacy Laboratory of Universitas Sumatera Utara. All selected rats were confirmed to be healthy and free from physical abnormalities. The acclimatization of test animals was conducted at the Animal Facility of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan. This process aimed to allow the rats to adapt to their new environment and reduce the risk of stress. Each rat was housed in a comfortable cage with adequate ventilation and maintained at room temperature. The cages were equipped with husk bedding along with food and water containers. The animals were fed 551 pellets ad libitum (Azhar et al., 2022)

### Alloxan induction

The alloxan solution was prepared by dissolving alloxan powder in NaCl 0.9%. The dosage of alloxan used in this study was 120 mg/kgBW. The rats were fasted for 8 hours prior to the intraperitoneal administration of alloxan at the predetermined dose. Three days after treatment, each rat underwent blood glucose measurement following another 8-hour fasting period. The rats in this investigation had more than 200 mg/dL of blood glucose.

### Experimental Design

This study employed a completely randomized design (CRD) of 5 treatment groups with 5 replications each. Prior to alloxan induction, the rats were fasted for 8 hours. After 72 hours, blood glucose levels were measured. Rats with blood glucose levels ≥200 mg/dL were considered diabetic. Subsequently, the rats received treatment with *Etlingera elatior* flower extract (EEF) according to Table 1. The extract was administered

orally, once daily, for a duration of 21 days (Delfita et al., 2021).

**Table 1.** Experimental treatments

| Group | Treatment |
|---|---|
| K(-) | Un-induced, but given standard feed and water |
| K(+) | Given alloxan 120 mg/kgBW + glibenclamide 0.09 mg/kgBW |
| P1 | Given alloxan 120 mg/kgBW + EEF 100 mg/kgBW |
| P2 | Given alloxan 120 mg/kgBW + EEF 200 mg/kgBW |
| P3 | Given alloxan 120 mg/kgBW + EEF 400 mg/kgBW |

### Creatinine Level Measurement

The determination of serum creatinine levels was carried out using the Jaffe method, which involves spectrophotometric analysis to observe the color change resulting from the reaction between creatinine and specific reagents in the serum. This process enables the quantification of creatinine concentration in each sample (Melisa et al., 2022).

### Kidney Damage Score

Histological slides stained with hematoxylin-eosin (HE) were examined under a light microscope at 400× magnification (Delfita et al., 2021). Observations were carried out across five microscopic fields, with 20 cells evaluated in each field, resulting in a total of 100 cells per slide. Cells exhibiting necrosis were counted and converted into percentages to facilitate damage scoring. A score of 0 was assigned if no necrotic cells were observed (normal category); a score of 1 was given if necrotic cells accounted for ≤50% (mild damage); a score of 2 for 51–70% necrotic cells (moderate damage); and a score of 3 if necrosis involved 71–100% of the observed cells (severe damage) (Melisa et al., 2022).

### Data analysis

Statistical analysis was performed using SPSS version 26. The creatinine levels data were analyzed through one-way analysis of variance (ANOVA) with a significance level set at $\alpha = 0.05$. If significant differences were observed, further comparison was conducted using Duncan's Multiple Range Test (DMRT). Meanwhile, the degree of kidney tissue damage was assessed using the Kruskal–Wallis non-parametric test at a 95% confidence interval, followed by the Mann–Whitney U test for post hoc pairwise analysis.

## RESULTS AND DISCUSSION

### Creatinine Levels

The observational data on creatinine levels in white rats are presented in Table 2.

**Table 2**. Mean Creatinine Levels of White Rats (Rattus norvegicus).

| Treatement Groups | Creatinine Levels ± STEDV |
|---|---|
| K (-) | $0.562 \pm 0.047^{a}$ |
| K (+) | $0.724 \pm 0.048^{b}$ |
| P1 | $0.734 \pm 0.09^{b}$ |
| P2 | $0.716 \pm 0.053^{b}$ |
| P3 | $0.622 \pm 0.037^{a}$ |

Note: Identical superscript letters within the same column indicate no significant difference between the control and treatment groups ($p \geq 0.05$), while different superscript letters indicate a statistically significant difference between the control and treatment groups ($p < 0.05$).

Table 2. presents the mean serum creatinine levels of white rats across different treatment groups, showing statistically notable variations. The results of the study demonstrated that, when compared with the negative control group (K-/without treatment), administration of glibenclamide at 0.09 mg/kgBW (K+) did not significantly reduce creatinine levels in alloxan-induced white rats. Similarly, the treatment group P1 showed no significant effect compared with the K- group, indicating that administration of torch ginger flower extract (EEF) at 100 mg/kgBW was ineffective in lowering creatinine levels. The administration of EEF at 200 mg/kgBW (P2) also yielded comparable outcomes to P1. In contrast, the P3 group, which received EEF at 400 mg/kgBW, exhibited a significant reduction in creatinine levels in alloxan-induced white rats when compared with the P1 and P2 groups.

### Kidney Histopathology

The observational data on renal necrosis tubular in white rats are presented in Table 3.

**Table 3**. Mean Renal Necrosis Tubular of White Rats (*Rattus norvegicus*).

| Treatement Groups | Necrosis Tubular ± STEDV |
|---|---|
| K (-) | $1 \pm 0.0^{a}$ |
| K (+) | $2 \pm 0.707^{b}$ |
| P1 | $1.8 \pm 0.447^{b}$ |
| P2 | $1.4 \pm 0.547^{ab}$ |
| P3 | $1 \pm 0.0^{a}$ |

Note: Identical superscript letters within the same column indicate no significant difference between the control and treatment groups ($p \geq 0.05$), while different superscript letters indicate a statistically significant difference between the control and treatment groups ($p < 0.05$).

Table 3. presents the differences in the level of renal tubular cell damage among the treatment groups. The Kruskal–Wallis test yielded a p-value of 0.012, indicating a statistically significant difference ($p \leq 0.05$). Based on the Mann-Whitney test analysis, it was found that there were significant differences in the degree of renal tubular damage among the groups. The results of the study demonstrated that, when compared with the normal group (K-), administration of glibenclamide at 0.09 mg/kgBW (K+) had no effect in reducing the degree of renal tubular necrosis in alloxan-induced rats.

Similarly, administration of torch ginger extract (EEF) at 100 mg/kgBW did not reduce tubular necrosis compared with the normal group (K-). A different outcome was observed in the P2 group, that at 200 mg/kgBW was able to lower the degree of renal tubular necrosis compared with the normal group (K-). However, the effect was not significant when compared with K+ and P1, indicating a moderate influence at this dose. In contrast, the P3 group, which received EEF at 400 mg/kgBW, demonstrated a significant reduction in renal tubular necrosis when compared with K+, P1, and P2.

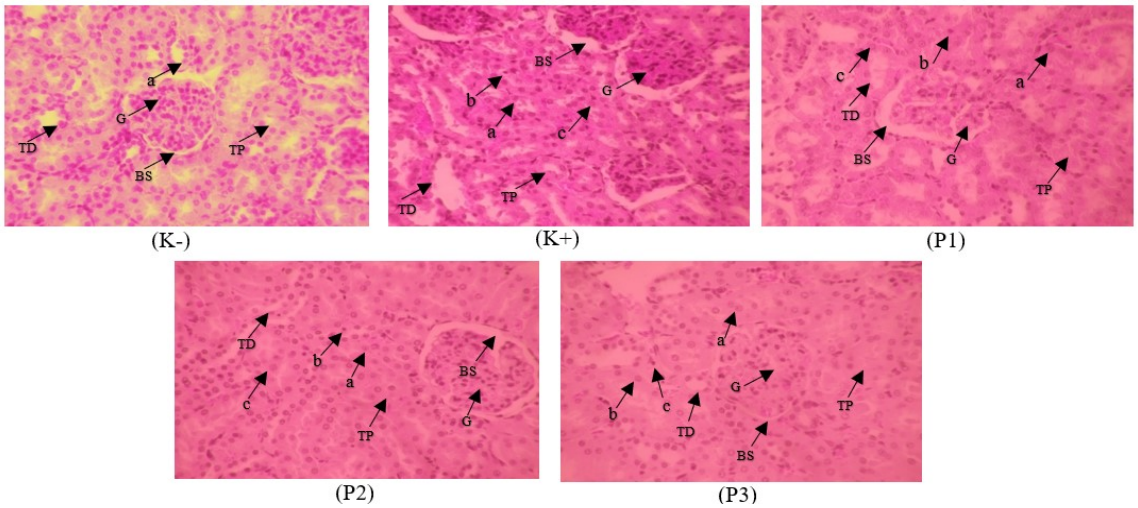


**Figure 2.** Histological appearance of white rat kidney tissues across treatment groups stained with Hematoxylin-Eosin (HE) at 400× magnification. Glomerular (G), Bowman's Space (BS), Proximal Tubule (PT), Distal Tubule (DT), normal cells (a), pyknotic nuclei (b), and karyorrhexis (c).

The average percentage of tubular necrosis in treatment groups K- (23.8%), P2 (49.5%), and P3 (37.2%) was below 50%, corresponding to a score of 1 and classified as mild damage. The K+ group exhibited a necrosis percentage of 59%, falling between 51% and 70%, thus receiving a score of 2, which indicates moderate damage. Similarly, group P1 showed an average necrosis percentage of 54% and was also assigned a score of 2, indicating a moderate level of injury. These findings suggest that administration of torch ginger flower extract at doses of 200 mg/kgBW and 400 mg/kgBW effectively reduced the degree of tubular necrosis to levels approaching normal.

## Discussion

Based on Table 2, the results of the Analysis of Variance (ANOVA) indicated that the treatments administered had a statistically significant effect on creatinine levels ($p<0.05$). The findings showed that the negative control group (K-) had an average serum creatinine level of 0.562 ± 0.047 mg/dL, which suggests normal kidney function without any decline in renal performance. In contrast, the groups induced with alloxan demonstrated elevated creatinine levels, indicating impaired kidney function as a result of alloxan toxicity. This is consistent with Attama et al. (2023), who reported that administration of alloxan in experimental animals resulted in an average creatinine level of 3.00 ± 0.15 mg/dL. Similarly, Ariani et al. (2024) observed that alloxan induction increased serum creatinine levels to 0.9275 ± 0.020 mg/dL. According to Sekiou et al. (2021), administration of alloxan elevated serum creatinine to 0.862 ± 0.07 mg/dL. This increase is likely attributed to the formation of Advanced Glycation End-Products (AGEs), which in turn trigger the generation of Reactive Oxygen Species (ROS). These ROS interact with polyunsaturated fatty acids (PUFAs) in kidney cell membranes, promoting lipid peroxidation and increasing malondialdehyde (MDA) levels. The resulting oxidative damage affects the glomeruli and renal tubules, compromising the kidneys' ability to filter creatinine efficiently.

The positive control group (K+), which received alloxan in combination with glibenclamide, showed a statistically significant difference compared to the negative control group (K-), with an average creatinine level of 0.724 ± 0.048 mg/dL. This suggests that glibenclamide alone was not sufficiently effective in providing renal protection. Delfita et al. (2021) similarly reported that administration of glibenclamide at 0.45 mg/kgBW for 21 days in alloxan-induced animals resulted in a reduction of creatinine to 1.12 ± 0.13 mg/dL, which still differed significantly from the normal control group. The average creatinine level in the positive control group (1.12 mg/dL) remained statistically comparable to the alloxan-only group (1.38 mg/dL), suggesting limited protective efficacy.

Treatment groups receiving torch ginger (Etlingera elatior) flower extract (EEF) showed varying responses. Groups P1 (alloxan + EEF 100 mg/kgBW) and P2 (alloxan + EEF 200 mg/kgBW) exhibited significantly

different creatinine levels compared to the negative control group (K-), with relatively high average values and no significant difference from the positive control group (K+). These findings indicate that the 100 mg/kgBW and 200 mg/kgBW doses were insufficient to restore renal function within the 21-day treatment period.

The most effective treatment in reducing serum creatinine was observed in group P3, which received the highest dose of the extract (400 mg/kgBW). This group showed no significant difference in creatinine levels compared to the negative control group, with an average of 0.622 ± 0.037 mg/dL, suggesting a nephroprotective effect of the extract against alloxan-induced kidney damage. This protective effect is likely due to the antioxidant properties of torch ginger, particularly its flavonoid content. Widyarini et al. (2022) demonstrated that administering torch ginger flower extract can reduce creatinine levels and repair cellular damage in renal tissues. The antioxidant activity of the extract neutralizes free radicals, helping to mitigate oxidative stress and inflammation in the kidneys. Flavonoids within the extract act by donating electrons to neutralize ROS and also inhibit the activity of peroxidase enzymes, thereby reducing further ROS formation (Ariani et al., 2024).

Based on Table 3, the Kruskal–Wallis test result yielded a p-value of 0.012, indicating a statistically significant difference ($p \leq 0.05$). Based on the Mann-Whitney test analysis, it was found that there were significant differences in the degree of renal tubular damage among the groups. Attama et al. (2023) reported that administration of alloxan in experimental animals led to tubular epithelial degeneration and necrosis. Similarly, Delfita et al. (2021) observed that alloxan induced tubular damage in diabetic rats, characterized by loss of the brush border (<25% and >25%), thickening of the basement membrane, inflammation, cast formation, and necrosis reaching up to 60% or even higher. Figure 2 illustrates the histological differences between the negative control group (normal) and the groups treated with alloxan (K+, P1, P2, and P3), which exhibited signs of necrosis, including pyknosis and karyorrhexis. Additionally, a noticeable widening of Bowman's space was observed in the alloxan-induced groups.

Alloxan induction at a dose of 120 mg/kgBW triggers an increase in free radical production and disrupts antioxidant defense mechanisms, ultimately leading to oxidative stress. This condition can damage various body tissues, with the kidneys particularly vulnerable. As the primary excretory organ, the kidneys are highly susceptible to oxidative damage due to their high blood perfusion and the abundance of mitochondria in renal cells—particularly in the proximal tubules, where solute reabsorption is an energy-intensive process. Free radicals are largely generated through the activity of the enzyme NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase. Excessive production of reactive species such as lipid peroxides and nitric oxide, accompanied by a decline in antioxidant enzymes like glutathione peroxidase (GSH-Px), results in an imbalance that drives oxidative stress (Delfita et al., 2021). Prolonged oxidative stress contributes to inflammation and further dysfunction in renal tissue, exacerbating tubular cell degeneration (Gordon et al., 2025).

The K+ treatment group (alloxan + glibenclamide) exhibited the highest degree of tubular necrosis, with a necrosis percentage of 59%, which falls under moderate damage and showed a statistically significant difference compared to the control group (K-). These findings indicate that glibenclamide administration was insufficient in providing a nephroprotective effect, despite its known efficacy in reducing blood glucose levels. This is supported by Delfita et al. (2021), who reported that glibenclamide at a dose of 0.45 mg/kgBW did not produce a significant difference in tubular necrosis compared to the negative control group (alloxan-induced).

Administration of torch ginger (*Etlingera elatior*) flower extract (EEF) at varying doses demonstrated differential protective effects. Group P1 (alloxan + EEF 100 mg/kgBW) was categorized under moderate damage, with a necrosis percentage of 54.9%, indicating that this dose was not sufficiently effective in exerting nephroprotective effects within the 21 days. In contrast, group P2 (alloxan + EEF 200 mg/kgBW) was classified under mild damage with a necrosis percentage of 49.5%. The most favorable outcome was observed in group P3 (alloxan + EEF 400 mg/kgBW), which exhibited the lowest degree of necrosis at 37.2%, approaching normal conditions. This group was also classified under mild damage, suggesting that the 400 mg/kgBW dose was the most effective in reducing tubular injury induced by alloxan.

The reduction in renal tubular damage may be attributed to the antioxidant activity of compounds present in torch ginger (*Etlingera elatior*) extract. Antioxidants function within mitochondria to suppress the formation of reactive oxygen species (ROS). ROS generation typically occurs due to electron leakage in the mitochondrial electron transport chain, which allows free electrons to react with molecular oxygen. Antioxidants such as flavonoids help stabilize these free radicals by converting them into more stable compounds, owing to the high reactivity of their hydroxyl groups (Putri, 2021). Flavonoids are bioactive compounds that support vascular health and exhibit significant anti-inflammatory effects. Their mechanism of action involves direct scavenging of free radicals, thereby inhibiting ROS formation and minimizing oxidative damage at the cellular level (Sholihah & Qomariyah, 2021). Additionally, flavonoids exert their protective role by inhibiting the activity of enzymes such as xanthine oxidase and NADPH oxidase, thus preventing redox reactions that lead to excessive free radical production, and consequently supporting cellular repair (Ariani et al., 2024).

## CONCLUSIONS

Based on the findings, it can be concluded that the administration of torch ginger extract (*Etlingera elatior*) significantly contributes to the reduction of serum creatinine levels and the extent of tubular necrosis in white rats (*Rattus norvegicus*) induced with alloxan. Among the tested doses, 400 mg/kgBW was the most effective in exerting a nephroprotective effect, as evidenced by creatinine levels approaching normal values and improvements in kidney tissue histology.

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***Competing Interests:*** The authors declare that there are no competing interests.

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