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Antihyperlipidemic Activity of Torbangun Extract (*Coleus amboinicus* Lour) on Diabetic Rats Induced by Streptozotocin

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***ABSTRACT** : Torbangun (Coleus amboinicus Lour) contains phenolic compounds, especially quercetin which act as antioxidants. This study aims to determine the antihyperlipidemic activity of torbangun leaf ethanol extract in diabetic rats induced by streptozotocin (STZ). Diabetes was induced in Sprague Dawley rats by single intraperitoneal administration of STZ (50mg/kg body weight). Normal as well as diabetic rats were divided into groups (n=5) receiving different treatments. Graded doses 620 mg/kg b.w. (T1) and 930 mg/kg b.w. (T2) of torbangun leaf ethanol extract were studied for a period of 14 days. Metformin hydrocloride (62.5 mg/kg b.w.) and quercetin (15 mg/kg b.w.) were used as a reference drug and antioxidant standart. Oral administration with graded doses T1 and T2 of torbangun leaf ethanol extract exhibited antihyperglycemic and antihyperlipidemic activity in streptozotocin-induced diabetic rats. Data were analyzed with test followed Post-Hoc test with 95% significance level. The daily oral treatment with torbangun leaf ethanol extract showed a significant reduction in blood glucose. There were decreased serum contents of triglycerides and significantly total cholesterol, whereas HDL-cholesterol was increased. Studies clearly demonstrated that torbangun leaf ethanol extract possesses antihyperlipidemic activity in diabettic rats.*

***KEYWORDS** – antihyperlipidemic, Coleus amboinicus Lour, streptozotocin.*

I. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease and endocrine metabolic disorder wich is characterized by high blood glucose consentration due to the defects of insulin secretion and insulin action or both. It is a growing health problem in most countries and its a incidence is considered to be high (4-5%) all over the world [1]. World Health Organization (WHO) suggests that worldwide the global population is in the midst of diabetes epidemic with people in Southeast Asia and Western Pacific being mostly at risk. The number of cases for diabetes that currently at 171 million is predicted to reach 366 million by year 2030 [2]. Diabetes mellitus is classified in two types, insulin dependent diabetes mellitus (IDDM, type 1) and non insulin dependent diabetes mellitus (NIDM, type 2). Type 1 diabetes is an autoimmune disease characterized by local inflammatory reaction in and round islets that is followed by selective destruction of insulin secreting β-cells. Type 2 diabetes is characterized by peripheral insulin resistenace and impaired insulin secretion [3].

Increased production of superoxides and lowered antioxidant enzyme activities compromising with body antioxidant defense systems in hyperglycemia is associated with the pathogenesis of diabetic dyslipidemia, micro- and macrovascular complications. Currently available for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs are not free from side effects [4] .

Plant based medicines are gaining prominence in treatment of metabolic disease like diabetes. Many flavonoid containing plants serve as a hidden wealth of diabetes control [5]. Approaches to the control of diabetes mellitus are aimed at prevention of hyperglycemia involving dietary manipulation and use of plant [6]. Torbangun (*Coleus amboini*cus Lour) is an aromatic shrub widely distributed in Indonesia. The literature survey revealed torbangun leaf extract to have an antioxidant property [7]. The purpose of the research was to evaluate the antihyperlipidemic activity of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract in diabetic rats induced by streptozotocin.

II. MATERIALS AND METHODS

2.1.Plant materials.

Leaves of torbangun (*Coleus amboinicus* Lour) were collected from a herbal garden in Cibeureum Bogor, West Java, Indonesia in the months of January to March 2014. The leaves separated, cleaned, air-dried, coarsely powdered, and subjected for Soxhlet-extraction by using ethanol. Powder weighing 70 g was extracted with 600 ml of 96% ethyl alcohol for 72 h for each batch. The solvent was recovered using rotovapour (Buchi, Switzerland). The semisolid mass obtained was concentrated under reduced pressure and stored in an air tight container [8].

2.2. **Chemistry :**

Streptozotocin (STZ) purchased from Sigma-Aldrich Chemie (Germany). Quercetin: extraced from Sophora Japonica L, was optained from Shanghai Seni Pharma-Tech Co, Ltd (Chika). Metformin Hydrochlorida, purchased from Sigma-Aldrich Chemie (Germany).

2.3. Animal

Healthy male *Sprague-Dawley* rats (180-200 gr) breed in BPOM Jakarta-Indonesia. Animals were kept at room temperature (26 ± 2^{o}C) with 12 h light/dark cycle for 1 week to acclimatize to laboratory conditions before starting the experiment. They were given free access to food with standart diet and water *ad libitum*.

2.4. Induction of diabetes in experimental animals.

The animals were fasted overnight and diabetes was induced by a single intra peritoneal (ip) injection of a freshly preparat solution of Streptozotocin (STZ) 50 mg/kg b.w. in 0.1 M sitrat buffer (pH 4.5). To avoid an early fatal hypoglycemic state, a 5% glucose solution was fed for 1 day to all the animals. Blood glucose of all rats was estimated after 48 hours. Rats with fasting blood glucose level of 220-415 mg/dl were considered as diabetic and selected for further study[9].

2.5. Experimental design

A total of 30 rats were divided into six groups of five animals each. The daily oral treatments using suspension of each extract were continued for 14 days, with destilled water serving as the vehicle. Suspensions were prepared using 0.3% w/v sodium carboxy methylcellulose in destillat water and used for the experimental purpose. The volume of the suspension or vehicle was 5 ml/kg. All the groups received daily treatment orally between 08.00 and 09.00 h : (i) Group 1, animal control (vehicle treated with 5 ml/kg *b.w.* of destillat water); (ii) Group 2, diabetic control (vehicle treated with 5 ml/kg b.w. of destillat water); (iii) Group 3, diabetic rats treated with torbangun leaf ethanol extract dose 620 mg/kg b.w. (T1); (iv) Group 4, diabetic rats treated with torbangun leaf ethanol extract dose 930 mg/kg b.w. (T2); (v) Group 5, diabetic rats treated with Metformin hydrocloride dose 62.5 mg/kg b.w. [10] ; (vi) group 6, diabetic rats treated with Quercetin dose 15 mg/kg b.w.[11]. At the end of the experimental period, the animals were deprived of food over night anesthesia, blood was collected immediately for biochemical analyses.

2.6. Sample collection

Blood samples were collected from tip of rat tail and blood glucose levels estimated using Gluco-check electronic glucometer (Gluco-check, Delhi) and body weight measured during treatment on weekly basis (i.e. 0, 1, 4, 7, 10 and 14 days). Blood was collected by cardiac puncture and immediately transferred into tubes containing EDTA. Blood was then centrifuged at 6.000 g for 8 min recover serum for the estimation contents of total cholesterol (TC), triglycerides (TGs) and HDL-cholesterol level were estimated using commercial diagnostic kits (Rajawali-Nusindo). All estimations were performed according to the kit manufacturer's instruction [12].

2.7. Statistical analysis:

The results were expressed as the mean±SEM. The results obtained from the present study were analyzed using One-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis using Graph Pad Prism Software. Differences between the data were considered significant at $P<0.05$.

III. RESULTS

Weight loss is a very serious issue in the management of diabetes mellitus may be due to degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose. Table 1 show the body weight of control and experimental animals on 14 days of treatment. Results showed no intra group variation in the basal body weight. There was reduction of

body weight in dose T2 diabetic control animals compared to test drug treated animals. Reduced body weight indicates the induction of diabetes. Oral administration of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract dose T1: 620 mg/kg b.w. to diabetic rats improved body weight significantly (P<0.01) and this could be due to a better control of hyperglycaemic state in diabetic rats. However, body weight in drug treated rats were lower than non diabetic rats [13].

Table 1: Effect of *Coleus amboinicus* Lour leaf ethanol extract on body weight

Group	Initial body weight(g)	Final body weight (g)
N	164.720 ± 13.262a	195.480 ± 18.097a
D	174.940 ± 11.968a	201.680 ± 17.235a
D-T1	171.720 ± 16.692a	188.340 ± 17.832ab
D-T2	170.980 ± 12.791a	161.420 ± 17.029bc
D-Mt	170.420 ± 24.549a	156.980 ± 39.301c
D-Q	167.720 ± 18.115a	162.860 ± 21.266bc
P-value	0.9474	0.015*

Data are expressed as mean ± S.E.M; n=5 animals in each group. Values are statistically significant at *P<0.05. Diabetic control was compared with control rats; Diabetic + torbangun (*Coleus amboinicus* Lour) T1 (620 mg/kg), Diabetic + torbangun (*Coleus amboinicus* Lour) T2 (930 mg/kg), Diabetic + Metformin hydrochloride and Diabetic + Quercetin were compared with diabetic control.

Streptozotocin-induced hyperglycemic rats showed a significant decrease (P<0.05) in blood glucose on the experiment. Table 2 showed oral treatment with extract dose of T1 : 620 mg/kg *b.w.*and T2: 930 mg/kg b.w. Diabetic control rats showed significant elevation (P<0.05) in fasting blood glucose on successive days of the experiment as compared to their basal values, which was maintained over a period of 14 days. Daily oral treatment with torbangun (*Coleus amboinicus* Lour) leaf ethanol extract dose of T1 showed significant reduction (P<0.05) in blood glucose on successive days of the experiment as compared to their basal values.

Table 2. Effect of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract on blood glucose level in diabetic rats

Group	Initial blood glocose (mg/dL)	Final blood glucose (mg/dL)
N	90.800 ± 5.718c	94.800 ± 3.421c
D	62.380 ± 50.381b	172.800 ± 53.336abc
D-T1	221.600 ± 106.971b	112.800 ± 53.336abc
D-T2	340.600 ± 53.017a	245.600 ± 115.833a
D-Mt	404.400 ± 22.345a	213.200 ± 116.294ab
D-Q	333.200 ± 80.819a	242.600 ± 113.015a
P-Value	<.0.001	0.0218*

Data are expressed as mean ± S.E.M; n=5 animals in each group. Values are statistically significant at *P<0.05. Diabetic control was compared with control rats; Diabetic + torbangun (*Coleus amboinicus* Lour) dose T1 (620 mg/kg), Diabetic + torbangun (*Coleus amboinicus* Lour) dose T2 (930 mg/kg), Diabetic + Metformin hydrocloride and Diabetic + Quercetin were compared with diabetic control.

STZ-induced hyperglycemic rats showed a significant elevation (P<0.05) in serum contents of TC, TGs whereas HDL-cholesterol were decreased (P<0.05) as compared to the control group. Increase in concentration of total cholesterol, triglycerides and decreased of HDL is observed in STZ untreated diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus and a risk factor for disease. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia and hypercholesterolemia caused by derangement of metabolic abnormalities [14]. The decreased levels of HDL in diabetic rats might be due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx [15]. Administration of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract and metformin hydrochloride normalized serum lipids, secondary to the diabetic state. Diabetes induced hyperlipidemia is attributable of excess mobilization of fat from the adipose tissue due to the under utilization of glucose.

Table 3 showed oral treatment with doses T1 showed reduction in serum contents of total cholesterol, triglycerides and simultaneously increased the HDL-cholesterol levels as compared to the diabetic control group. Administration of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract at dose T1 620 mg/kg b.w.; dose T2 930 mg/kg b.w. and standard drug metformin hydrochloride, quercetin resulted fall of these serum lipoproteins when compared to diabetic rats. After 14 days treated of the extract, metformin hydrochloride and quercetin, there was a elevation in HDL level in serum and the results were found to be comparable to that of control rats.

Table 3. Effect of torbangun (*Coleus amboinicus* Lour) leaf ethanol extract on lipids level in diabetic rats

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)
N	51.2 ± 16.485^{b}	84.4 ± 8.849^{a}	50.4 ± 7.392^{a}
D	98.4 ± 22.966^{a}	115.2 ± 59.439^{a}	22.2 ± 13.014^{b}
D-T1	66.4 ± 17.534^{b}	78 ± 16.309^{a}	38.2 ± 12.812^{ab}
D-T2	77.4 ± 8.163^{ab}	99.4 ± 24.532^{a}	41.4 ± 13.276^{ab}
D-Mt	76.4 ± 19.085^{ab}	93.6 ± 19.926^{a}	40.4 ± 11.128^{ab}
D-Q	74.6 ± 17.828^{ab}	81.8 ± 21.738^{a}	43.6 ± 14.277^{a}
P-value	0,0275*	0,5231	0,0696

Data are expressed as mean ± S.E.M; n=5 animals in each group. Values are statistically significant at *P<0.05. Diabetic control was compared with control rats; Diabetic + torbangun (*Coleus amboinicus* Lour) dose T1 (620 mg/kg), Diabetic + torbangun (*Coleus amboinicus* Lour) dose T2 (930 mg/kg), Diabetic + Metformin hydrochloride and Diabetic + Quercetin were compared with diabetic control. Lipids levels were expressed as mg/dl.

IV. DISCUSSION

Streptozotocin is well known for its selective pancreatic islets β-cell cytotoxicity and has extensively used to induce diabetes in experimental rat model. It interferes with cellular metabolic oxidative mechanism. Metformin hydrochloride is often used as a standard antidiabetic drug in STZ induced moderate diabetes to compare the efficacy of variety of hypoglycemic compounds or plant extract. The present study was conducted to assess the antihyperlipidemic activity of torbangun (*Coleus amboinicus* Lour) extract in STZ induced diabetic rats. Results shows of torbangun extract dose T1 significantly improving the body weight and controlling the blood glucose level in diabetic rats. Dose T1 is more effective than dose T2 and comparable to metformin hydrochloride and quercetin. There was improvement in their body weights, indicating that the extract had beneficial effect in preventing loss of body weight of diabetic rats.

Results from this work also indicate that the extract, especially the dose T1 620 mg/kg b.w., produced more alleviating effects. This observation confirms the fact that ethanol extract of plants are generally known for their high contents in chemical compounds capable of producing biological activities [16]. With regard to the lowering blood glucose concentrations, it could be proposed that torbangun (*Coleus amboinicus* Lour) may act by (1) stimulating insulin secretion similarly to metformin hydrochloride [17], (2) triggering progressive regeneration of the damaged β-cells after sequential injection of streptozotocin or (3) potentiating glucose uptake and use by various tissues [18]. The improvements observed in the body weight as well as in the relative weights of the pancreas and liver of diabetic animals after plant extract applications further support these proposed pancreatic and extra-pancreatic mechanisms of action of torbangun (*Coleus amboinicus* Lour) [19].

As one of the complications that followed diabetic hyperglycemia is dyslipidemia, the serum lipid profile of rats was evaluated in this study. As expected, untreated diabetic animals showed a significant increase in serum TC and TGs concentrations against low levels of HDL-C [20]. This increase in serum lipids is mainly due to the increased fatty acid mobilization from adipose tissue. Since insulin has an inhibitory action on HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl coenzyme A reductase), the key enzyme in cholesterol biosynthesis [21], insulin deficiency or insulin resistance may therefore be responsible for hyperlipidemia. Treatment of type 2 diabetic rats with torbangun (*Coleus amboinicus* Lour) leaf ethanol extract dose T1 620 mg/kg b.w. reversed although not completely dyslipidemia as evidenced by the significant TC decrease ($p<0.05$) and TGs, whereas the increased in HDL-C. These alleviating effects clearly denote the antihyperlipidemic potential of torbangun [22]. It could also be suggested that this antihyperlipidemic effects of torbangun pass through a decrease in intestinal cholesterol absorption or a decrease in the biosynthesis of cholesterol specifically by decreasing the activity of HMG-CoA reductase inhibitors [23].

V. CONCLUSION

In conclusion, the present study indicates treatment of STZ-treated rats with torbangun (*Coleus amboinicus* Lour) for two consecutive weeks could restore the biotransformation by shifting the balance of lipid and carbohydrate metabolism. The extract showed significant antihyperglicemic with very crucial effects on lipids levels as antihyperlipidemic.

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