นิพนธ์ต้นฉบับ • Original Article

ผลปลอดพิษต่อระบบสืบพันธุ์ของสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าวในหนูไมซ์เพศผู้

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Non-toxic Effect of *Momordica cochinchinensis* Spreng Aril Extract on Reproductive System of Male Mice

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หลักการและวัตถุประสงค์: ฟักข้าว (*Momordica cochinchinensis* Spreng) เป็นพืชในเขตร้อนชนิดหนึ่งที่มีสารพฤกษเคมีต่างๆ โดยเฉพาะอย่างยิ่งแคโรทีนอยด์และองค์ประกอบของฟีนอลิก แม้ว่าฟักข้าวจะถูกศึกษาเกี่ยวกับฤทธิ์ทางเภสัชวิทยาเป็นจำนวนมาก แต่ผลของสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าวต่อพารามิเตอร์ต่างๆ ของระบบสืบพันธุ์เพศผู้ยังไม่มีการศึกษามาก่อน การศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อศึกษาผลความเป็นพิษของเยื่อหุ้มเมล็ดฟักข้าวต่อฮอร์โมนเพศ น้ำหนักและโครงสร้างอวัยวะของระบบสืบพันธุ์ รวมทั้งคุณภาพของสเปิร์มในหนูไมซ์เพศผู้

วิธีการศึกษา: หนูไมซ์เพศผู้ สายพันธุ์ ICR ถูกแบ่งเป็น 3 กลุ่ม ประกอบด้วยกลุ่มควบคุม กลุ่ม vehicle ที่ได้รับน้ำกลั่นและกลุ่มที่ได้รับสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าว (1,000 มก./กก. ต่อน้ำหนักตัว) เป็นเวลา 35 วันอย่างต่อเนื่อง เมื่อสิ้นสุดการทดลอง ทำการตรวจสอบระดับของฮอร์โมนเทสโทสเตอโรน น้ำหนักของอวัยวะสืบพันธุ์ต่างๆ คุณภาพของสเปิร์มและโครงสร้างของอัณฑะและหลอดเก็บสเปิร์มด้วยกระบวนการตัดย้อมเนื้อเยื่อแบบธรรมดา

ผลการศึกษา: การศึกษาครั้งนี้พบว่าหนูที่ได้รับสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าวมีน้ำหนักตัว น้ำหนักของอัณฑะ หลอดเก็บสเปิร์ม และต่อมสร้างน้ำเลี้ยงสเปิร์ม ความเข้มข้นของสเปิร์มและระดับของฮอร์โมนเทสโทสเตอโรนไม่แตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) เมื่อเปรียบเทียบระหว่างกลุ่มควบคุมและกลุ่ม vehicle นอกจากนี้ยังไม่พบการทำลาย

Background and Objective: *Momordica cochinchinensis* (MC) Spreng, a tropical plant, has various phytochemical components especially carotenoid and phenolic compounds. Although MC was mostly studied about pharmacological properties, its effects on male reproductive parameters have never been reported. This study aimed to investigate the toxic effects of aril MC on sex hormone, body weight, reproductive organ structures, and sperm quality in male mice.

Methods: ICR male mice were randomly divided into three groups (control, vehicle, and aril MC [1000 mg/kg BW] groups) for 35 consecutive days. At the end of experiment, testosterone level, weights of reproductive organs, sperm quality, and structures of testis and epididymis by using routine light microscope histology were examined.

Results: This study found that weights of body, testis, epididymis, and seminal vesicle, sperm concentration, and testosterone levels in aril MC received mice were not significantly different ($P>0.05$) as compared to those of control and vehicle groups. Additionally, the damages of histological structure of testicular and epididymal tissues in MC treated group were not observed as compared to the control.

Conclusion: Aril MC extract was not toxic to the reproductive organs of male mice.

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ของโครงสร้างทางจุลกายวิภาคของเนื้อเยื่ออัณฑะและหลอดเก็บสเปิร์มในกลุ่มที่ได้รับสารสกัดเมื่อเปรียบเทียบกับกลุ่มควบคุม

สรุป: สารสกัดจากเยื่อหุ้มเมล็ดฟักข้าวไม่มีความเป็นพิษต่ออวัยวะต่างๆ ในระบบสืบพันธุ์ของหนูไมซ์เพศผู้

Keywords: *Momordica cochinchinensis* Spreng, reproductive parameters, testis, epididymis, male mice

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Introduction

Recently, medicinal plants are widely used in traditional treatments to relieve severity of many diseases. The use of alternative medicine tends to increase in the modern societies because of their antioxidant capacity and less toxicity. Although medicinal plants have many advantages in treatments, long-term use may affect physiological systems, including male reproductive system.

Momordica cochinchinensis Spreng (MC) is botanically classified in the Cucurbitaceae family and a tropical plant found in China, Myanmar, Laos, Cambodia, Vietnam, Malaysia, Bangladesh and Philippines including Thailand[1]. Previous studies showed that this plant has various phytochemical components[2-9]. Pharmacological properties of MC consist of antioxidant activities[7], anti-inflammatory effect[10], immunomodulatory activity[11], anti-tumor properties[12], and anti-cancer activity[13,14]. Additionally, aril part of MC was documented to have anti-cancer activity[15] and anti-hyperglycemia[16,17]. In Thailand, this plant is most popular in consumption and research to be developed as skin care products and food supplements. Since many reports showed the toxic sensitivity of various plants on male reproductive system[19,20], such effects of aril MC have never been studied. Therefore, this study aimed to investigate the toxic effects of aril MC on reproductive parameters in adult male mice.

Methods

Plant collection and extraction

M. cochinchinensis (MC) Spreng was cultured at Kuchinarai district, Kalasin province, Thailand from January to May, 2014. The plants were authenticated for its actual species by Prof. Dr. Pranom Chantaranothai, Department of Biology, Faculty of Science, Khon Kaen University, Thailand and kept in the Herbarium (#Apichakan Sampannang 01 [KKU]), Khon Kaen University, Thailand. The fresh-ripe MC arils (8000 g) were mixed with distilled water in the ratio (1:1) and then filtered by the nylon cloth. The aril MC filtrate was dried using Spray dryer. Percentage (%) yield of MC aril extract was 10.50.

Animals and treatment regime

Twenty-one ICR male mice, aged 6-8 weeks, were purchased from National Laboratory Animal Center (NLAC), Mahidol University, Salaya, Nakhon Pathom, Thailand. Mice were acclimatized at Northeast Laboratory Animal Center (NELAC), Khon Kaen University, Thailand. This study was approved by the Animal Ethics Committee of NELAC, Khon Kaen University, Thailand, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (ref. No. 0514.1.12.2/35 with record No. AEKKU-NELAC 29/2557). Mice were randomly divided into three groups (control, vehicle, and MC treated groups). In control group, mice were not treated with DW or MC extract. Mice in vehicle group were administered with only DW. In MC treated group, animals were orally received with aril MC 1000 mg/kg BW for 35 consecutive days (one spermatogenesis cycle). At the end of experiment, all animals were euthanized by cervical dislocation. Blood samples by cardiac puncture were centrifuged by microcentrifuge (Microfuge 22R Centrifuge), 800 rpm at 4°C for 5 minutes to collect serum. The serum was delivered to the radio-chemical unit of Srinagarind hospital, Department of Radiology, Faculty of Medicine,

Khon Kaen university, Thailand for measurement the levels of testosterone hormones by using radioimmuno assay.

Epididymal sperm concentration

Sperm concentration analysis was performed as previously described by Iamsaard et al[19]. Briefly, sperm fluid was collected from epididymis and vas deferens. Its fluid was dipped and re-suspended in 1 ml of phosphate buffer saline (PBS). Then re-suspended sperm was centrifuged (3000 rpm, 2 min) to wash and separate the mature sperm pellet from supernatant. To analyze the sperm concentration, the sperm pellets were re-suspended with fresh 1 ml PBS before dilution. The diluted sperm (1:20 dilution) were triplicately counted in each animal using a Neubauer's counting chamber.

Histopathological examinations of the testes and epididymes

Testes and caudal epididymes were fixed with 10% formalin and embedded with paraffin and sectioned by microtome about 5 μm thicknesses. The tissue sections were deparaffinized and stained by hematoxylin and eosin (H&E) before histological observations under light microscope.

Statistical analysis

All quantitative results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan test were used SPSS program (version 16) software to examine the significance of differences among sets of data. The level of statistical significance was $p<0.05$.

Results

Aril MC extract did not affect final body weight, absolute and relative weights of testis, seminal vesicle, and epididymis plus vas deferens as compared to control and vehicle groups (Table 1). Additionally, sperm concentration and testosterone levels in MC treated group were not changed as compared to the control as shown in Table 1 ($p>0.05$).

Table 1 Effects of aril MC on body weight, reproductive organ weights, sperm concentration, and testosterone levels

Parameters	Control	Vehicle	MC treated
Initial body weight (g)	32.8 ± 1.5	33.9 ± 1.3	33.8 ± 1.2
Final body weight (g)	42.6 ± 1.6	41.8 ± 2.3	41.9 ± 2.0
Absolute weight of testis ($x10^{-1}$g)	2.4 ± 0.4	2.8 ± 0.3	2.7 ± 0.3
Relative weight of testis ($x10^{-3}$g)	6.7 ± 0.9	6.7 ± 0.8	6.6 ± 0.8
Absolute weight of seminal vesicle ($x10^{-1}$g)	3.4 ± 0.4	3.5 ± 0.6	3.5 ± 0.6
Relative weight of seminal vesicle ($x10^{-3}$g)	7.9 ± 1.0	8.3 ± 1.4	8.5 ± 1.2
Absolute weight of epididymis plus vas deferens ($x10^{-2}$g)	7.4 ± 0.8	7.7 ± 0.6	7.7 ± 0.4
Relative weight of epididymis plus vas deferens ($x10^{-3}$g)	1.7 ± 0.2	1.9 ± 0.2	1.8 ± 0.1
Sperm concentration ($x10^{-6}$cell/ml)	64.4 ± 8.4	60.3 ± 4.3	62.0 ± 9.1
Testosterone levels (ng/ml)	0.7 ± 0.3	0.9 ± 0.3	0.7 ± 0.2

* Significant difference ($p<0.05$). Data are represented as mean ± S.D. (n = 7 mice each group).

In Figure 1, the results showed that gross morphologies of testis, seminal vesicle and epididymis plus vas deferens were not obviously altered as compared to that of control and vehicle groups, which they related to the results of absolute and relative weights of male reproductive organ as shown in Table 1.

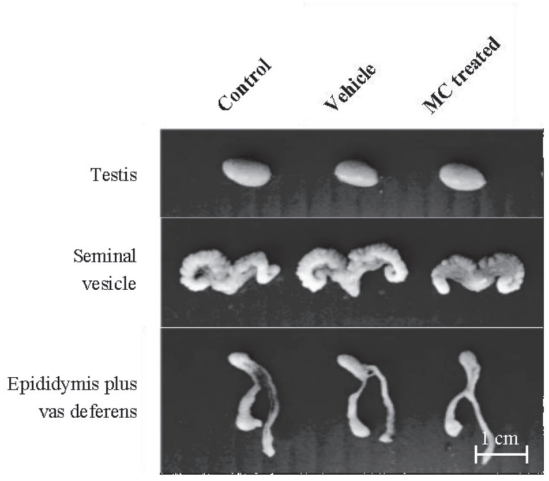

Figure 1 Representative gross morphologies of testis, seminal vesicle, and epididymis plus vas deferens compared among the control, vehicle, and MC treated groups.

In testicular histological observations, seminiferous tubules in MC treated group were found to have a normal arrangement of spermatogenic and Sertoli cells with no histopathological lesions (Fig. 2C). Similar to control and vehicle groups (Fig. 2A & B), interstitial compartment in MC treated group was not altered (Fig. 2C). Furthermore, the aril MC extract also did not damage the epididymal-epithelial cells and density of sperm mass within epididymal lumen (Fig. 2F) as compared to that of control and vehicle groups (Fig. 2D & E).

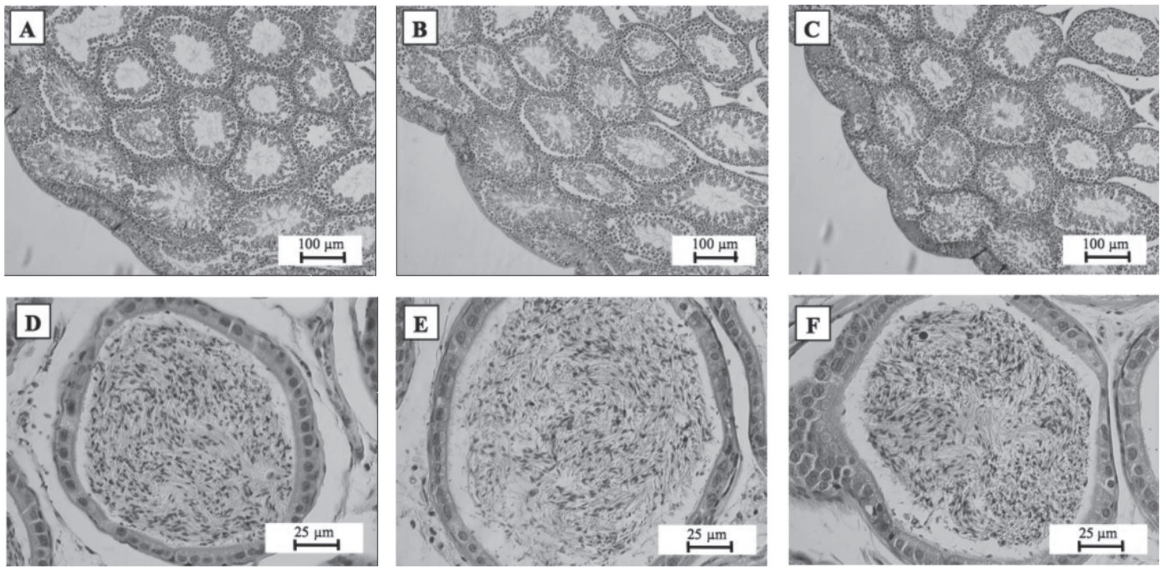

Figure 2 Showing representative histologies of testis and caudal epididymis among control (A & D), vehicle (B & E), and MC treated (C & F) groups, respectively.

Discussion

Since many studies have previously reported the pharmacological properties of aril *Momordica cochinchinensis* (MC) Spreng in both animal models and cell cultures[15-17], this study has demonstrated for the first time about the non-toxicity effect on male reproductive system. The results showed that aril MC extract was not toxic to the weights of body and reproductive organs, sperm concentration, testosterone levels, and histological structures of testis and epididymis (shown in Table 1, Fig. 1 & 2) in adult male mice. Our results may be similar to that of previous studies which also found that the aril MC extract did not affect morphological vital organs and behavioral changes[16-18]. Although the dose of MC extract (1000 mg/kg BW) used in this study was higher than that of previous investigations (200 and 400 mg/kg BW)[16,17], it did not adversely affect male reproductive parameters. It is possible that dose of the MC extract was lower than its LD_{50} documented in an acute toxicity study (2000 mg/kg BW)[18]. Recent findings indicate that a dose of aril MC extract used in this study is not toxic to male reproductive parameters in mice. In addition, it supports the safe dose of use for other pharmacological properties demonstrated previously[7, 10-18]. However, to investigate the chronic toxic effects on male reproductive system, the longer-experimental study of aril MC administration should be performed in further study to observe the side effects of this plant in prolonged consumptions.

Conclusion

Aril-MC extract did not affect the changes of testosterone levels, body weight, reproductive organ structures, and sperm quality in adult male mice.

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