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ลิโมนินฟื้นฟูการสร้างอสุจิและการสร้างฮอร์โมนเทสโทสเตอโรนในหนูอ้วน

ธนพัฒน์ สาระรัตน์[1], อนุสรณ์ เปาะสะเกษ[1,2], พวงรัตน์ ภักดีโชติ[1,2], พัชรวิภา มณีไสย[1,2], วรรณภา อิชิดะ[1,2], โสภิดา ภู่ทอง[1*]
[1]ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น 40002
[2]กลุ่มวิจัยหัวใจและหลอดเลือด มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น 40002

Limonin Improves Testicular Spermatogenesis and Testosterone Production in Obese Rats

Thanapat Sararat[1], Anuson Poasakate[1,2], Poungrat Pakdeechot[1,2], Putcharawipa Maneesai[1,2], Wannapa Ishida[1,2], Sophida Phuthong[1*]
[1]Department of Physiology, Khon Kaen University, Khon Kaen, Thailand, 40002
[2]Cardiovascular Research Group, Khon Kaen University, Khon Kaen, Thailand, 40002

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บทคัดย่อ

หลักการและวัตถุประสงค์: โรคอ้วนมีความเกี่ยวข้องกับความผิดปกติของอัณฑะ ส่งผลให้การสร้างอสุจิและฮอร์โมนเทสโทสเตอโรนลดลง จนเกิดภาวะมีบุตรยากในเพศชาย การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของลิโมนินต่อการสร้างอสุจิและเทสโทสเตอโรนในหนูอ้วน

วิธีการศึกษา: หนูทดลอง Sprague-Dawley เพศผู้ถูกแบ่งออกเป็น 3 กลุ่ม ได้แก่ กลุ่มควบคุม กลุ่มโรคอ้วน และกลุ่มโรคอ้วนที่ได้รับลิโมนิน ใน 4 สัปดาห์สุดท้ายของการทดลอง (LM 100) เมื่อสิ้นสุดการทดลอง วัดน้ำหนักตัว ไขมันในช่องท้อง เทสโทสเตอโรนในซีรัม จำนวนและการเคลื่อนที่อสุจิ และตัวชี้วัดความเครียดออกซิเดชันในอัณฑะ

ผลการศึกษา: โรคอ้วนส่งผลเพิ่มน้ำหนักและมวลไขมัน ลดจำนวนและการเคลื่อนที่ของอสุจิ และเทสโทสเตอโรนในซีรัม อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม ($p<0.05$) การให้ลิโมนินช่วยลดน้ำหนักและมวลไขมันได้อย่างมีนัยสำคัญ ($p<0.05$) และเพิ่มจำนวนและเปอร์เซ็นต์การเคลื่อนที่ของอสุจิและระดับเทสโทสเตอโรนในซีรัม ($p<0.01$) ตัวชี้วัดความเครียดออกซิเดชั่น; MDA ในอัณฑะของหนูอ้วนเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ และการได้รับลิโมนินช่วยลดระดับ MDA ในอัณฑะอย่างมีนัยสำคัญ ($p< 0.01$)

สรุป: ลิโมนินสามารถใช้เป็นผลิตภัณฑ์เสริมอาหารเพื่อการป้องกันความผิดปกติของอัณฑะที่เกิดจากโรคอ้วน

คำสำคัญ : ลิโมนิน, โรคอ้วน, ความผิดปกติของอัณฑะ, เทสโทสเตอโรน

Abstract

Background and Objective: Obesity has been found to be associated with testicular dysfunction, resulting in decreased sperm quality, testosterone production and consequently male infertility. The aim of this study was to determine the effects of limonin on sperm count and motility and serum testosterone level in obese rats.

Methods: Male Sprague-Dawley rats were divided into the following groups; the control, the obesity (Obesity), the obesity treated with limonin at dose 100 mg/kg (LM 100) groups at the last four week of the experiment. At the end of experiment, body weight and retroperitoneal fat were measured. Sperm count and motility measurement was performed. Serum testosterone and testicular MDA level were investigated.

Results: Obesity significantly elevated body weight and fat accumulation while decreased the caudal epididymal sperm concentrations and percentages of sperm motility and serum testosterone compared to the control groups. ($p<0.05$). Treatment with limonin significantly reduced body weight and fat mass ($p<0.05$). An improvement of sperm concentrations and percentages of sperm motility and serum testosterone was observed in LM 100 group ($p<0.01$). Testicular MDA significantly increased in Obesity, while limonin treatment significantly diminished testicular MDA level ($p<0.01$).

Conclusion: Limonin treatment potentially represents as a supplementation for the prevention of testicular dysfunction induced by obesity.

Keywords: Limonin, obesity, testicular, dysfunction, testosterone

*Corresponding author: Sophida Phuthong, E-mail: sophiph@kku.ac.th

Introduction

Obesity, a medical condition characterized by increased body weight with excessive fat accumulation may have several adverse health conditions including cardiovascular diseases, metabolic disorder, and reproductive dysfunction[1]. Numerous studies have been demonstrated that obesity can lead to male infertility by changes in testicular spermatogenesis and hormone production as evidence by decline of semen quality and hypogonadism[2-4]. Exposure to diet containing high fat has been considered as a cause of obesity development due to an imbalance between energy intake and energy expenditure. Previous studies in animal model have found that obese rat showed a reduced semen quality and diminished testosterone level compared to control diet-fed rat[5, 6]. The mechanism by which obesity causes impaired spermatogenesis and testosterone production may associated with oxidative stress[7].

Limonin, a natural tetracyclic triterpenoid compound is widely found in citrus and other plants. It exerts numerous biological effects such as anti-cancer[8], anti-inflammation[9], antibacterial[10], and anti-oxidant[11, 12]. In the animal model of obesity, limonin showed anti-obesity effect as evidence by reduced fat accumulation, plasma triglyceride, and cholesterol[13]. Moreover, limonin can reduced the elevated serum levels of oxidative stress marker; malondialdehyde (MDA) in metabolic syndrome mice[14]. Thus, limonin might have beneficial effect on obesity-induced testicular impairment by ameliorated oxidative stress. However, it is still unknown if limonin can improve testicular dysfunction through the reduction of oxidative damage in the testis. Thus, this study was designed to confirm the alteration in testicular spermatogenesis and testosterone production in obesity and to evaluate the effect of limonin on testicular dysfunction in obesity.

Methods

Chemicals

Limonin was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China).

Animals

Male Sprague-Dawley rats weighing 220-250 grams were used in the experiments following the ethical guideline for work with animals which was approved by the ethics committee of Khon Kaen University (No. AEKKU-NELAC IACUC-KKU-51/64). The animals were housed in the HAVC (Heating, Ventilation and Air-Conditioning) system under well-controlled condition of temperature (23±2 °C), and a 12:12 h. light-dark cycle and were given standard chow diet (SCD) with tab water or diet containing high fat with 15% fructose water at Northeast Laboratory Animal Center.

Experimental designs

Fifteen rats were randomly distributed into 3 groups (n=5/group) including 1) Control group; rats fed with SCD with tab water, diet-induced obesity group (Obesity); rat fed with diet containing high fat with 15% fructose for 16 weeks, limonin treated group (LM 100); rats fed with diet containing high fat with 15% fructose for 16 weeks and were intragastrically administered with limonin at dose 100 mg/kg BW/day for the last 4 weeks (at week 12^{th}).

Body weight was monitored weekly. At the end of experiment, rats were sacrificed. Blood sample was collected for testosterone level analysis. Semen was collected from cauda epididymis for sperm concentration and motility assessment.

Measurement of sperm concentration and motility

Semen was place in microtube and diluted with 4 mL of PBS solution (pre-warm at 37 °C). 10 µL of semen solution was dropped onto hematocytometer for sperm motility and concentration assessment. Evaluation of sperm motility and concentration was done under light microscope at 400× magnification. Sperm concentrations were expressed as number x 10^6 cells/ml. The motility was expressed as percentage of sperm motility.

Serum testosterone analysis

Blood sample was collected form abdominal aorta. Serum sample was obtained by centrifugation (3000 rpm for 15 min). Serum testosterone concentration was measured by using a Cayman's Testosterone ELISA Kit according to the manufacturer's instruction (Cayman Chemical, Michigan, USA). In brief, 50 µL of sample testosterone-acetylcholinesterase tracer and testosterone ELISA antiserum were added to each well of the ELISA plate. The ELISA plate was incubated at room temperature for 2 hours on an orbital shaker. The wells were then aspirated and washed five times with wash solution. 200 ul of Ellman's reagent was added to each well and the plate was incubated in a dark place for 90 minutes at room temperature. The quantification of testosterone concentration was obtained by a microplate reader at absorbance of 405 nm.

Oxidative stress marker assessment

Testes was homogenized following the protocol described by Wang and coworkers[15]. In brief, 150 µL of supernatant form tissue was incubated for 10 min then 0.6 % TBA will be added to supernatant. The mixture was boiled in a water bath for 15 min. After cooling to room temperature, the mixture was centrifuged at 1,000 g for 15 min. The absorbance of the supernatant was measured at 532 nm against blank containing all the regents excepts test sample.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and post-hoc Tukey test were performed to determine the differences among the groups. A p-value < 0.05 was considered to be statistically significant.

Results

Body and organ weights

Table 1 The effect of limonin on body weight and fat accumulation in diet-induced obesity

	Control	Obesity	LM 100
Body weight (g)	678.80 ± 8.31	949.20 ± 46.96**	768.80 ± 23.5#
Retroperitoneal fat/BW	3.09±0.28	7.51±0.69**	4.41±0.43##

Data are expressed as mean ± SEM, n = 5. ** p < 0.01 compared to Control, # p < 0.05, ## p < 0.01 compared to Obesity

Table 1 presented the effect of limonin on body weight and fat accumulation on diet-induced obesity in rats. In comparison to control rat, obesity in rat significantly increased body weight and retroperitoneal fat/body weight (p<0.01). Treatment with limonin significantly decreased the body weight (p<0.05) and retroperitoneal fat/body weight (p<0.01).

Body weight gain

Figure 1 presented the effect of limonin on body weight gain during 16 week of the experiment. Obesity group showed significantly difference in body weight at the week 12th compared to the control (p<0.05). However, treatment with limonin demonstrated lower body weight compared to obesity at week 16th.

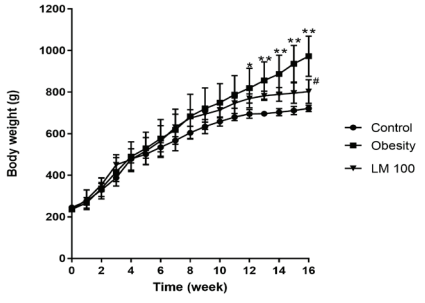

Figure 1 Effect of limonin on body weight gain during 16 weeks of the experiment. Data are presented as mean ± SEM, n = 5. * p < 0.05, ** p < 0.01 compared to Control, # p < 0.05 compared to Obesity.

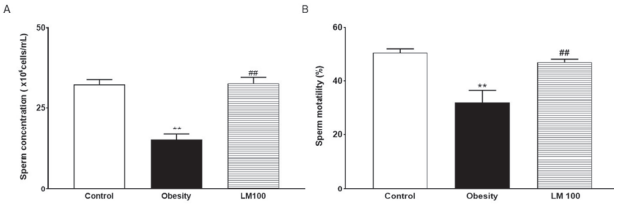

Figure 2 Effect of limonin on A) sperm concentration and B) sperm motility in obese rats. Data are presented as mean ± SEM, n = 5. ** p < 0.01 compared to Control, ## p < 0.01 compared to Obesity.

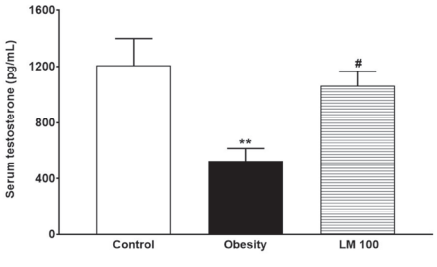

Figure 3 The effect of limonin on testosterone level in obese rats. Data are presented as mean ± SEM, n = 5, ** p < 0.01 compared to Control, # p < 0.01 compared to Obesity.

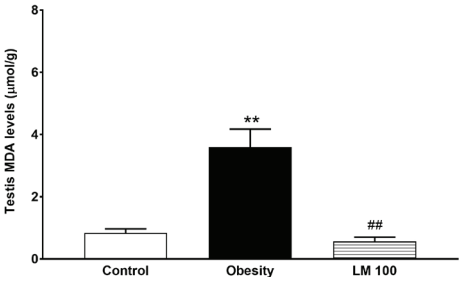

Figure 4 The effect of limonin on testicular malondialdehyde (MDA) level in obese rats. Data are presented as mean ± SEM, n = 5, ** p < 0.01 compared to Control, ## p < 0.01 compared to Obesity.

Sperm quality parameters

Figure 2 presented the effect of limonin on sperm concentration and motility in diet-induced obesity. In obese rats, the caudal epididymal sperm concentrations

and percentages of sperm motility significantly decreased compared to the control (sperm concentration; 32.32 ± 1.50 vs. 15.2 ± 1.50 × 10^6 cells/mL, sperm motility; 50.54% ± 1.41 vs. 31.89% ± 4.71). Limonin treatment significantly restored sperm concentrations and motility (sperm concentration; 32.68 ± 1.68 × 10^6 cells/mL, sperm motility; 47.14% ± 1.09).

Serum testosterone level

A significantly decreased in plasma testosterone level was observed in diet-induced obesity in rats compared to the control (1207.08 ± 187.08 vs. 526.45 ± 89.68 pg/mL) (p< 0.01). Treatment with limonin significantly improved testosterone level (1063.53 ± 100.00 pg/mL) (p<0.05) as presented in Figure 3.

Testicular malondialdehyde (MDA) assay

Figure 4 presented the effect of limonin on testicular malondialdehyde (MDA) level in diet-induced obesity. The levels of MDA in testicular tissue were significantly elevated in obese rats compared to the control (0.742 ± 0.10 vs. 3.99 ± 0.51 μmol/g) (p<0.01). Interestingly, testicular MDA were significantly attenuated by limonin treatment (0.576 ± 0.08 μmol/g) (p<0.01).

Discussion

The objective of this study was to investigate the alteration in testicular spermatogenesis and testosterone production in obese rat and to evaluate the improving effect of limonin supplementation. Diet containing high fat fed rats showed an increased body weight and fat accumulation compared to the control which confirmed the effect of a diet containing high fat on the development of obesity. Limonin treatment in obesity in rats showed a reduction in body weight and fat pad deposition indicating an improving effect of limonin on diet-induced obesity.

In addition, this study demonstrated that along with an increased body weight and fat accumulation, obesity group presented a decreased sperm concentration and motility accompanied by declined in serum testosterone. These finding were consistent with previous studies in obese and/or metabolic syndrome rats[16-20]. It is well known that Sertoli cell acts as supporting cell for spermatogenesis while Leydig cell contributes to testosterone production that is an essential hormone for testicular and Sertoli cell development, and spermatogenesis[17, 18]. Previous studies showed that Leydig cell and Sertoli cell dysfunction have been observed in metabolic syndrome, obesity and diabetes mellitus[21-23]. Moreover, reduction of testosterone observed in obese rats could be related to decline in number of Leydig and Sertoli cells in the testis[24]. Thus, our results indicated that obesity might impaired semen quality by disrupting the production of testosterone.

Several studies have been explained the connection between obesity and impaired spermatogenesis as a result from obesity-induced oxidative stress. Diet-induced obesity is related to oxidative imbalance showing excessive production of reactive oxygen species (ROS) and/or impaired antioxidant capacity[25-28]. As shown in the present study, the level of testicular MDA, marker for lipid peroxidation were significantly elevated in obese rat indicating oxidative stress in the testis. Previous studies have postulated that oxidative damage to testicular cell might lead to an increased rate of germ cell and Leydig cells apoptosis resulting in impaired spermatogenesis[29, 30]. Therefore, this study suggested that testicular function regarding spermatogenesis and testosterone production might be compromised by obesity-induced oxidative damage in the testis.

Limonin, a bioactive compound found in citrus plants has been proved to hold an antioxidant property which showing a diminished ROS production and decreased plasma MDA level along with an increased glutathione S-transferase (GST) activity in several tissues[12, 14, 28, 31]. In the present study, treatment with limonin for four weeks significantly restored the sperm concentration and motility and testosterone level. Moreover, limonin supplementation significantly decreased the testicular MDA level which confirms beneficial effect of limonin on testicular oxidative stress. This finding is also consistent with a previous report presenting that limonin can reduced the elevated serum levels of MDA in metabolic syndrome mice[14] and increased the glutathione and glutathione dependent enzymes in the testes[32]. Therefore, beneficial effect of limonin on testicular dysfunction in this study could be due to its antioxidant capacity. However, to confirm the precise mechanism, antioxidant parameter remains need to be elucidated in further study.

Conclusion

This study indicated that limonin dramatically improves diet-induced metabolic disorder and has beneficial effect against the testicular dysfunction through ameliorated oxidative stress. This finding provides a theoretical basis for the use of limonin as a supplementary food for obesity-associated testicular dysfunction

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