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Research Article
Effects of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one from *Swietenia macrophylla* King Seed on Oxidized LDL, HOMA Beta and Glucagon like Peptide 1 (GLP-1) Gene Expression in Type 2 Diabetic Rats

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Abstract

Background and Objective: Type 2 diabetes is characterized by hyperglycemia leads to increased production of reactive oxygen species. There is increasing evidence that active compounds of medicinal plants may be used to reduce oxidative stress. The aims of this study were to investigate anti-diabetic effects of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one derived from *Swietenia macrophylla* King seed on oxidized LDL, homeostatic model assessment beta-cell function (HOMA-β) and glucagon like peptide 1 (GLP-1) gene expression in diabetic rats. **Materials and Methods:** A total of 30 rats were used. They were divided into 6 groups as follows: (A) Normal rats, (B) Diabetic rats, (C) Diabetic rats with metformin, (D), (E) and (F) Diabetic rats with 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one *Swietenia macrophylla* King seed dose 10, 30 or 90 mg/200 g b.wt., respectively. Oxidized LDL and HOMA-β value were analyzed twice, before and after treatment. The GLP-1 gene expression in intestine was analyzed at the end of study. Data were analyzed by paired t-test and one-way ANOVA followed by Games-Howell test. **Results:** Administration of three different doses of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one from *Swietenia macrophylla* King seeds in diabetic rats significantly reduced oxidized LDL and increased HOMA-β value ($p<0.001$) and also GLP-1 gene expression ($p<0.05$). **Conclusion:** These findings demonstrate that administration of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one from *Swietenia macrophylla* King seeds, improved beta cell function through reducing oxidized LDL and increased GLP-1 expression.

Key words: 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one, *Swietenia macrophylla* King, oxidized LDL, GLP-1, beta cell function

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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

High level of glucose or hyperglycemia in type 2 diabetes mellitus (T2DM) leads to increased production of reactive oxygen species (ROS)[1]. The abundance of ROS levels may induce the cellular damage that involved oxidation of glucose, lipid peroxidation and non-enzymatic glycation of protein[2]. The imbalance between ROS and antioxidant defense may occurs the condition called oxidative stress[3].

Increased oxidative stress can stimulate oxidation of LDL[4] and, thereby influences complication in T2DM[5]. In addition, elevated concentration oxidized LDL (ox-LDL) has a negative effect for beta-cell pancreas[6]. High levels of Ox-LDL mediates increasing apoptosis beta-cell pancreas and decreasing insulin synthesis via activation JNK pathway[7], thereby leading to beta-cell dysfunction[8].

The deterioration beta-cell function in T2DM has been reported directly associated with disrupted action of incretin hormones[9]. Freeman[10] reported this disruption may occurs due to primarily by decreased glucagon like peptide 1 (GLP-1) levels in circulation and secondly by decreased GLP-1 secretion. In the pancreas GLP-1 has several important functions such as stimulate insulin secretion, reduce apoptosis beta-cell pancreas and improve proliferation and neogenesis beta-cell pancreas[9]. Preserving beta-cell pancreas functions have potential effects to stabilize T2DM and delaying the progression of T2DM. Specific regulator homeostatic of beta cell pancreas such as nuclear factors, cell cycle mediators and growth factors are needed to it[11]. According to Buteau[12], GLP-1 may act as a growth factor to induces survival and proliferation of beta-cell pancreas and it become a promising therapeutic agent against T2DM.

Currently using antioxidants to prevent progression of T2DM have been studied. Ruhe and McDonald[13] have shown that antioxidants, especially natural antioxidants have a beneficial effects to reduce oxidative stress. Antioxidants have various mechanism to combat oxidative stress such as enzyme inhibition and free radical scavenging activity[14]. There are several compounds of natural antioxidant, one of them that is known is flavonoids. Flavonoids have a characteristic multiple hydroxyl groups so that become an effective antioxidant[15].

Seeds of *Swietenia macrophylla* King (mahogany, familia Meliaceae) are widely used as a traditional medicine for patients with diabetes mellitus[16]. Research has shown that a mahogany seed extract has antioxidant effects and potentially stops oxidative stress cycle in diabetes mellitus[17]. Kalaivanan and Pugalendi[18] reported that alcoholic seed extract of *Swietenia macrophylla* has an antihyperglycemic effect on diabetic rats, induced by streptozotocin. This finding makes mahogany seeds a promising object of research related to its function as an effective treatment for diabetes mellitus. The active chemical compounds of mahogany seeds include flavonoids, saponins and alkaloids according to Mursiti[19]. In this study, the effect of a compound from mahogany seed in diabetic rats induced by streptozotocin and nicotinamide was studied.

MATERIALS AND METHODS

These study was carried out at Universitas Gadjah Mada from December, 2016 until May, 2017. 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one isolated from *Swietenia macrophylla* King seeds was isolated and characterized by Mursiti[19]. Thirty male Wistar rats (*Rattus norvegicus*), 8 weeks old and weight range 150-200 g. They were housed in cages in an animal room (22-25°C room temperature on a 12 h daylight cycle), food and water were given ad libitum during the experimental period using standard diet (AIN 93M). This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, 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). Oxidized -LDL levels were assayed by the enzymatic method (enzyme linked immunosorbent one-step process assay), were purchased from Qayee-Bio (Shanghai, China) HOMA beta was calculated according to the formula 20 × fasting insulin level (μ IU mL^{-1}) /fasting glucose (mmol mL^{-1})-3.5[20]. All other chemicals were of analytical grade.

Induction of type 2 diabetes mellitus: Type 2 diabetes mellitus was induced by intraperitoneal injection of a 65 mg kg^{-1} b.wt., of streptozotocin (NacalaiTesque, Inc, Japan) 15 min after injection of 230 mg kg^{-1} b.wt., of nicotinamide (NA) (Sigma-Aldrich, USA) following the method outlined by Masiello *et al.*[21]. Fasting blood glucose levels were measured 5 days after induction and the rats were categorized as diabetic if the fasting blood glucose was $\geq$170 mg dL^{-1} [21].

Experimental procedure: The rats were housed in individual cages and acclimatized to the laboratory condition for 7 days and had free access to food and water during the experimental period. The standard diet was AIN 93 M consisting of (g kg^{-1} mix), cornstarch 465.692, casein 140, dextrinized cornstarch 155, sucrose 100, soybean oil 40, alphacel 50, AIN-93-M-MX 35, L-cysteine 1.8, AIN-93-VM 10,

choline bitartrate 2.5 and tert-butylhydroquinone 0.008. The present study was approved by the Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada.Twenty five rats were divided into groups.

The rats were divided into 6 groups: A (control) and all animals received food and water given *ad libitum*. Group B were diabetic rats without treatment. Group C were diabetic rats treated with metformin. Groups D, E and F were diabetic rats treated with 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one isolated from *Swietenia macrophylla* King seeds with doses 10, 30 or 90 mg/200 g b.wt., respectively). The extracts were administered orally by gavages for 4 weeks, after which all of the animals were euthanized.

GLP-1 expression with quantification PCR (q-PCR): cDNA was synthesized using the iScript mix Biorad® kit according to the protocol of the producer. SsoFast™ Evagreen®supermix Biorad® was used for q-PCR on a Biorad iCycler model CFX 96 Real-Time System. The primers used for cDNA amplification were forward 5′-CAACATTGCCAAACGTCATGAT-3′ and reverse 5′-TTGATGAAGTCTCTGGTGGCA-3′ for the GLP-1 peptide, encoded by the *Gcg* gene, (249 bp);The q-PCR reaction was conducted individually with each gene using the same internal control beta actin gene (240 bp). Forward 5′-ACGGT CAGGTCATCACTATCG3' Reverse 5′-GGCATAGAGGTCTTT ACGGATG3'. The program for cDNA amplification was 5 min at 95°C, followed by 40 cycles at 95°C for 60 sec, 57°C for 60 sec.

Data analysis: The results were expressed as the Mean±SE. One way ANOVA was used to analyze blood glucose, oxidized LDL, HOMA beta and GLP-1 gene expression between the groups. Paired t-test was used to evaluate the blood glucose, oxidized LDL and HOMA beta before and after treatment. Data were considered statistically significant if p values were lower than 0.05.

RESULTS

The results showed that rats, induced with streptozotocin and nicotinamide have blood glucose levels higher than normal, indicating type 2 diabetes. Blood glucose levels of these type 2 diabetes rats decreased significantly ($p<0.01$) after administration of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one at doses of 10, 30 or 90 mg /200 g b.wt., for 4 weeks data not shown.

Ox-LDL increased after administration of streptozotocin and nicotinamide as shown in Table 1. The compound 7-Hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one doses 10, 30 or 90 mg/200 g b.wt., for 4 weeks lowered ox-LDL significantly ($p<0.001$). The highest reduction of Ox-LDL in diabetic rats were found with a dose of 10 mg/200 g b.wt., ($p<0.001$).

HOMA-β values decreased after induction with streptozotocin and nicotinamide (Table 2). Giving 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one in doses of 10, 30 or 90 mg/200 g b.wt., for 4 weeks resulted in significantly increased HOMA-β values ($p<0.002$). The highest increase of HOMA-β value was observed with a dose of 90 mg/200 g b.wt., ($p<0.002$).

An increase in the relative expression of GLP-1 in rats' intestines after the administration of the 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one doses 10, 30 or 90 mg/200 g b.wt., for 4 weeks as shown in Fig. 1.

DISCUSSION

In the present study, the Ox-LDL were significant decrease and HOMA beta and relative expression of GLP-1 adipose tissue were increase after diabetic rat were treated with 7-Hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chromen-4-one.

Table 1: Oxidized-LDL level and their mean differences

	Oxidized LDL			
Groups	Pre-test	Post-test	Mean difference	p-value
A	382.10±87.05[a]	395.84±84.64[a]	-13.75 (-71.49 ; 43.99)	0.545
B	616.90±98.78[b]	727.76±119.16[b]	-116.86 (-252.87 ; 19.15)	0.076
C	576.53±46.76[b]	422.36±73.60[a]	154.17 (77.35; 231.00)	0.005
D	684.55±63.01[b]	444.03±68.27[a]	244.52 (147.60 ; 341.43)	0.002
E	613.85±84.29[b]	486.19±62.44[a]	127.66 (-0.51 ; 255.83)	0.051
F	583.41±87.94[b]	416.47±47.43[a]	166.94 (77.65 ; 256.23)	0.007
p-value	<0.001	<0.001		

A: Normal rats, B: Diabetic rats, C: Diabetic rats+metformin, D: Diabetic rats+10 mg/200 g b.wt., E: Diabetic rats+30 mg/200 g b.wt. and F: Diabetic rats+90 mg/200 g b.wt., of 7-Hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one. Values are presented as Mean±SE. [a,b,c,d,e]indicate $p<0.05$ according to one-way ANOVA test, followed by Games-Howell test. p-value in row indicate the differences of Ox-LDL levels before and after treatment. $p<0.05$ according to paired sample t-test

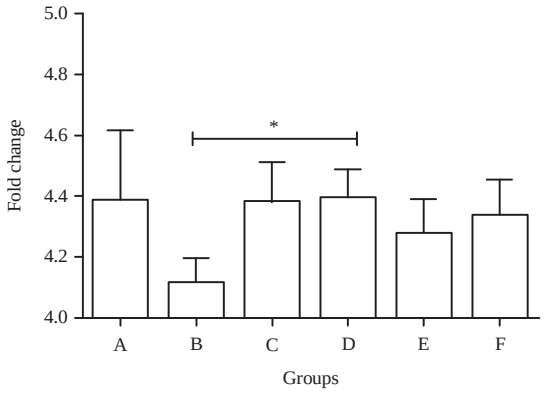

Fig. 1: GLP-1 expression after 4 weeks of treatment. A: Normal rats, B: Diabetic rats, C: Diabetic rats+metformin, D: Diabetic rats+10 mg/200 g b.wt., E: Diabetic rats+30 mg/200 g b.wt. and F: Diabetic rats+90 mg/200 g b.wt., of 7-Hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one. Values are presented as Mean±SE, significant difference is represented by *according to one-way ANOVA test followed by Tukey HSD test

Table 2: HOMA beta values and their mean differences

	HOMA beta			
Groups	Pre-test	Post-test	Mean difference	p-value
A	36.65±1.54[a]	43.42±0.96[a]	6.77	0.045
B	6.38±0.50[b]	6.75±0.54[b]	0.36	0.723
C	6.78±0.39[b]	26.81±2.25[c]	20.03	0.001
D	6.98±0.60[b]	17.79±0.60[c]	10.81	0.001
E	8.45±1.17[b]	21.89±1.17[c]	13.45	0.002
F	8.71±0.80[b]	23.49±0.80[c,d]	14.78	0.002
p-value	<0.001	<0.001		

A: Normal rats, B: Diabetic rats, C: Diabetic rats+metformin, D: Diabetic rats+10 mg/200 g b.wt., E: Diabetic rats+30 mg/200 g b.wt. and F: Diabetic rats+90 mg/200 g b.wt., of 7-Hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one. Values are presented as Mean±SE. [a,b,c,d]indicate $p<0.05$ according to one way ANOVA test, followed by Games-Howell test. [c,d]indicate no significantly different between [c] nor [d]. p value in row indicate the differences of HOMA beta before and after treatment. $p<0.05$ according to paired sample t-test

The presence of free radicals, low density lipoprotein easily and readily oxidized. In diabetic patients, oxidized LDL increased and contributed to atherogenesis and a potent inducer of inflammatory molecules. Oxidized low density lipoprotein cause macrophages phagocytosis, eating Ox-LDL, causing the death of macrophages. Macrophages that have died will stick to the walls of blood vessels, it happens over and over so that over time there will be a buildup of dead cells called foam cells. These form cells in the presence of calcium form plaque so far minimize elasticity and surface area. The condition is compensated by increases in blood pressure, so that plaque rupture and form a thrombus. Thrombus will cause blockage of small blood vessels that can lead to ischemic heart disease[22].

Flavonoids are hydrophilic as inhibitors of LDL oxidation[23]. The ability of flavonoids in protecting LDL from oxidation through two ways, direct and indirect interaction. Direct interaction occurs between flavonoid with LDL thus inhibiting LDL oxidation. Indirect interaction through the accumulation of flavonoids in the arteries and protects macrophage cells from oxidative stress. According to Nijveldt *et al.*[24] the ability of flavonoids lowering oxidized-LDL by its activity as an antioxidant that hydroxyl groups of flavonoid capable of stabilizing the ROS by react with reactive compound of radicals so that it becomes inactive compounds.

The activity of the anti-oxidative defense system are depleted in diabetic animal models caused by persistent and chronic hyperglycemia and promotes free radical generation[25]. Pancreatic β-cells may be particularly susceptible to oxidative[26]. One effort to improve function of pancreatic beta-cells is through the stimulation of incretin hormones. Glucagon like peptide 1 is an incretin hormone which is secreted by endocrine L-cells in the mucosa of the cecum and colon. An important role of GLP-1 hormone in the stimulation of pancreatic β-cell to produce insulin[27,28]. Glucagon like peptide 1 acts through the GLP-1 receptor, a G-coupled-protein receptor[29]. In theβ-cells, ncretins (GLP-1 and GIP) bind their incretin receptors (GLP-1 and GIP receptors) and increase the level of cAMP intracellular, leading to stimulation of insulin secretion, suppression of β-cell apoptosis and increase of β-cell growth[26]. Under diabetic conditions, expression of incretin receptors in β-cells is down-regulated, leading to decrease of insulin secretion, increase of β-cell apoptosis and decrease of β-cell growth[23]. In this study the increase in HOMA–β can be explained by the up-regulation of GLP-1R expression on pancreatic beta cells after administration of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one. In summary, the results of the present study demonstrate that administration of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one decreased Ox-LDL, increased HOMA beta and had a tendency to increase GLP-1 expression. This finding indicates that the 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one has ability to increase beta-cell pancreas function through upregulating GLP-1 gene expression. However further study is needed to elucidate the mechanism.

CONCLUSION

In summary, the result of the present study demonstrate that administration of 7-Hydroxy-2-(4-hydroxy-3-

methoxyphenyl)-chromen-4-one has ability to increase pancreatic beta cell function through upregulating GLP-1 gene expression and reducing oxidized LDL.

SIGNIFICANCE STATEMENTS

This study discovers the effects of 7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one from *Swietenia macrophylla* King seed that can be beneficial for improving pancreatic beta cell in diabetic rat. This study will help the researcher to uncover critical areas of diabetes that many researchers were not able to explore. Thus a new theory on antidiabetic agent, may be arrived at.

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