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Seven-Hydroxy-2- (4-Hydroxy-3-Methoxy-Phenyl) -Chromen-4-one of *Swietenia Macrophylla* King Seed Improves Lipid Profile, Atherogenic Index, and Upregulates Adipose Tissue Peroxisome Proliferator-Activated Receptor Gamma Expression in Type 2 Diabetic rats

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Abstract

Background and Objective: Diabetes mellitus is a group of metabolic diseases with characteristic hyperglycemia. Hyperglycemia leads to excessive free radical production. There is increasing evidence that active compounds of medicinal plants may be used to treat diabetes. This study investigated the effects of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one* from *Swietenia macrophylla* King seed on lipid profile, atherogenic index and adipose tissue Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) expression in type 2 diabetic rats.

Methods and Materials: A total of 36 rats with age of eight weeks and weighing an average of 200 grams were used. Rats were divided into 6 groups as follows: A) normal, B) diabetic rats, C) diabetic rats with metformin, D), E) and F) diabetic rats with *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one* (*Swietenia macrophylla* King) seed doses 10, 30 or 90mg/200g body weight (BW), respectively, were administered treatment orally by gavages. Blood samples were collected for assessment of lipid profile, atherogenic index, before and after 4 weeks administration and at the end of the study, test animals were euthanized. The adipose tissue was used for PPAR-γ expression assessment. Data were analyzed by paired *t* test and One-Way ANOVA followed by Games-Howell test.

Results: Supplementation with three different doses of extract from *Swietenia macrophylla* King seed in streptozotocin induced diabetic rats for four weeks significantly ($p<0.001$) reduced cholesterol, triglyceride, low density lipoprotein and increased high density lipoprotein, reduced atherogenic index and increased PPAR-γ expression.

Conclusions: These findings demonstrate that supplementation with *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one* from *Swietenia macrophylla* King seed, improves lipid profile, atherogenic index and increases PPAR-γ gene expression.

Key words: atherogenic Index, Diabetes mellitus**,** free radical**,** lipid profile, *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*, PPAR γ

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease that is commonly found around the world. DM is a group of metabolic diseases with characteristic hyperglycemia that occurs due to abnormal insulin secretion, abnormal insulin action, or both[1]. Hyperglycemia induces the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2] (Tiwari.2013) The condition of hyperglycemia in diabetes leads to excessive production of free radicals [3]. Increased formation of free radicals can increase oxidative stress which results in damage to cellular proteins, lipids contained in cell membranes, nucleic acids, and leads to cell death [4]. Imbalance between oxidative stress and antioxidant defense mechanisms in diabetes can lead to cell and tissue damage [5]. In patients with diabetes, abnormal regulation of insulin can cause abnormalities of lipid metabolism. Defects in disposal of lipids from the bloodstream is a common problem that happens and this lipid metabolism abnormality is a cause of the increase in triglyceride levels. Abnormalities of lipid metabolism can lead to the occurrence of macrovascular complications [6]. According Zaman *et al*. (2000) [7], ischemic heart disease is caused by thrombus that occurs due to blockage of small blood vessels. Peroxisome Proliferator-Activated Receptors (PPARs) are transcription factors that are superfamily ligands which can be induced by hormone receptors including core, along with thyroid hormone receptors, retinoid, steroid hormones and vitamin D [8]. PPAR-gamma are activated by fatty acids, eicosanoids and thiazolidinediones (TZDs) [9].

PPAR-γ genes play a role in the regulation of adipogenesis, insulin sensitivity and energy homeostasis [8]. PPAR-γ also can be used as a therapeutic target for type 2 diabetes [10]. Many metabolic activities are regulated by PPAR ligands such as long chain fatty acids or their derivatives. Currently a more natural approach is used in the treatment of metabolic disorders due to excessive production of free radicals. The use of natural antioxidants has become the chosen alternative because there are concerns about side effects of synthetic antioxidants that are toxic to the liver and mutagenesis [11].

Research shows that mahogany seed (*Swietenia macrophylla* King, Linn.) extract has antioxidant effects and can potentially stop the cycle of oxidative stress in DM [11. In addition, mahogany seeds also have the the positive effects of anti-inflammatory, anti-tumor, and anti-mutagen properties [12]. Reported by Kalaivanan and Pugalendi (2011) [13], *Swietenia macrophylla* King seeds had an anti-hyperglycemia effect on DM rats that were induced by streptozotocin. This finding makes mahogany seeds as a promising object of research related to its function as a treatment drug for DM. Mahogany seeds' chemical compounds include flavonoids, saponins, alkaloids, steroids and tannins [14,15]. This study investigated the effects of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one* extracted from *Swietenia macrophylla* King seeds on lipid profile, atherogenic index and adipose tissue PPAR-γ expression in type 2 diabetic rats.

MATERIALS AND METHODS

These study was conducted at Universitas Gadjah Mada from December 2016 until May 2017. 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one extracted from *Swietenia macrophylla* King seeds was isolated as described by Mursiti (2008)[15]. Thirty male Wistar rats (*Rattus norvegicus*), 8 weeks old, weight range 150-200g were obtained as test animals. They were housed in cages in an animal room (22-25°C room temperature on a 12-hour daylight cycle), while food and water were given *ad libitum* during the experimental period using standard diet (AIN 93M). The standard diet in the experiment consisted of casein 24%, DL-methionine 0.30%, cornstarch 61%, vitamin mix 1%, mineral mix 3.5%, choline chloride 0.2%, alpha cell 5% and corn oil 5%. This study was approved by the Medical

and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

Reagents

Assay kits for measuring plasma glucose level by the enzymatic glucose-peroxidase (GOD-PAP) method were purchased from Diasys® (Holzheim, Germany). Lipid profile level were assayed by Dyasis® (Holzheim, Germany). Atherogenic Index was calculated according to the formula: log[TG/HDL][16]. All other chemicals were of analytical grade.

Induction of Type 2 Diabetes Mellitus

Type 2 Diabetes mellitus was induced by intraperitoneal injection of 65 mg/kg BW of streptozotocin (NacalaiTesque, Inc, Japan) 15 minutes after injection of 230 mg/kg BW of nicotinamide (NA) (Sigma-Aldrich, USA) following the method outlined by [17]Masiello *et al*., 1998. Fasting blood glucose levels were measured 5 days after induction, and the rats were categorized as diabetic if the fasting blood glucose levels were ≥170 mg/dL[17].

Experimental Design

The rats were divided into 6 groups A (control): Animals received food and water given *ad libitum* and were euthanized after 4 weeks. Group B (diabetic rats, untreated) were euthanized after 4 weeks. Group C (diabetic rats treated with metformin) were also euthanized after 4 weeks. Groups D, E and F (diabetic rats) treated with *7-hidroxy-2-(4-hidroxy-3-methoxy-phenyl)-chroman-4-one* doses 10, 30 or 90mg/200gBW/daily, respectively, were administered treatment orally by gavages for 4 weeks, after which the animals were euthanized.

PPAR-γ Expression with quantification PCR (q-PCR)

cDNA was synthesized using the iScript mix Biorad® kit according to the protocol of the producer. SsoFast™Evagreen® with Supermix Biorad® was used for q-PCR. The primers used for cDNA amplification were forward 5'-ACACCATGCTGGCCTCCCTGA-3' and reverse 5'-A GTGGAGACCGCCCAGGCTTG-3' for PPAR-Y (220 bp); The q-PCR reaction was conducted individually with each gene using the same internal control beta actin gene (240 bp). *Forward* 5'-ACGGTCAGGTCATCACTATCG3' *Reverse* 5'–GGCATAGAGGTCTTTACGGATG-3'. The cycle for the cDNA amplification was 5 minutes at 95^{o}C, 95^{o}C at 1 minute, followed by 60.2^{o}C and 40 cycles. Then, the final extension step at 72^{o}C for 5 minutes was performed, followed by a melt-curve analysis at 65-95^{o}C. The products of PCR were subjected to gel electrophoresis in 2% agarose. The cycle of threshold (C_T) value was obtained automatically from the machine's calculation.

Data Analysis

The results were expressed as the mean ± SE. Lipid profile and atherogenic index were analyzed before and after treatment by Paired t test. PPAR-gamma gene expressions were analyzed by one-way ANOVA. Data were considered statistically significant if *p* values were lower than 0.05.

RESULTS

The effect of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one on cholesterol in diabetic rats is shown in **Table 1**. Cholesterol levels decreased significantly after administration of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one with doses 10, 30 or 90 mg/200gBW. The highest reduction of cholesterol in diabetic rats was observed with the dose 90 mg/200gBW (p<0.001). As shown in **Table 2**, triglyceride levels increased after administration of streptozotocin and nicotinamide. The compound 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one in doses of 10, 30 or 90 mg / 200gBW for 4 weeks reduced serum triglyceride levels significantly (p<0.001). The highest reduction of triglyceride levels in diabetic rats was found with a dose of 90 mg/200gBW (p<0.001). **Table 3** shows increased serum LDL levels after administration of streptozotocin and nicotinamide. The compound 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one in doses of 10, 30 or 90 mg/200gBW for 4 weeks reduced serum LDL levels significantly (p<0.001). The highest reduction of LDL levels in diabetic rats was found with a dose of 90 mg/200gBW (p<0.001). **Table 4** shows decreases in serum HDL levels after induction with streptozotosin and nicotinamide. Giving compound 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one in doses of 10, 30 or 90 mg/200gBW for 4 weeks increased serum HDL levels significantly (p<0.001). The highest increase of HDL levels in diabetic rats was found in diabetic rats with 90 mg/200gBW (p<0.001). The atherogenic index is shown in Table 5. After administration of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one doses of 10, 30 or 90mg/200gBW for 4 weeks, atherogenic index significantly decreased (p<0.002). The highest decrease of atherogenic index was observed with a dose of 10 mg/200gBW (p<0.001).

There were also increases in the relative expression of PPAR-γ in rats adipose tissue after the administration of the 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one doses 10, 30 or 90mg/ 200g BW for 4 weeks as shown in **Figure 1**. The highest increase in the relative expression of PPAR-γ was observed with a dose of 10 mg/200gBW (p<0.001).

Table 1. Serum cholesterol levels

Group	Serum cholesterol levels (mg/dL)		Mean difference	p
	before	after		
A	103.10 ± 2.20^{a}	103.6 ±1.77^{a}	0.48	0.151
B	153.03 ± 3.71^{b}	153.7 ±3.49^{b}	0.69	0.127
C	149.42 ± 3.50^{b}	107.6 ±3.82^{a}	-41.83	<0.001
D	147.64 ± 4.73^{b}	128.2 ±4.64^{c}	-19.40	<0.001
E	151.60 ± 2.29^{b}	127.4 ±1.84^{c}	-24.47	<0.001
F	154.83 ± 3.92^{b}	112.9 ±3.60^{a}	-41.94	<0.001
p	<0.001	<0.001		

A: Normal rats; B: Diabetic rats; C: Diabetic rats + Metformin; D: Diabetic rats + 10 mg/200 gBW E: Diabetic rats + 30 mg/200 gBW, and F: Diabetic rats + 90 mg/200 gBW of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*. Values are presented as mean ± SE. $^{a, b, c}$ indicate p<0.05 according to One Way ANOVA test, followed by Tukey HSD test. P value in rows indicate the differences of serum cholesterol levels before and after treatment. P<0.05 according to paired sample t test.

Table 2. Serum triglyceride levels

Group	Serum triglyceride levels (mg/dL)		Mean difference	p
	before	after		
A	53.32 ± 1.97[a]	44.2 ± 1.01[a]	-9.10	<0.001
B	106.54 ± 0.23[b]	86.5 ± 2.13[b]	-19.94	<0.001
C	105.81 ±1.95[b]	51.2 ± 1.74[c]	-54.61	<0.001
D	105.80 ± 0.60[b]	69.9 ± 1.64[d]	-35.91	<0.001
E	104.61 ± 0.12[b]	60.7 ± 1.67[e]	-43.86	<0.001
F	107.53 ± 0.25[b]	51.3 ± 3.78[c]	-56.19	<0.001
p	<0.001	<0.001		

A: Normal rats; B: Diabetic rats; C: Diabetic rats + Metformin; D: Diabetic rats + 10 mg/200 gBW E: Diabetic rats + 30 mg/200 gBW, and F: Diabetic rats + 90 mg/200 gBW of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*. Values are presented as mean ± SE. [a, b, c] indicate p<0.05 according to One Way ANOVA test, followed by Tukey HSD test. P value in row indicate the differences of serum triglyceride levels before and after treatment. P<0.05 according to paired sample t test.

Table 3. Serum LDL levels

Group	LDL levels (mg/dL)		Mean difference	p
	before	after		
A	43.72 ± 3.71[a]	45.9 ± 3.04[a]	2.28	0.002
B	90.23± 2.91[b]	93.0 ± 2.84[b]	2.80	<0.001
C	85.84 ± 3.31[b]	46.8 ± 3.48[a]	-39.07	<0.001
D	84.70 ± 3.49[b]	73.2 ± 2.87[c]	-11.54	<0.001
E	88.43 ± 2.26[b]	62.6 ± 1.44[d]	-25.88	<0.001
F	94.31 ± 5.28[b]	53.6 ± 2.11[e]	-40.72	<0.001
p	<0.001	<0.001		

A: Normal rats; B: Diabetic rats; C: Diabetic rats + Metformin; D: Diabetic rats + 10 mg/200gBW. E: Diabetic rats + 30 mg/200 gBW, and F: Diabetic rats + 90 mg/200 gBW of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*. Values are presented as mean ± SE. [a, b, c] indicate p<0.05 according to One Way ANOVA test, followed by Tukey HSD test. P value in row indicate the differences of serum LDL levels before and after treatment. P<0.05 according to paired sample t test.

Table 4. Serum HDL levels

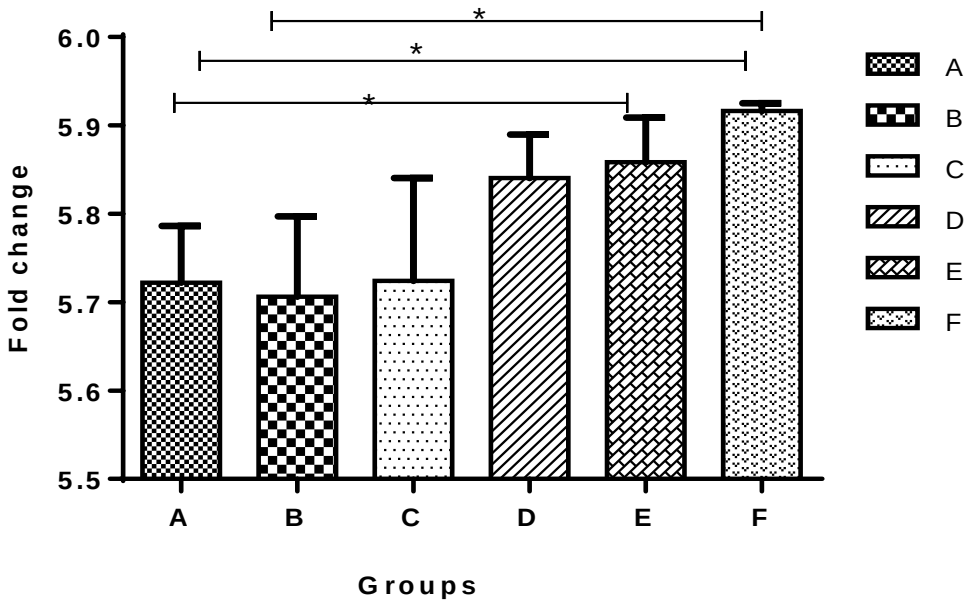
Group	HDL levels (mg/dL)		Mean difference	p
	before	after		
A	74.90 ± 2.61[a]	73.9 ± 2.08[a]	-0.90	0.098
B	26.74 ± 2.99[b]	24.7 ± 2.71[b]	-1.94	0.002
C	31.82 ± 5.23[b]	67.3 ± 4.39[c]	35.54	<0.001
D	32.60 ± 4.78[b]	45.8 ± 3.67[d]	13.12	<0.001
E	29.51 ± 4.44[b]	57.7 ± 2.98[e]	28.15	<0.001
F	25.42 ± 2.75[b]	65.3 ± 2.93[c]	39.89	<0.001
p	<0.001	<0.001		

A: Normal rats; B: Diabetic rats; C: Diabetic rats + Metformin; D: Diabetic rats + 10 mg/200 gb.wt E: Diabetic rats + 30 mg/200 gb.wt, and F: Diabetic rats + 90 mg/200 gBW of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*. Values are presented as mean ± SE. [a, b, c] indicate p<0.05 according to One Way ANOVA test, followed by Tukey HSD test. P value in row indicate the differences of serum HDL levels before and after treatment. P<0.05 according to paired sample t test.

Table 5. Atherogenic Index

Group	Atherogenic index		Mean Difference	p
	Pre test	Post test		
A	-0.14 ± 0.02[a]	-0.02 ± 0.01[a]	0.08	<0.001
B	0.58 ± 0.05[b]	0.52 ± 0.48[b]	0.06	<0.001
C	0.49 ± 0.07[b]	-0.14 ± 0.04[c]	0.62	<0.001
D	0.62 ± 0.09[b]	-0.11 ± 0.02[d]	0.73	<0.001
E	0.52 ± 0.57[b]	0.01 ± 0.03[e]	0.51	<0.001
F	0.49 ± 0.06[b,c]	0.17 ± 0.03[c]	0.32	<0.001
p	<0.001	<0.001		

A: Normal rats; B: Diabetic rats; C: Diabetic rats + Metformin; D: Diabetic rats + 10 mg/200 gb.wt E: Diabetic rats + 30 mg/200 gb.wt, and F: Diabetic rats + 90 mg/200 gBW of *7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one*. Values are presented as mean ± SE. [a, b, c] indicate p<0.05 according to One Way ANOVA test, followed by Tukey HSD test. [b,c] indicate no difference between [b] nor [c]. P value in row indicate the differences of atherogenic index before and after treatment. P<0.05 according to paired sample t test.



A, normal; B, diabetic rats; C, diabetic rats + metformin; D, diabetic rats +10mg/200gBW Flavonoid ; E, diabetic rats + 30mg/200gBW Flavonoid dan F, diabetic rats + 90mg/200gBW Flavonoid

Figure 1. PPAR gama relative expression with beta-actin.

DISCUSSION

In the present study, the lipid profile, atherogenic index and relative expression of PPAR-gamma in adipose tissue were significant improved after diabetic rats treated with three different doses of 7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)–chroman-4-one for four weeks.

In diabetes, long-term hyperglycemia and insulin resistance increases the lipogenesis and triglyceride level and decreases the HDL level and then can cause increasing Aterogenic Index of Plasma (AIP) level. The AIP level is defined as log(TG / HDL-C), indicating that people with high AIP have a higher risk of coronary artery disease than those with low AIP [16]. In our study we found that in diabetic rats the total cholesterol, triglyceride, and low density lipoprotein increased and high density lipoprotein decreased significantly. Dyslipidemia and DM are highly related with insulin resistance and hyperglycemia[18] and this condition can increase protein glycation [3,19] and reactive oxygen species (ROS) formation and together contribute to LDL oxidation and atherosclerosis [20.

Pharmacological interventions by some medications which influence primary glucose metabolism (metformin and acarbose) or induced weight loss (orlistat, combined with dietary intervention) can also effectively delay progression to type 2 diabetes [21,22,23]. PPARγ is an attractive pharmacological target for the development of drugs to treat metabolic disorders such as insulin resistance [24] type II diabetes [25] and chronic inflammation [26]. PPARγ is the potent function modulator found in adipose tissue, endothelial cells and vascular smooth muscle cells [27], but is expressed predominantly in adipose tissues. PPARγ controls adipocyte differentiation and is activated by endogenous agonists such as fatty acids [28] and xenobiotics such as rosiglitazone[29]. The treatment drug, thiazolidinedione was used as an antidiabetic drug until it became evident that its use was associated with increased risk of myocardial infarction [30]. PPARγ is a regulator of lipid and glucose metabolism and therefore its synthetic ligands such as glitazones –the derivatives of thiazolidinediones (e.g., troglitazone, rosiglitazone and pioglitazone) - improve insulin and glucose levels and increase whole body insulin sensitivity. Therefore, they are called insulin-sensitizing medications used in the treatment of DM. [31]. PPARγ protects non-adipose tissues against excessive lipid overload and maintains normal organ function (liver, skeletal muscle). Activated PPARγ in adipocytes guarantees a balanced and adequate secretion of adipocytokines (adiponectin and leptin) that are mediators of insulin action in peripheral tissues. In consequence, the insulin sensitivity of the whole body is maintained. [32].

Flavonoids were shown as agonists or partial agonists of PPARγ such as the flavonols: kaempferol and quercetin, the flavones: luteolin and apigenin, and the isoflavones: daidzein and genistein [33]. It has been hypothesized that plant-derived PPARγ modulators may be able to improve insulin sensitivity without detrimental side effects. In contrast, flavonoids such as quercetin inhibited the activation of all three isoforms of PPAR [34], but its metabolites upregulated PPAR-γ expression [35] and could alleviate hepatic fat accumulation [36]. Apart from polyunsaturated fatty acids, phytanic acid is also a natural PPARγ agonist in the human diet that reveals a similar activity to omega-3 PUFA and increases glucose uptake and insulin sensitivity; however, it has less capacity to differentiate adipocytes [37].

CONCLUSION

In conclusion**,** the results of the present study demonstrate that administration of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one reduces atherogenic index and increases adipose tissue PPAR-γ expression. The highest effect occurred at dose 10mg/200gBW. These findings justify further research to provide more evidence to support the inclusion of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one as a part of healthy dietary practices for patients with diabetes mellitus.

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