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Original Research Article

Seven-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one reduces atherogenic index and Nrf2 and GPx gene expressions in hyperlipidemic rats

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Abstract

Purpose: *To investigate the effect of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one isolated from mahogany (Swietenia macrophylla King) seeds on atherogenic index, expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and expression of the glutathione peroxidase (GPx) genes in hyperlipidemic rats.*
Methods: *A total of 25 rats male aged 8 weeks and weighing an average of 200 g were used. They were divided into five groups as follows: (I) normal (N), (II) hyperlipidemic (HL), (III) hyperlipidemic rats treated with simvastatin (HL+SV), (IV and V) hyperlipidemic rats treated with 30 or 90mg, respectively, of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200 g body weight per day for 4 weeks. Atherogenic index (AI) was calculated from the levels of triglyceride (TG) and high-density lipoprotein (HDL) while Nrf2 and GPx gene expressions were determined by quantitative real-time polymerase chain reaction (qRT-PCR).*
Results: *Two different doses of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one in hyperlipidemic rats significantly reduced their atherogenic index ($p < 0.05$). Nrf2 and GPx expression levels were lower than ($p > 0.05$) those of hyperlipidemic group.*
Conclusion: *Seven-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one reduces the atherogenic index and expression levels of Nrf2 and GPx genes in hyperlipidemic rats. Thus, this compound has potential as an antihyperlipidemic agent*

Keywords: *Hyperlipidemia, Oxidative stress, Nrf2, GPx, Gene expression*

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INTRODUCTION

Hyperlipidemia, a form of dyslipidemia, is a lipid metabolism disorder characterized by increased levels of cholesterol and/or triglycerides. Hyperlipidemia is caused by many factors, including an unhealthy lifestyle, low physical activity, and a high-fat diet [1].

Oxidative stress causes translocation of the nuclear factor erythroid 2-related factor 2 (Nrf2) from the cytoplasm to the nucleus, where it combines with the antioxidant-responsive

element (ARE) and induces the expression of antioxidant genes such as *superoxide dismutase* (*SOD*) and *glutathione peroxidase* (*GPx*) [2]. Nrf2 is a regulator of oxidative stress in the cytoplasm and is removed by Kelch-like ECH-associated protein 1 (Keap1) for proteosomal degradation [3,4]. Keap1 regulates the expression of Nrf2 and enzymes involved in the Nrf2-Keap1 downstream signaling pathway, such as GPx. In hyperoxic conditions, the conformation of the Keap1 protein is changed and its binding to Nrf2 is prevented. Then, *Nrf2* expression is increased, which also increases the expression of downstream antioxidants and resistance to oxidative damage [5].

Oxidative stress can be prevented by endogenous and exogenous antioxidants. Endogenous antioxidants protect cells from damage caused by oxidative stress. In hyperlipidemia, endogenous antioxidants are not sufficient to prevent cell damage. Therefore, exogenous antioxidants are needed to protect cells from oxidative stress–related diseases.

Many studies have reported that flavonoid compounds reduce oxidative stress. A previous study found that 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one isolated from mahogany seeds (*Swietenia macrophylla* King) influences the expression of some genes involved in carbohydrate metabolism in a rat Type 2 diabetes mellitus (T2DM) model [6]. This study aimed to evaluate the effects of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one flavonoid groups on the atherogenic index and expression of *Nrf2* and *GPx* genes in hyperlipidemic rats.

EXPERIMENTAL

Animals

Twenty-five male rats (*Rattus norvegicus*), aged 8 weeks old, with an average weight of 200g, were obtained from the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were housed in individual cages and acclimatized to laboratory conditions (22–25°C) and a 12-h daylight cycle for 7 days with free access to food and water. The standard diet was AIN 93 M consisting of (g/kg): casein 24 %, DL-methionine 0.30 %, corn starch 61%, vitamin mixture 1 %, mineral mixture 3.5 %, and choline chloride 0.2 %, with 5 % alpha cells and 5 % corn oil. This study was approved by the Health and Medical Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (approval no.KE/FK/0729/EC/2018) and was conducted in accordance with the guidelines of Declaration of Helsinki issued in 1964 and amended in 1996 [7]

Experimental design

Twenty-five rats were divided into five groups: normal (N), hyperlipidemic (HL), hyperlipidemic rats treated with simvastatin (HL+SV), hyperlipidemic rats treated with 30 or 90mg 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen -4-one per 200g body weight per day (HL+30 or HL+90, respectively). The drugs were administered orally by gavage for 4 weeks.

Atherogenic index measurement

Assay kits for serum triglyceride (TG) and high-density lipoprotein (HDL) were purchased from Dyasis® (Holzheim, Germany). The atherogenic index (AI) was calculated using the following equation: AI=log (TG/HDL) [8].

Gene expression analysis using quantitative polymerase chain reaction (qPCR)

The cDNAs were synthesized using the iScript cDNA Synthesis kit (Bio-Rad) according to the manufacturer's protocol. The SsoFast™ Evagreen® Supermix (Bio-Rad) was used for qPCR on an iCycler Model CFX 96 Real-Time System (Bio-Rad). The qPCR reaction was conducted for each gene (*Nrf2* and *GPx*) using the same internal control *Beta actin* gene (Table 1). The program for cDNA amplification was 5 min at 95°C, followed by 40 cycles at 95°C for 60 sec, and 57°C for 60sec.

Table 1: Primer sequences for cDNA amplification

Nrf2	F 5′-GCCTTCCTCTGCTGCCATTAGTC-3′ R 5′-GTGCCTTCAGTGTGCTTCTGGTT-3′
GPx	F5′-GCTGCTCATTGAGAATGTCG-3′ R 5′-GAATCTCTTCATTCTTGCCATT-3′
Beta actin	F 5′-ACGGTCAGGTCATCACTATCG- 3′ R 5′- GGCATAGAGGTCTTTACGGATG-3′

Statistical analysis

The results are expressed as mean ± standard deviation (SD). Differences in atherogenic indices among the groups before and after treatment with 30 or 90mg 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day (HL+30 or HL+90, respectively) were analyzed by one-way ANOVA followed by the Games-Howell test. The expression levels of liver *Nrf2* and *GPx* genes after treatment were compared by one-way ANOVA followed by the Games-Howell tests. Paired *t*-tests were used to analyze the

atherogenic index before and after treatment. Differences were considered significant at $p<0.05$.

RESULTS

After 4 weeks of daily administration of 30 or 90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight, serum triglyceride levels decreased significantly ($p<0.05$). The results are shown in Table 2. As shown in Table 3, HDL levels increased significantly ($p<0.05$) after administration of 30 or 90 mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day for 4 weeks.

Administration of 30 or 90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day for 4 weeks significantly reduced the atherogenic index ($p<0.05$). The greatest decline was observed in the group treated with 90 mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight (Table 4).

Administration of 30 or 90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day for 4 weeks significantly reduced the relative gene expression levels of *Nrf2* in rat liver tissue ($p>0.05$, Figure 1).

Administration of 30 or 90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day for 4 weeks significantly reduced the relative gene expression levels of *GPx* in rat liver tissue ($p>0.05$, Figure 2).

DISCUSSION

This study showed that administration of 30 or 90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one per 200g body weight per day for 4 weeks lowered the levels of triglycerides, increased HDL levels, and reduced the atherogenic index ($p<0.05$). Based on these results, we conclude that 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one has the potential to treat dyslipidemia.

Mallick and Khan [9] reported that sweet oranges (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) produce antioxidants that have hypolipidemic effects in rats fed with a cholesterol-rich diet. Another study showed that chrysin flavonoid from honey, propolis, and plant extracts exerted

Table 2: Effect of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one on serum triglyceride levels (mg/dL) in a hyperlipidemic rat

Group	Mean (mg/dL) ± SD		Mean difference (95% CI)	*P*-value
	Before	***After***		
Normal	67.47 ± 2.75	68.12 ± 2.41	–0.65 (–1.07; –0.23)	0.013
Hyperlipidemic	135.97 ± 9.49	138.05 ± 9.02	–2.08 (–3.85; –0.30)	0.031
HL + SV	132.88 ± 5.55	79.25 ± 3.04	53.63 (43.25; 64.01)	0.000
HL + 30	126.52 ± 7.86	92.78 ± 3.13	33.74 (21.85; 45.64)	0.001
HL + 90	133.05 ± 7.60	86.17 ± 3.04	46.88 (38.91; 54.85)	0.000

Table 3: Effect of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one on HDL levels (mg/dL) in a hyperlipidemic rats

Group	Mean (mg/dL) ± SD		Mean difference (95% CI)	*P*-value
	Before	After		
Normal	73.63 ± 2.46	75.21 ± 2.92	–1.58 (–3.03; –0.13)	0.039
Hyperlipidemic	24.25 ± 2.69	23.32 ± 2.86	0.93 (0.35; 1.50)	0.011
HL + SV	25.48 ± 2.83	61.31 ± 3.73	–35.83 (–42.14; –29.52)	0.000
HL + 30	27.08 ± 2.04	46.79 ± 1.86	–19.72 (–23.20; –16.23)	0.000
HL + 90	26.37 ± 3.83	55.44 ± 1.85	–29.07 (–35.24; –22.91)	0.000

Table 4: Effect of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one on atherogenic index in a hyperlipidemic rat

Group	Atherogenic index		Mean difference (95%CI)	*P*-value
	Before	***After***		
Normal	–0.04 ± 0.023	–0.04 ± 0.018	0.005 (–0.006; 0.016)	0.269
Hyperlipidemic	0.75 ± 0.070	0.77 ± 0.070	–0.024 (–0.036; –0.011)	0.006
HL + SV	0.72 ± 0.057	0.11 ± 0.037	0.607 (0.507; 0.707)	0.000
HL + 30	0.67 ± 0.030	0.30 ± 0.010	0.373 (0.340; 0.406)	0.000
HL + 90	0.71 ± 0.091	0.19 ± 0.003	0.515 (0.402; 0.628)	0.000

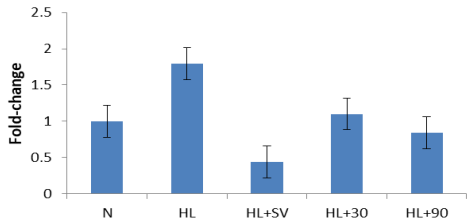

Figure 1: *Nrf2* relative expression level. N, normal rats; HL, hyperlipidemic rats; HL+SV, hyperlipidemic rats+simvastatin; HL+30, hyperlipidemic rats+30mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one; and HL+90, hyperlipidemic rats+90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one

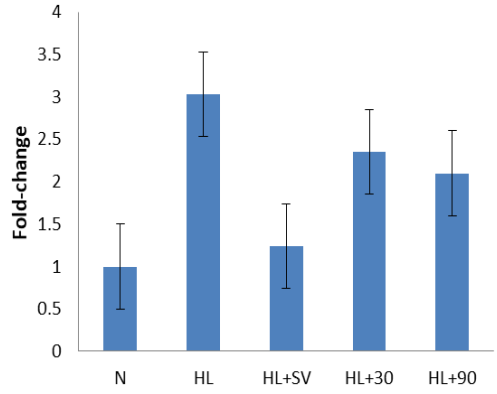

Figure 2: *GPx* relative expression levels. N, normal rats; HL, hyperlipidemic rats; HL+SV, hyperlipidemic rats+simvastatin; HL+30, hyperlipidemic rats+30mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one; and HL+90, hyperlipidemic rats+90mg of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one

antioxidant and hypolipidemic effects on Triton WR-1339–induced hyperlipidemia in female C57BL/6 mice [10]. Zeni *et al*. [11] reported that black mulberry (*Morus nigra*) leaf extracts contained abundant polyphenols, particularly chlorogenic acid. Chlorogenic acid had beneficial effects by reducing cholesterol and controlling fatty accumulations in the liver by increasing peroxisome proliferator–activated receptor alpha (PPAR-α) [12].

In the present study, rats with dyslipidemia had higher *Nrf2* expression levels than normal rats, and treatment with 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one isolated from mahogany (*Swietenia macrophylla* King) seeds reduced the Nrf2 expression levels. These results suggest that 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one has antioxidant properties that reduce dyslipidemia-induced oxidative stress. Polyphenolic compounds and antioxidant activity also were detected in extracts of white (*Morus alba*) and black (*Morus nigra*) mulberry leaf [13-15].

GPx is localized in the cytoplasm, in the mitochondrial matrix, and in insoluble forms associated with membranes involved in the neutralization of lipid hydroperoxides [16]. The GPx function is responsible for lowering hydrogen peroxide (H_2O_2) levels and converting lipoperoxides and organic hydroperoxides into suitable hydroxylation compounds, which are less reactive. Quercetin has *in vivo* antioxidant properties, and quercetin treatment increases hepatic GPx expression in older rats [17]. Research by Martin *et al* [18] showed that cocoa polyphenolic extract was an effective inducer of GPx. These reports are consistent with the study of Phachonpai *et al* [19], which reported that quercetin added to rat diet significantly increased the superoxide dismutase (SOD), catalase (CAT), and GPx activities [19,20].

CONCLUSION

The results of this study indicate that 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chromen-4-one lowers the atherogenic index and expression levels of *Nrf2* and *GPx* genes in hyperlipidemic rats, and there may be suitable for management of hyperlipidemia but further investigations are required to ascertain this.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". All authors read and approved the manuscript for publication.

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