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Mob: +92 300 3008585, Fax: +92 41 8815544
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Research Article

Effect of Alkaloid Compound 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline of *Swietenia macrophylla* King Seed on Lipid Profile and Liver Tissue RBP4 Expression on Type 2-diabetes Rats

[1]Prasetyastuti, [2]Singgih Setiawan, [2]Yuwinda Prima Ardelia, [2]Tsurayya Fatma Hanifah, [2]Yunardi Singgo and [1]Sunarti

[1]Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Indonesia
[2]Faculty of Medicine, Universitas Gadjah Mada, Indonesia

Abstract

Background: Retinol Binding Protein 4 (RBP4) is adipocytokine that it's secretion and expression in adipose tissue is highly correlated with glucose uptake and insulin sensitivity. Increasing of RBP4 in type 2-diabetes mellitus correlate with insulin resistence. *Swietenia macrophylla* king contain alkaloid, saponin and flavonoid. **Objective:** The aim of this study is to investigate the effect of 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline alkaloid compound of *Swietenia macrophylla* king seed to lipid profile and liver tissue RBP4 expression in type 2-diabetes rats. **Methodology:** Twenty five adult rattus norvegicus were used, five in each group. Group I: Diabetic rat (untreated), group II: Diabetic rat receive glibenclamid, group III: Diabetic rat receive 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10 mg/200 g b.wt., day^{-1}, group IV: Diabetic rat receive 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 20 mg/200 g b.wt., day^{-1} and group V: Diabetic rat receive 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 40 mg/200 g b.wt., day^{-1}. Blood samples were analysed for blood glucose, cholesterol, low density lipoprotein, triglyceride and high density lipoprotein level before and after 4 weeks treatment. At the end of the experiment sample of liver tissue were removed for immunohistochemical analysis. **Results:** Blood glucose level decreased significantly ($p<0.05$) in group II, III, IV and V. Cholesterol level decreased non-significantly ($p>0.05$), Low Density Lipoprotein (LDL) decreased significantly ($p<0.05$) at dose 40 mg/200 g b.wt. High Density Lipoprotein (HDL) level increased significantly ($p<0.05$) at doses 10 and 20 mg/200 g b.wt. **Conclusion:** However, the expression of Retinol Binding Protein 4 (RBP4) decreased after treatment with 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline. These results demonstrated that 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline increased HDL and decreased LDL and RBP4 expression.

Key words: Diabetes melitus, *Swietenia macrophylla* king, retinol binding protein 4, lipid profile

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Corresponding Author: Prasetyastuti, Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia
Tel: (62+) 81227882379

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder, characterized by hyperglycemia caused severe complicated as microvascular and macrovascular[1]. Insulin resistance and β-cell damage are two mechanism that contribute to DM type-2 pathogenesis[2]. Insulin resistance caused decreased mitochondria function in energy metabolism. Retinol Binding Protein 4 (RBP4) is adipocytokine that its excretion and expression in adipose tissue correlate with glucose uptake and insulin sensitivity. Incresing serum level of RBP4 is associated with decreased GLUT4 expression in adipose tissue that caused insulin signaling damage in skeletal muscle and induced glucose production by liver[3]. Retinol binding protein 4 inhibit insulin receptor substate-1 phosphorylation that contribute to insulin resistance[4]. Decreased in RBP4 expression is effective strategic for preventive and therapy type-2 diabetes.

Swietenia macrophylla king used as traditional medicine for diabetes patient in Indonesian. *Swietenia macrophylla* king seed contain alkaloid compound has hypoglycemic effect[5]. The aim of this study is to investigate antidiabetic activity of alkaloid compound 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline to serum blood glucose, lipid profile and RBP expression in liver tissue.

MATERIALS AND METHODS

Alkaloid compound 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline from *Swietenia macrophylla* king seed characterized by Mursiti[6].

Experimental animal: Adult male *Rattus norvegicus* rats at 8 weeks old and average weight 200 g were performed in this experiments. Animals were housed in animal room at 23-25°C and maintained with standard feeding and water *ad libitum*.

Induction of diabetes: Diabetes was induced in rat by intraperitonial (i.p.) injection of streptozotocin (STZ) at dose 60 mg kg^{-1} b.wt. and nicotinamide[7] 230 mg kg^{-1} b.wt. Rats with blood glucose level of 150 mg dL^{-1} or higher were considered to be diabetic[8,9]. The blood glucose level were estimated by GOD-PAP. Blood samples were collected from plexus retro-orbitalis hematocrit tube contain 40 IU heparin.

Experimental protocol: The animals were randomly divided into five groups with five animals in each group. Group I: Diabetic rats, group II: Diabetic rats receiving glibenclamide, group III: Diabetic rats receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10 mg/200 g b.wt., group IV: Diabetic rats receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 20 mg/200 g b.wt. and group V: Diabetic rats receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 40 mg/200 g b.wt. Five days after STZ-injection, rats with blood glucose level of 150 mg dL^{-1} were included in the study. Treatment with 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline were started 5 days after STZ-injection. Blood glucose, cholesterol, low density lipoprotein, triglyceride and high density lipoprotein measurement were done on day 5 after STZ-injection and the end of the study (4 weeks). Serum cholesterol, low density lipoprotein, triglyceride and high density lipoprotein were estimated by enzymatic colorimetric methode. At the end of this experiment all animals were killed under ether anaesthesia and liver tissue was removed for immunohistochemical analysis (RBP4 expression examined) (Fig. 1).

Histopathological procedure: Liver tissue were fixed in 10% neutral buffered formalin solution, embedded in paraffin and then stained with haematoxylin and eosin. The preparations were evaluated by means of a bright-field microscope and photographed.

Statistical analysis: All the values of blood glucose level, cholesterol, low density lipoprotein, triglyceride and high density lipoprotein were expressed as Mean±Standard Deviation (SD) and analyzed for ANOVA and *post hoc* test. Differences between groups were considered significant at $p<0.05$ levels.

RESULTS

The effect of the alkaloid compound 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline on blood glucose level of diabetic rats is shown in Table 1. The results clearly indicated that group II (diabetic rats receiving glibenclamid), group III (diabetic rats receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10 mg/200 g b.wt.), group IV (diabetic rats receiving 3,6,7-trimethoxy-4-methyl-

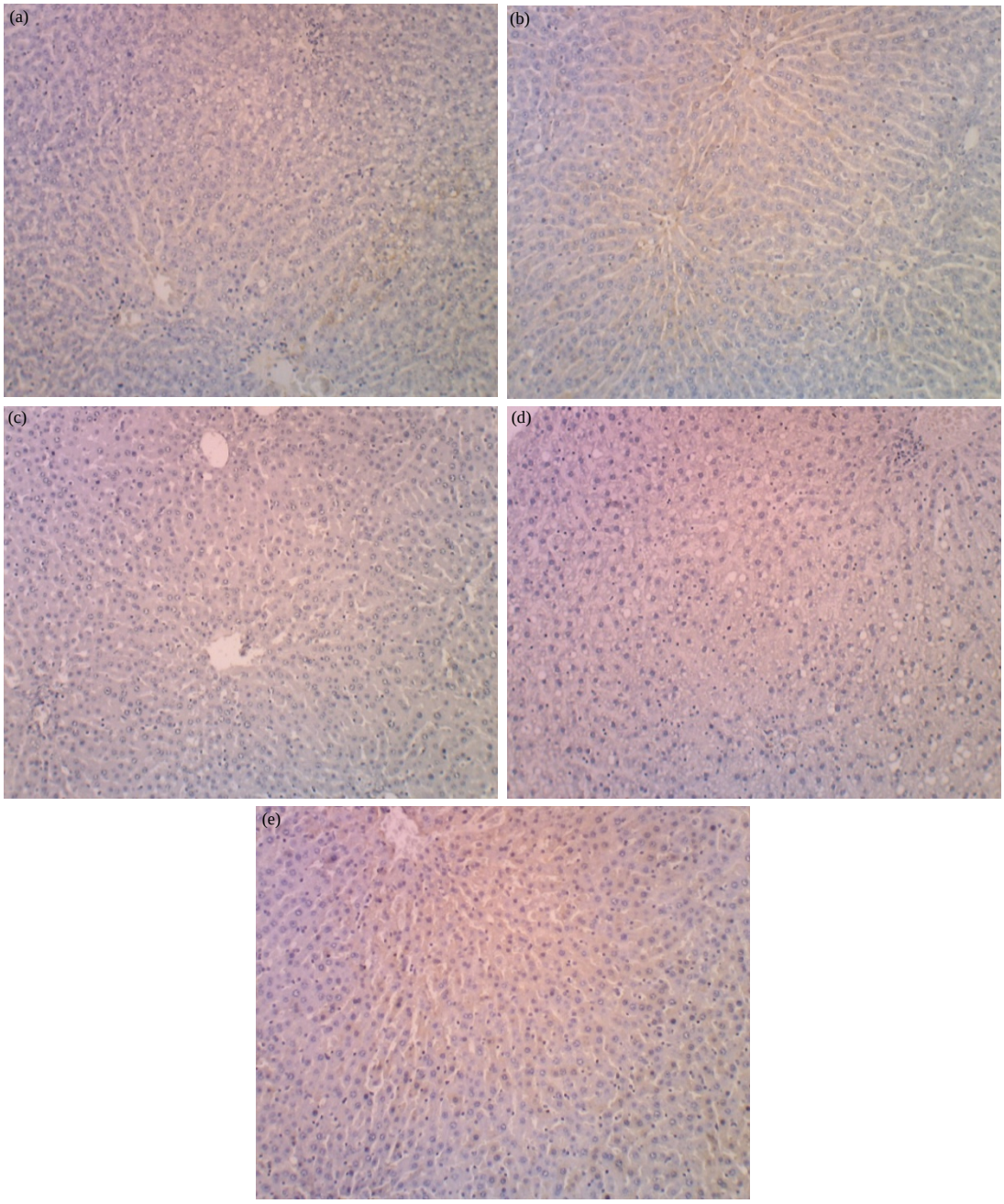

Fig. 1(a-e): Hepatic RBP4 expression in diabetic rats, (a) Diabetic control group, (b) Diabetic rats received glibenclamide 0.18 mg/200 g b.wt., day^{-1}, (c) Diabetic rats received alkaloid 10 mg day^{-1}, (d) Diabetic rats received alkaloid 20 mg day^{-1} and (e) Diabetic rats received alkaloid 40 mg day^{-1}

Table 1: Blood glucose level pre and post treatment (Mean±SD mg dL^{-1})

Groups	Before treatment (Means±SD)	After treatment (Means±SD)	p-value
DM	242.68±21.07	258.77±20.55	0.236
DM+glibenclamide	234.01±16.90	112.76±6.99	0.001
DM+alkaloid 10	324.89±18.95	181.12±5.48	0.002
DM+alkaloid 20	240.49±21.32	140.85±8.57	0.001
DM+alkaloid 40	233.70±6.160	132.75± 4.78	0.001

1,2,3,4-tetrahydro-isoquinoline dose 20 mg/200 g b.wt.) and group V (diabetic rats receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 40 mg/200 g b.wt.) decreased blood glucose level 52, 44, 41.43 and 43.2%, respectively.

The cholesterol is low density lipoprotein and triglyceride rats treated with 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-

Table 2: Cholesterol concentration pre and post treatment (Mean±SD)

	Cholesterol level (mg dL^{-1})			
Groups	Pre-test	Post-test	Mean difference	p-value
DM	43.78±8.74	56.02±6.07	-12.23 (-21.10, -3.37)	0.016
DM+glibenclamide	58.38±18.09	59.57±8.94	-1.18 (-17. 88, 15.51)	0.863
DM+alkaloid 10	50.35±13.06	44.48±11.47	5.87 (-13.83, 25.56)	0.478
DM+alkaloid 20	57.15±11.03	53.34±7.02	3.72 (-5.88, 13.31)	0.365
DM+alkaloid 40	67.93±6.92	58.12±16.27	9.81 (-7.34, 26.97)	0.201
p-value	0.026	0.142		

Table 3: Low density lipoprotein concentration pre and post treatment

	LDL level (mg dL^{-1})			
Groups	Pre-test	Post-test	Mean difference	p-value
DM	20.66±2.24	23.72±8.32	-3.06 (-13.25, 7.13)	0.451
DM+glibenclamide	28.24±4.37	30.88±13.91	-2.64 (-24.48, 19.20)	0.754
DM+alkaloid 10	33.54±7.38	20.88±6.38	12.66 (-3.39, 28.71)	0.094
DM+alkaloid 20	22.42±2.48	20.12±3.83	2.30 (-3.72, 8.32)	0.349
DM+alkaloid 40	35.5±1.66	25.90±2.46	9.60 (5.32, 13.88)	0.003
p-value	<0.001	0.254		

Table 4: Triglyceride level pre and post treatment

	Triglyceride level (mg dL^{-1})			
Groups	Pre-test	Post-test	Mean difference	p-value
DM	94.78±12.24	174.74±19.31	-79.96 (-103.36, -56.55)	0.001
DM+glibenclamide	112.96±31.91	66.47±17.31	46.49 (4.01, 88.98)	0.038
DM+alkaloid 10	88.06±32.87	55.76±29.52	32.31 (-15.85, 80.47)	0.136
DM+alkaloid 20	100.60±27.35	78.89±48.24	21.71 (-65.17, 108.59)	0.526
DM+alkaloid 40	44.16±12.23	59.88±21.33	-15.72 (-30.56, -0.88)	0.042
p-value	0.004	<0.001		

Table 5: High density lipoprotein pre and post treatment

	HDL level (mg dL^{-1})			
Groups	Pre-test	Post-test	Mean difference	p-value
DM	25.00±4.36	20.70±2.86	4.30 (-3.82, 12.42)	0.215
DM+glibenclamide	11.44±1.43	31.68±6.22	-20.24 (-28.78, -11.70)	0.003
DM+alkaloid 10	15.66±3.21	33.82±3.35	-18.16 (-24.38, -11.94)	0.001
DM+alkaloid 20	16.18±3.10	31.78±6.57	-15.6 (-26.71, -4.49)	0.018
DM+alkaloid 40	21.16±3.45	27.12±6.39	-5.96 (-12.92, 1.00)	0.076
p-value	<0.001	0.007		

isoquinoline dose 10 mg/200 g b.wt., for 4 weeks caused reduction on these parameters, about 11.66, 37.7 and 36.7% ($p>0.05$), respectively. At dose 20 mg/200 g b.wt., decreased 6.7, 10.26 and 21.58% ($p>0.05$) for cholesterol, low density lipoprotein and triglyceride, respectively, treatment at dose 40 mg/200 g b.wt., decreased about 14.4% ($p>0.05$) for cholesterol, 27.04% ($p<0.05$) for low density lipoprotein and increased 26.25% significantly ($p<0.05$) for triglyceride (Table 2-4). Serum level high density lipoprotein (Table 5) was elevated significantly ($p<0.05$) in the group that receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline doses 10 mg and 20 mg/200 g b.wt., about 53.7 and 49.1%, respectively and 22% increased non-significantly ($p>0.05$) that receiving 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline doses 40 mg/200 g b.wt. The RBP4 expression decreased non-significantly ($p>0.05$) after treatment with 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline doses 10, 20 mg and 40 mg/200 g b.wt. (Table 6).

DISCUSSION

This study examined the effect of 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline on plasma glucose, lipid profile and RBP4 expression during 4 weeks to

Table 6: RBP4 expression on liver (%)

Groups	RBP4 expression
DM	37.13±12.56
DM+glibenclamide	26.43±13.11
DM+alkaloid 10	8.65±4.95
DM+alkaloid 20	9.29±5.94
DM+alkaloid 40	4.92±2.53
p-value	0.434

STZ-induced diabetic rats. The blood glucose level significantly reduction and a little significant for lipid profile. The 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10, 20 mg and 40 mg/200 g b.wt., displayed a same activity with glibenclamid for reduced blood glucose level. These observation is agree with those of Maiti *et al.*[10] and Falah *et al.*[11] glibenclamide is Sulfonylurea family that its effect increased insulin release from β-cell and improve blood glucose level[12]. From the results it is assumed that the 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline could be responsible for stimulation of insulin release and the observed restoration of metabolic activities.

In the present study, treatment of rats with 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10 and 20 mg/200 g b.wt., for 4 weeks resulted in marked increased HDL level and dose 40 mg/200 g b.wt., decreased LDL level significantly. These observation is agree with De *et al.*[13]. However, substances as antioxidant or prooxidant depending on consentration and metal transition[14]. In this study, 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline at dose 40 mg/200 g b.wt., act as pro-oxidant so increased triglyceride level significantly. Decreased free fatty acid transport to liver caused increasing lipolysis in type-2 diabetes for inhibit triglyceride synthesis[15].

CONCLUSION

The alkaloid compound 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydro-isoquinoline dose 10 and 20 mg/200 g b.wt., significantly ($p<0.05$) decreased blood glucose level and increased HDL serum level and reduce RBP4 expression in liver ($p>0.05$).

REFERENCES

1. American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care, 37: S81-S90.
2. Cernea, S. and M. Dobreanu, 2013. Diabetes and beta cell function: From mechanisms to evaluation and clinical implications. Biochem. Med. (Zagreb), 23: 266-280.
3. Yang, Q., T.E. Graham, N. Mody, F. Preitner and O.D. Peroni *et al.*, 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature, 436: 356-362.
4. Ouchi, N., J.L. Parker, J.J. Lugus and K. Walsh, 2011. Adipokines in inflammation and metabolic disease. Nat. Rev. Immunol., 11: 85-97.
5. Hajra, S., A. Mehta, P. Pandey and S.P. Vyas, 2011. Antioxidant and antidiabetic potential of ethanolic extract of *Swietenia mahagoni* (Linn.) seeds. Int. J. Pharm. Res. Dev., 3: 180-186.
6. Mursiti, S., 2004. Identifikasi senyawa alkaloid dalam biji mahoni bebas minyak (*Swietenia macrophylla* king) dan efek biji mahoni terhadap penurunan kadar glukosa darah tikus putih (Rattus Novergicus). Universitas Gadjah Mada, Yogyakarta.
7. Masiello, P., 2006. Animal models of type 2 diabetes with reduced pancreatic β-cell mass. Int. J. Biochem. Cell Biol., 38: 873-893.
8. Mohammed, A., A.B. Adelaiye, M.S. Abubakar and E.M. Abdurahman, 2007. Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats. J. Med. Plant Res., 12: 034-037.
9. Iqbal, M., Kalsoom and S.A. Jafri, 2011. Effect of *Punica granatum* flowers extract on hypercholesterolemic and alloxan induced diabetic rats. Global J. Biotechnol. Biochem., 6: 83-86.
10. Maiti, A., S. Dewanjee, G. Jana and S.C. Mandal, 2008. Hypoglycemic effect of *Swietenia macrophylla* seeds against type II diabetes. Int. J. Green Pharm., 2: 224-227.
11. Falah, S., M. Safithri, T. Katayama and T. Suzuki, 2010. Hypoglycemic effect of mahogany (*Swietenia macrophylla* king) bark extracts in alloxan-induced diabetic rats. Wood Res. J., 1: 89-94.
12. Lullman, H., M. Klaus, Z. Albrecht and B. Detlef, 2000. Color Atlas of Pahrmmacology. 2nd Edn., Thieme, New York.
13. De, D., K. Chatterjee, K.M. Ali, T.K. Bera and D. Ghosh, 2011. Antidiabetic potentiality of the aqueous-methanolic extract of seed of *Swietenia mahagoni* (L.) Jacq. in streptozotocin-induced diabetic male albino rat: A correlative and evidence-based approach with antioxidative and antihyperlipidemic activities. Evid. Based Complem. Altern. Med., 10.1155/2011/892807
14. Carocho, M. and I.C.F.R. Ferreira, 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol., 51: 15-25.
15. Mooradian, A.D., 2009. Dyslipidemia in type 2 diabetes mellitus. Nat. Rev. Endocrinol., 5: 150-159.