

Effect *Ulomoides dermestoides* extract to TNF- α expression and kidney histopathology in diabetic rat model

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ABSTRACT: Type 1 diabetes mellitus is a degenerative disease that often occurs, that is caused by impaired insulin production and damage to the cell β pancreas. This disease is characterized by increased levels of glucose in the blood, which interferes with insulin production in the body. *Rattus norvegicus* was used as an animal model of diabetes mellitus with streptozotocin 20 mg/kg and five experimental groups with three therapeutic doses of 2.3 mg/kg, 4.6 mg/kg, and 9.2 mg/kg. The total flavonoid equivalent contained in *Ulomoides dermestoides* was 1.48 g/mL. Immunohistochemistry results showed therapeutic doses of 2.3 mg/kg and 4.6 mg/kg can reduce TNF- α expression in kidney tissue, but a dose of 9.2 mg/kg had no effect when compared to the positive control. Necrosis and protein deposits were seen in the renal tubules in the four treatment groups compared with the negative control. A dose of 2.3 mg/kg was the best treatment with high TNF- α expression and can repair kidney tissues.

Keywords:

diabetes mellitus type 1, histopathology renal, *Rattus norvegicus*, renal tubular necrosis, streptozotocin.

■ INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by increased blood glucose levels which is often called hyperglycemia. Type 1 is caused by an inflammatory reaction in the pancreas called insulinitis. Inflammation occurs as a result of mediators of the immune response of T-lymphocytes (Matthew C 2020). *Ulomoides dermestoides* is a darkling beetle native to Papua and the Indomalaya region. It used in traditional medicine to cure asthma, arthritis and diabetes (Crespo *et al.* 2011).

Streptozotocin is a diabetogenic agent that is applied in diabetic animal models for research. Streptozotocin inhibits insulin secretion and induces necrosis in pancreatic β cell (Hikmah *et al.* 2015). Diabetes was the main focus of this study, using adult beetle therapy's stimulation of pancreas regeneration and *U. dermestoides* effects on glycemia. So that *U. dermestoides*, which is still little studied in Indonesia, can be used as a potential treatment for diabetes.

■ MATERIALS AND METHOD

Animal Preparation: This experimental using 20 male *Rattus norvegicus* wistar strain with body weight 195 + 5g. Treatment groups used different doses of 2.3 mg/kg, 4.6 mg/kg, 9.2 mg/kg. *Rattus norvegicus* was injected with multiple low dose streptozotocin 20 mg/kg intraperitoneally for five days. ***U. dermestoides* Extraction:** *U. dermestoides* extraction used maceration method from the 6 grams of *U. dermestoides* which is extracted 20 mL of liquid extract. **Sampling of Kidney tissue:** Euthanization was performed

by cervical dislocation, necropsy and isolation kidney. **Histopathology preparation with Hematoxylin and Eosin staining:** Kidney histopathology preparation begins with fixation, followed by filtration using liquid paraffin and then embedding. Hematoxylin and eosin staining with deparaffinization using xylol, rehydration using ethanol, and soaking in hematoxylin for 1 minute and eosin for 5 minutes according to Hwitzen and Darby (2010) protocol. **Immunohistochemistry staining:** Deparaffinization was carried out using xylol and rehydration using ethanol with graded concentrations. TNF- α primary and secondary antibodies were added and mixed with tris buffer and H₂O₂. The procedures were based on Pantin-Jackwood (2014) method. **Data analysis:** The histopathologic features of the kidneys were analyzed qualitatively.

■ RESULT AND DISCUSSION

Immunohistochemistry of TNF- α expression in the kidney of diabetic rat model

The TNF- α and kidney tissue appear to provide an immunological response, according to immunohistochemistry (Fig. 1). The presence of brownish color indicated a higher TNF- α antibody binding reaction in kidney tissue. Increased expression of TNF- α is a response to the high tissue inflam-

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mation that occurred in the diabetic states that showed in the positive control. *U. dermestoides* treatment showed a good recovery with dose of 2.3 mg/kg.

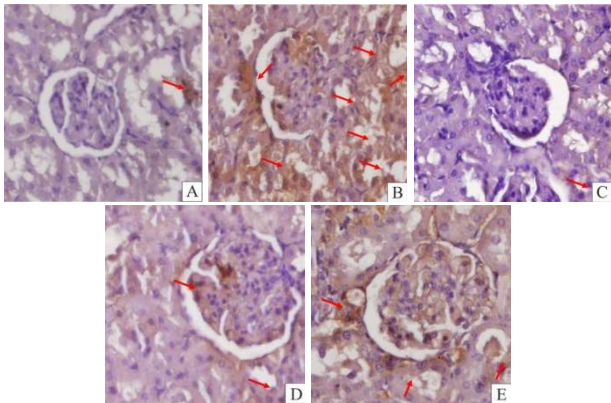


Figure 1. Renal tissue of *Rattus norvegicus* induced streptozotocin after administration of *U. dermestoides* (immunohistochemistry staining). A) negative control; B) positive control; C) dose of 2.3 mg/kg; D) dose of 4.6 mg/kg; E) dose of 9.2 mg/kg. Immunohistochemical reactions caused brownish color (→) of the tissue indicating TNF- α expression.

Histopathology on the kidney *Rattus norvegicus* model of type 1 diabetes mellitus

The kidney histopathological examination using H&E staining was performed to detect abnormalities in tubular epithelial cells. The prevalence of protein deposits in the tubules and the morphological pattern of tubular epithelial cells were seen during examination of the kidneys.

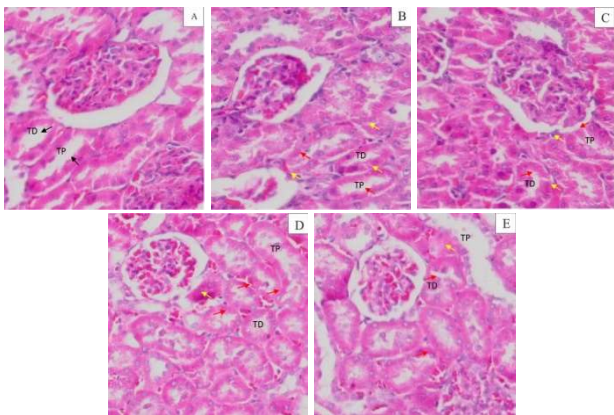


Figure 2. Renal tissue from *Rattus norvegicus* induced streptozotocin after administration of *U. Dermestoides* (H&E stain). A) negative control; B) positive control; C) Dose of 2.3 mg/kg; D) Dose of 4.6 mg/kg; E) Dose of 9.2 mg/kg; (→) normal renal tubular epithelium; (→) renal tubular necrosis; (→) renal tubular protein deposits.

In the negative control of the group (Fig. 2A), there was no necrosis of the tubules indicated by the fact that the epithelial and interstitial boundaries were still clearly visible. Whereas, in the positive control of the group (Fig. 2B) the epithelial and intercellular (interstitial) boundaries were invisible due to the presence of protein deposits in the lumen. This peroxidation affects the membrane cross-linking, the membrane structure, and the fluidity of interstitial cells in the regulation of osmolarity (Akash *et al.* 2018).

A group of rats induced by streptozotocin and given *U. dermestoides* extract at a dose of 2.3 mg/kg (Fig. 2C) revealed cell regeneration with marked cell boundaries and

decreased necrosis. Cell regeneration is also present in Fig. 2D, but not as clear as in Fig. 2C, necrosis is still shown and the intercellular spaces are not visible. While the group of Fig. 2E, showed necrosis and epithelial cell damage. This indicates that 2.3 mg/kg is the effective dose for therapy.

Necrosis in the cortex area was observed in the kidney histopathologically. Protein coagulation causes a partial conversion of tissue into an eosinophilic mass, which is known as coagulative necrosis. Protein accumulation appears to be less common morphologically, but there is an increase in the reabsorption of protein pinocytosis in disorders with substantial protein leakage through kidney filtration. The presence of protein accumulation in the lumen increases glomerular capillary permeability so that protein molecules can penetrate the filter the tubule epithelial cells are very sensitive to toxins (Mapuskar *et al.* 2019; Tonnus *et al.* 2019).

CONCLUSION

The results revealed that the administration of *Ulomoides dermestoides* extract can reduce TNF-expression and inhibit renal tubular necrosis in *Rattus norvegicus* induced by streptozotocin with an effective dose of 2.3 mg/kg.

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