DOI: 10.7860/JCDR/2018/35710.12102

Hypoglycemic Effect of 70% Ethanolic Extract of *Tinosporacrispa* L. (*Bratawali*) Stem from Indonesia in Wistar Rat Induced by Alloxan

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ABSTRACT

Introduction: Indonesian people often use *Bratawali* (*Tinosporacrispa* L.) to treat Diabetes Mellitus (DM) empirically. Based on literature, the active compound in the plant is thought to initiate insulin secretion by modulating the β-cell of Ca^{2+} concentration.

Aim: The aim of the present study is to evaluate hypoglycemic effect 70% ethanolic extracts of *Tinosporacrispa* L. (EETC) in Wistar rats induced by alloxan.

Materials and Methods: Twenty five rats were divided into 5 groups. The group I was negative control treated by aqua bidest, group II was positive control treated by glibenclamide at dose of 0.63/kg bw/day, group III was treated by EETC at dose of 500 mg/kgbw/day, group IV was treated by EETC at dose of 1000 mg/kgbw/day and group V was treated by EETC at dose of 2000 mg/kgbw/day. The fasting Blood Glucose Level (BGL) of all rats were measured and then they were injected by alloxan at dose of 150 mg i.p. On fourth day BGL were re-measured. All rats with BGL>200 mg/dL were treated by extract/medicine appropriated their group. This treatment was for ten days. The Fasting BGL between groups was analysed using ANOVA followed by LSD.

Results: The results of this research shows that the EETC at dose of 500 mg/kgbw/day and 2000 mg/kgbw/day can reduce fasting BGL in Wistar rats induced by alloxan significantly ($p<0.05$). The percentage of reduction of fasting BGL at dose of 500 mg/kgbw/day and 2000 mg/kgbw/day are 44.78±5.27 and 43.12±4.25 respectively. EETC at dose of 500 and 2000 mg/kg bw/day allegedly able to repair the pancreatic islet cell.

Conclusion: It is concluded that the EETC reduce fasting BGL in Wistar rats induced by alloxan.

Keywords: Antidiabetic, Diabetes mellitus, Fasting blood glucose level

INTRODUCTION

Diabetes Mellitus (DM) is a disease caused by a metabolic disorder characterized by hyperglycaemia. The causes are multifactorial and include genetic, environmental and lifestyle factors [1].

Indonesia is one of the developing countries in Asia. Number of patients with DM in Indonesia was ranked fourth highest in the world after India, China and the United States. WHO Predicts an increasing number of people with diabetes mellitus from 8.426 million in 2000 to 21.257 million in 2030 [2].

Tinosporacrispa (L.) is a traditional Indonesian medicinal plant commonly planted in the yard or grows wild in the forest [3]. Empirically, Indonesian people often use *Bratawali* (*Tinosporacrispa* L.) to treat DM. Indonesian people use leaves and stems of this plant by boiling. In Indonesia this plant was known as *Bratawali*, *Andawali*, *Antawali* or *Putrawali*. This plant is large and belongs to Menispermaceae family [3].

MATERIALS AND METHODS

This research was experimental study with animal test. The study was performed in Department of Pharmacology of Medical Faculty of Universitas Muhammadiyah, Surakarta, Indonesia from September 2015 to August 2016. The study protocol was approved by the Health Research Ethics Committee of Medical Faculty of University of Muhammadiyah Surakarta with no: 182/A.1/KEPK-FKUMS/I/2016.

Plant Collection

Tinosporacrispa L. was collected from Tawangmangu, Karanganyar, Jawatengah, Indonesia on October 2015. The plant was confirmed by Biology of Universitas Muhammadiyah, Surakarta, Indonesiain November 2015.

Extract Preparation

Tinosporacrispa L. Stems were washed and then dried for two weeks in ambient temperature. A suitable weight of this stem was macerated by 70% ethanol for 4 days. The filtrates were evaporated by vacuum evaporation at Pharmacology laboratory of Faculty of medicine of Universitas Muhammadiyah, Surakarta.

Induction of Diabetes

The method of diabetic induction was conducted by Sutrisna research on 2015 and others research previously [4-8]. The Wistar strain rats were injected by alloxan monohydrate at dose of 150 mg/kg intra peritoneal (i.p). On fourth days, fasting BGL was measured and compared with pre injection of alloxan.

Experimental Design

Twenty five male Wistar rats weighting 175-225 g were brought from animal house of Pharmacology laboratory of faculty of Medicine of Universitas Muhammadiyah, Surakarta. These rats were divided by into five groups of five rats each. The group I was negative control (aquabidest), group II was positive control (treated by glibenclamide 0.63 mg/kg/day), group III (treated by EETC at dose of 500 mg/kg/day), group IV (treated by EETC at dose of 1000 mg/kg/day) and group V (treated by EETC at dose of 2000 mg/kg/day). The fasting BGL of all rats were measured and then they were injected alloxan at dose of 150 mg i.p. This dose refers to our previous research [6]. On day four fasting BGL were re-measured. All rats with fasting BGL ≥200 mg/dL were treated by extract/medicine according to their group. This treatment was done for ten days. On day 15, all rats were measured for fasting BGL and then sacrificed by cervical dislocation. The pancreas was subjected to histopathological examination with Haematoxylin-eosin staining. The observation was done by light microscope with 400x magnifications. Histopathology of pancreas was evaluated qualitatively.

STATISTICAL ANALYSIS

The fasting BGL are presented as mean±SD. Fasting BGL between groups was analysed using ANOVA followed by LSD. The Histopathology of pancreas is evaluated qualitatively.

RESULTS

Effect of EETC on Blood Glucose Level

The effect of EETC on fasting BGL is documented in [Table/Fig-1]. Based on [Table/Fig-1], it can be seen that the fasting BGL increased on day 4. This is caused by alloxan injection. On day 14 (after 10 days treatment), the fasting BGL of all groups reduced.

Groups	Fasting BGL		
	Day 0 (before alloxan injection)	Day 4 (after alloxan injection)	Day 14 (after 10 days of treatment)
Negative control	103.67±6.44	219.00±86.26	159.6±4.68
Positive control	92.50±9.18	202.33±13.73	89.30±11.36*
EECT at dose of 500 mg/kg/day	128.33±14.98	257.33±18.70	103.8±9.15*
EECT at dose of 1000 mg/kg/day	128.67±10.28	148.33±29.65	96.80±8.99
EECT at dose of 2000 mg/kg/day	140.50±10.07	293.67±99.40	121.3±4.79*

[Table/Fig-1]: The effect of EETC on fasting blood glucose level.
Values of fasting BGL are expressed mean±SD.
*significant difference with negative control on p<0.05 by LSD test.

Based on [Table/Fig-1] the fasting BGL on day 14 are analysed by ANOVA test. There is significant difference in fasting BGL with p-value <0.05.

From [Table/Fig-1], the percent of reduction of BGL was calculated.

Groups	The percent of reduction of fasting BGL (%)
Positive control	39.43±4.62
EECT at dose of 500 mg/kg/day	44.78±5.27**
EECT at dose of 1000 mg/kg/day	10.65±2.81
EECT at dose of 2000 mg/kg/day	43.12±4.25**

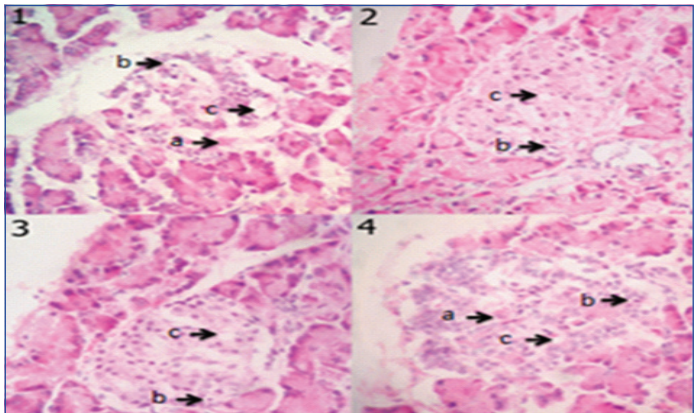
[Table/Fig-2]: The percentage of reduction of BGL.
**: Not significantly difference with negative control on p<0.05 by LSD test.

The result of percent of reduction of BGL can be seen in [Table/Fig-2].

From [Table/Fig-2], it can be concluded that the percent of reduction of BGL EECT at dose of 500 mg/kg BW/day and EECT at dose of 2000 mg/kg BW/day is equivalent to positive control.

The Effect of EETC on Histopathology of Pancreas

After measurement of fasting BGL on day 14, rats were killed by cervical dislocation. The pancreas was taken for histopathology examination with H&E staining. The result of this can be seen in [Table/Fig-3].



[Table/Fig-3]: The histopathological staining by HE of rat pancreas. 1. Negative control group, 2. EETC at dose of 500 mg/kg/day, 3. EETC at dose of 1000 mg/kg/day, 3. EETC at dose of 2000 mg/kg/day. a) Necrosis, b) inflammation cell, c) Vacuole (H&E,400X)

From [Table/Fig-3], It can be seen that it there was no islet cell necrosis in group I & III (EECT at dose of 500 and 2000 mg/kg bw/day). EETC at dose of 500 and 2000 mg/kg bw/day was allegedly able to repair the pancreatic islet cell.

DISCUSSION

The results of this research show that 70% of EETC reduces fasting BGL in Wistar strain rats induced by alloxan. This research uses alloxan monohydrate for induction of diabetes. Alloxan as an inducing agent of diabetes refers to research conducted by several previous studies [4-8].

Based on histopathological results, this extract can repair pancreas islet cell. Various studies have showed that the T. *crispa* reduced blood glucose level in animal test and patient [9-12]. The aqueous extract of *Tinosporacrispa* stems at dose of 4 g/L in drinking water for 2 weeks improve glucose tolerance in alloxan diabetic rats [9]. The research by Sari SI at 2014 stated the tinokrisposid (compound of *T. crispa* stem) at dose of 5, 10 and 20 mg/kg can decrease blood glucose level in diabetic rats significantly (p<0.05) [10]. Research by Umamaheswari S and Prince BM, 2007 demonstrated that the *Tinosporacordifolia* root extract lowers plasma thiobarbituric acid reactive substances hydroperoxides, glycosylated hemoglobin (HbA1c) and ceruloplasmin levels in diabetic rats models [11]. Noipha K et al., found that *T. crispa* extract increases the level of GLUT1, AMPKα, and PPAR transport transporters, thus increasing glucose uptake in L6 myotubes [12]. The treatment with dry powder of *T. crispa* at dose of 250 mg twice daily on patients with metabolic syndrome decreased fasting blood glucose significantly from the baseline [13].

Several compounds suspected in *Tinosporacrispa* are flavones and flavonoids, tannin [14,15], terpenoids [16], and alkaloides [17]. Flavonoids are natural antioxidants that can act as a reducing hydroxyl radicals, superoxide and peroxyl radicals. The mechanism of antioxidants of flavonoid is by capturing the ROS directly and preventing the regeneration of ROS. Indirectly, flavonoids can increase activity of cellular antioxidant enzyme. Flavonoids effectively act as Scavanger reactive species, such as superoxide, peroxyl radicals and peroxynitrite by transferring atomic H + [18]. The mechanism of flavonoid antioxidant is thought to be: a) scavenging ROS, b) inhibiting enzymes involved in ROS formation c) protection of antioxidant defenses [19,20]. Research by Noor H, Ashcroft SJ found that the active compound *T. crispa* L was suspected to initiate insulin secretion by modulating the β-cell of Ca^{2+} concentration [9].

The stems of *T. crispa* L. Contain striterpens. Two triterpenes of *T crispa* L. are cycloeucalenol and cycloeucalenone [21]. Triterpenoids or steroids are compounds that have a role as an antioxidant. The Antioxidant mechanism of triterpenoids is to scavenging of reactive species, such as superoxide, and metal chelate (Fe^{2+} and Cu^{2+}) [22]. Triterpenoids have also the ability to maintain of the stability of liver cell membrane [23].

LIMITATION

The present study only measures the pancreatic histopathological changes. Biochemical changes due to pancreatic damage (serum amylase or lipase) need to be evaluated.

CONCLUSION

EETC at dose of 500 mg/kgbw/day and 2000 mg/kgbw/day can reduce fasting blood glucose level in Wistar rats induced by alloxan. EETC at dose of 500 and 2000 mg/kg bw/day allegedly able to repair the pancreatic islet cell. It is concluded that the EETC can be developed as antidiabetic agent.

ACKNOWLEDGEMENTS

We thank to Universitas Muhammadiyah, Surakarta, Indonesia as funder of this research.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 04, 2018**
Date of Peer Review: **Mar 20, 2018**
Date of Acceptance: **May 26, 2018**
Date of Publishing: **Sep 01, 2018**