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Pancreatic Protection Effects of Butterfly Pea (*Clitoria Ternatea*) Flower Extract Against White *Rattus Novergicus* Induced By Alloxan

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ABSTRACT

The pancreas is an endocrine gland that produces the peptide hormones insulin, glucagon, and somatostatin, and also an exocrine gland that produces digestive enzymes. These hormones play an important role in regulating the body's metabolic activities, especially blood glucose homeostasis. The aim of this study was to investigate the ethanolic extract of butterfly pea flower (*Clitoria ternatea* L) against white rats (*Rattus novergicus*) induced by alloxan and to I the level of its damage. Fifteen male wistar rats (150-200 g) body weight (BW) were divided into 5 groups. The control healthy group, the placebo group and the extract treated group that received 150 mg/kg BW (group I), 300 mg/kg BW (group II) and 450 mg/kg BW (group III) orally. Placebo (Na-CMC) and extract were given for 5 consecutive days before alloxan administration (150 mg/kg BW) on days 6 and 7. Blood samples were taken to determine the glucose and albumin levels on day 0 (before treatment), on day 5 (after treatment of the extract but before the administration of alloxan), and on 6 and 7 after 24 h administration of alloxan. Alloxan induced gives a significant increase in glucose and albumin levels. The average of glucose level was 236.6±37.6 mg/dl in the health control, 252±28.1 mg/dl in the placebo group, 137±35.5 mg/dl in the extract of group I, 115.9±19.1 mg/dl in the extract of 300 mg of group II and 145.2±58.6 mg/dl of group III. Meanwhile the average of albumin level in the healthy group was 3.39±0.8 g/dl, the placebo group was 2.7±0.2 g/dl, the group I was 1.9±0.04 g/dl, the group II was 1.77±0.16 g/dl, and the group III was 1.85±0.19 g/dl. This study showed that at highest dose of *C. ternatea* was able to significantly prevent the elevation pancreatic damage biomarkers and this finding was associated with the result of histopathological analysis of the pancreas. These results suggest that the ethanol extract of *C. ternatea* at a dose of 450 mg/kg BW (group III) has a good protective effect on the function and structure of pancreatic tissue.

Keywords: Pancreas; Protective effect; *Clitoria ternatea;* Alloxan.

INTRODUCTION

Diabetes mellitus (DM) is considered as a main challenge in both developing and developed countries, as lifestyle has changed and its management seems to be vital (Entezari M, *et al*., 2021). This chronic metabolic disease appears to have an increasing trend in coming years, as lifestyle has changed a lot in modern world. Currently, DM is considered as a life-threatening disease and it has various complications such as retinopathy, nephropathy, neuropathy and infertility as well as cardiovascular diseases that should be addressed in DM treatment (Beckman, 2002; Forbes,

2013). These complications seriously threaten the life of diabetes mellitus patients. Diabetes mellitus can be mainly divided into type 1 diabetes mellitus (T1DM) (~5%) and type 2 diabetes mellitus (T2DM) (~95%) according to the pathology features (Vatere, 2014; Shrestha, 2014).

Diabetes Mellitus is a metabolic disease with characteristics form of hyperglycemia caused by disturbances in insulin secretion, work insulin or both (American Diabetes Association, 2013). Overall, DM is categorized into two types including type I diabetes (T1D) and type II diabetes (TIID). There are significant differences between TID and TIID. In TID, β cells of pancreas are destructed and it is considered as an autoimmune disease, leading to insulin secretion interference. However, the insulin levels are high in TIID and cells are resistance to insulin (Ashrafizadeh, 2019; Yaribeygi, 2020).

The pathology of T1DM is insulin deficiency own to the apoptosis and loss of insulin-secreting pancreatic β-cells that are destroyed by the T and B cells of the autoimmune system (Santin, 2013; Elzirik, 2009). So far, a cure for T1DM is almost not available. T2DM is a complex metabolic disorder featured by insulin resistance in liver and muscle tissues, and excessive hepatic glucose production associated with inappropriately high level of glucagon (Zhang, 2019; Stumvoll, 2005). Chronic hyperglycemia further impairs pancreatic β-cells, which induces the deterioration of the disease (Haythorne, 2019; Aguayo, 2018).

Clitoria ternatea, commonly known as butterfly pea, is an herbaceous perennial climber plant (Mukherjee, 2008). The *C. ternatea* flower is rich in blue anthocyanin, and possesses numerous benefits for humans' health and well-being. The chemical composition induce *C. ternatea* flower to possess beneficial properties such as a therapeutic agents due to its bioactive compounds are responsible as antidiabetic, anti-cholesterol, antidepressant, anticonvulsant, memory enhancing, anti-inflammatory, and antioxidant activities(Jeyaraj, 2021; Rahman, 2006). In addition to their function as antioxidants, flavonoids can also aid cell signaling, which has biological effects to modulate signaling pathways in cells or signal transduction pathways (El-Shafe et al, 2015). The antioxidant potential of *Clitoria ternatea* extract with flavonoid content is reported to be able to inhibit lipid peroxidation, so that it can counteract free radicals in the body (Francenia S, 2019). According to research that has been done (Al Sanafi, 2016), C. ternatea flower contains chemical compounds such as tannin, carbohydrat, saponin, terpenoid, phenol, anthraquinon, anthoxyanin, cardiac glycosid, stigmast-4-ene-3, 6-dione, essential oil and steroid.

METHODS

Materials

The flower of *Clitoria ternatea* were obtained from Sumberejo district of Blitar. Male rats (Wistar strain) were provided and cared for in Biopharmacy Laboratory, Faculty of Pharmacy, Hasanuddin University. Other chemicals, such as ethanol 70%, alloxan, ether, sodium carboxymethyl cellulose (Na CMC) and formaldehyde 10% were purchased from local chemical distributors.

Animal preparation

Fifteen male rats, 2-3 months of age with 150-200 grams of weight, were used in this study. The rats were ensured to have no anatomical abnormalities and show no sign of illnesses. The rats were caged with a 12-hour lighting cycle and were given standard food and drink ad libitum. This study has been approved by the health research etichs committee Sekolah Tinggi Ilmu Farmasi Makassar with the protocol number of 065/EC.1.1.B/V/KEPK/2022.

Sample Preparation And Extraction

The flowers of *Clitoria ternatea* were sorted and washed with running water until clean, then cut into small pieces and dried. As much as 500 grams of dry powder of *Clitoria ternatea* flowers was put into a maceration vessel. Ethanol (70%) was added with a ratio of 1:7.5 then left for 3 days while stirring

occasionally. After 3 days, the extract was filtered using flannel cloth, and the residue obtained was re-macerated with 70% ethanol. The treatment was repeated until the solvent was colorless. The yield obtained was collected and evaporated with a rotary evaporator to obtain a thick extract.

Experimental Procedures

Rats were divided into 5 groups, including healthy control group, placebo group, and 3 extract treatment groups that received either 150 mg/kg, 300 mg/kg or 450 mg/kg dose. The placebo (Na-CMC) or extract was given in 5 consecutive days prior to alloxan (150 mg/kg) administration on day 6 and day 7. Blood samples were withdrawn before treatment was initiated (day 0), after treatment before alloxan administration (day 6) and 24-hour after alloxan administration (day 7). The rat's blood samples were taken via the lateral vein. These blood samples were analyzed to obtain the baseline, post-treatment and post-alloxanl induction levels of glucose and albumin. Following blood withdrawal, the rats were euthanized with cervical dislocation and the pankreas were removed.

Blood Analysis

The collected blood was placed in tubes containing EDTA and then centrifuged at a speed of 2500 rpm for 15 minutes. The serum was separately collected from the blood cells, placed into Eppendorf tubes and stored in the refrigerator (4^{o}C) until analyzed. The creatinine and urea analysis were performed based on kit's instruction as previously described in Djabir et al (2021) study.

Histopathological Analysis

The specimens of pankreas organs were immediately fixed with 10% formalin buffer and were cut into a thickness of 0.5-1 cm. The embedding cassette containing the cut specimen was processed on a tissue processor. When the specimen was ready to embed in paraffin, it was thinly sliced using a microtome with a thickness of 4-5 m. The staining process was carried out using Mayer's hematoxylin and eosin. Histopathological analysis of rat kidney was measured qualitatively using the Mitchel method (2001) and the parameters observed including the level of inflammatory cell, vacuolysis and pancreas cellular necrosis.

Statistical Analysis

The statistical analysis was performed using spss 25 software. The normality of the data is tested using a shapiro-wilk analysis. If the data obtained were normal, it was continued by the analysis of variance, then followed by post hoc analysis. If the data was not normally distributed, then the data was analyzed using a Kruskall-Wallis analysis, followed by the Mann Whitney U test.

RESULTS AND DISCUSSION

Metabolism is a process that describes macromolecular changes, especially in organic compounds due to biological chemical interconversion. This metabolic process is greatly assisted by enzymes that work specifically. The impact of biochemical metabolism is the formation and decomposition of organic macro molecules such as proteins, fats, carbohydrates to nucleic acids (Wali, 2021).

The presence of pancreas dysfunction can be indicated by a marked increase in plasma glucose and albumin levels. In this study, the glucose and albumin levels of rats before treatment (day 0), after treatment (day 6), and after alloxan induction (day 8) can be seen in Figure 1.

Plasma glucose and albumin levels increase with decreased pancreatic function. Plasma glucose levels are a good indicator of pancreatic injury because the pancreas produces hormones needed for metabolism and utilization of carbohydrates, proteins. glucose and albumin levels in plasma showed higher the level of pancreatic cell damage.

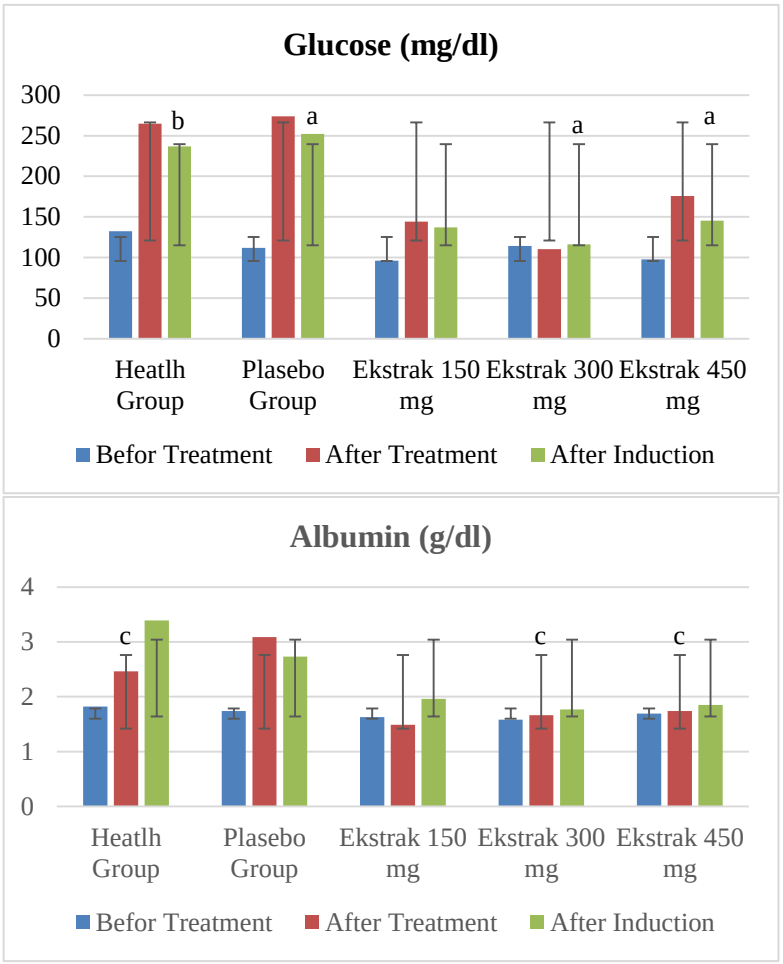
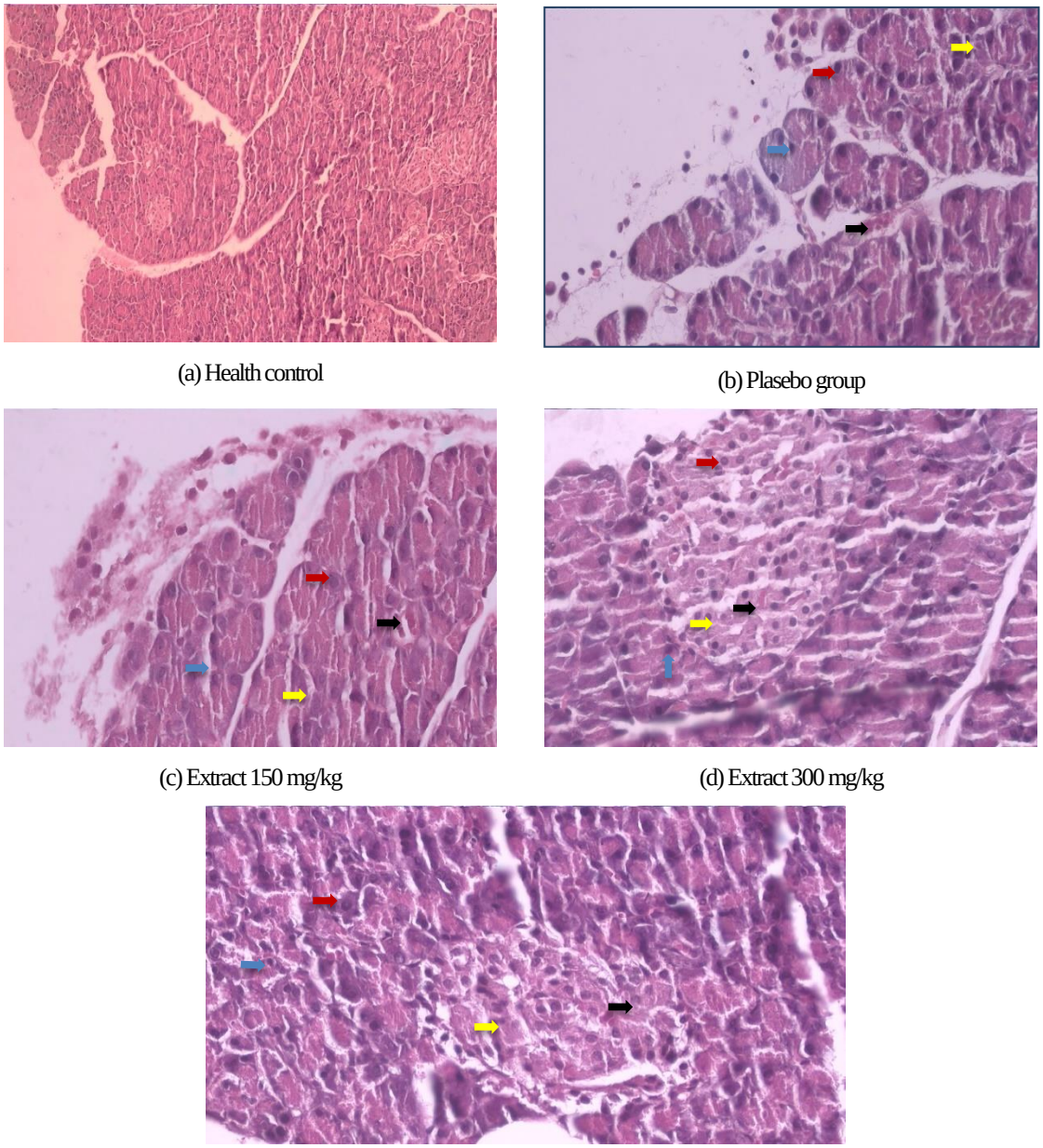


Figure 1. The plasma glucose and albumin levels of rat groups before and after receiving treatments and after induction of alloxan. a: p<0.05 vs placebo group after alloxan induction, b: p<0.05 vs health group after alloxan induction, c: p<0.05 vs health control after treatment.

Table 1. Level of Pancreatic Histological Damage

Group	Damage		
	Degeneration	Necrosis	Inflammation Cell
Healthy Control	0	0	0
	0	0	0
	0	0	0
Placebo Group	1	3	2
	1	3	2
	1	1	1
Ekstract 150 mg/kg	2	2	1
	1	2	1
	0	0	0
Ekstract 300 mg/kg	1	1	0
	3	3	2
	1	1	3
Ekstract 450 mg/kg	1	0	1
	0	0	0
	1	1	0

0 / - = Normal; 1 = < 25% damage; 2 = 26-50% damage; 3 = 51-75% damage; 4 = >75% damage



(a) Health control

(b) Plasebo group

(c) Extract 150 mg/kg

(d) Extract 300 mg/kg

(e) Extract 450 mg/kg

Figure 2. Normal pancreatic cells (➡) appear to be round and have a nucleus in the middle, degeneration pancreatic cells (➡) show the presence of vacuoles of varying size, necrosis cells (➡) are characterized by the loss of cell nucleus and inflammatory cells (➡) appear as blood spots.

From the results, before treatment the mean value of glucose levels was 132 mg/dL in healthy control, 111.7 mg/dL in placebo group, 96.2 mg/dl in extract 150 mg group, 114.3 mg/dL in extract 300 mg/kg group and 97.5 mg/dL in extract 450 mg/kg group. Meanwhile, the average of albumin level in the healthy group was 1.8 g/dL, the placebo group was 1.7 g/dL, the 150 mg extract group was 1.63 g/dL, the 300 mg extract group was 1.58 g/dL and the 450 mg extract group was 1.69 g/dL. From statistical analysis, there was no significant difference on the baseline values among groups. This indicates that the pancreas conditions of all rats were still

normal and has no difference before treatment. After day 8, following aloksan administration at a dose of 150 mg/kg, there was an increase in glucose and albumin levels. This indicates that aloksan is capable of causing pancreas damage at the dose given in rats. This elevation of glucose and albumin was experienced in all treatment groups except for rats receiving Clitori ternatea extract at a dose of 450 mg/kg ($p<0.05$).

The result of the histopathological examination of the pancreas can be found in Table 1. It was found no abnormality or cellular injury in the healthy control as seen in Figure 2. In contrast, in the placebo group, all rats experienced pancreas damage, including cell degeneration, necrosis, and inflammation cell in the pancreas.

At a dose of 150 mg/kg extract, pancreas damage still occurred in almost all rats in the group. The visible damage included degeneration, necrosis and ilnflammation cells with the level of damage ranging from 26-50%. In the extract dose group of 300 mg/kg, the damage was characterized by degeneration, necrosis and inflammation cells with a smaller percentage of damage, ranging from 51-75%.

While in the extract dose group of 450 mg/kg, the damage occurred almost diminished in all rats, although some histological changes were found, such as degeneration, necrosis and inflammation cells. However, the percentage of damage was quite low (<25%), and there was even 1 rat in this group that did not experience abnormalities in the pancreas structure.

The results of glucose and albumin level measurement was supported by the histopathological examination of the pancreas, showing that the protective pancreas effect of the ethanolic extract of C. ternatea flowers was most effective when administered at the dose of 450 mg/kg. This is because the Telang Flower (C. ternatea L.) contains anthocyanin compounds with high antioxidant activity (Al Sanafi, 2016).

CONCLUSION

The ethanolic extract of *C. ternatea* flowers at a dose of 450 mg/kg can protect the function and the histology of the pancreas in the alloxan-induced rats as the glucose and albumin levels decreased, and pancreas tissues were improved compared to the placebo group.

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