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Momordica cochinchinensis (L.) Spreng. Aril Extract Prevents Adverse Reproductive Parameters of Male Rats Induced with Valproic Acid

El Extracto del Arilo *Momordica cochinchinensis* (L.) Spreng. Previene los Parámetros Reproductivos Adversos Inducidos con Ácido Valproico en Ratas Macho

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SUKHORUM, W.; SAMPANNANG, A. & SRIPANIDKULCHAI, B. & IAMSAARD, S. *Momordica cochinchinensis* (L.) Spreng aril extract prevents adverse reproductive parameters of male rats induced with valproic acid. *Int. J. Morphol., 34(3)*:870-876, 2016.

SUMMARY: This study aimed to investigate protective effect of *Momordica cochinchinensis* (MC) aril extract on adverse reproductive parameters of male rat induced with valproic acid (VPA) commonly used in treatment for antiepileptic diseases. Male Wistar rats were divided into 6 groups (control, VPA, 200 mg/kg BW of PE only, and 50, 100, 200 mg/kg BW MC+VPA, respectively). Animals were pretreated with aqueous MC extract for 23 days before co-administered with VPA induction for 10 days. At the end of experiment, all male reproductive parameters and testicular histology were examined. The results showed all doses of PE significantly protect the decrease the weights of epididymis and seminal vesicle but not of body and testicular weights. MC extract also increased sperm concentration and seminiferous tubular diameters in MC+VPA co-administrative groups. Moreover, testicular histology of MC+VPA groups showed significant declining of testicular histopathologies as compared to VPA group. It was concluded that *M. Cochinchinensis* aril extract can prevent adverse male reproductive parameters and testicular damage induced with VPA.

KEY WORDS: *Momordica cochinchinensis*; Valproic acid; Male reproductive parameters; Testicular damage; Rats.

INTRODUCTION

Momordica cochinchinensis (MC) Lour. Sprengel or Fag Kaow is a kind of melon; its fruit (also called Gac fruit) has been used as a traditional medicinal plant and alternative food in many countries especially in Asia, including Thailand. Recently, various scientific studies have shown that MC fruit extract has antioxidant and pharmacological properties (Kubola & Siriamornpun, 2011). MC has been demonstrated to have anti-cancer activities (Zheng *et al*., 2014; Jung *et al*., 2013a; Liu *et al*., 2012; Chuethong *et al*., 2007; Tien *et al*., 2005). In addition, it was also reported for its anti-inflammatory effect (Jung *et al*., 2013b) and angiogenesis (Kang *et al*., 2010). Moreover, MC extracts have been shown to have immunomodulatory activity (Tsoi *et al*., 2006) against H5N1 avian influenza (Rajput *et al*., 2007), foot and mouth disease (Xiao *et al*., 2007), Newcastle disease (Xiao *et al*., 2009), and infectious bursal disease (Rajput *et al*., 2010), respectively.

Valproic acid (VPA) is a common antiepileptic drug recently used in the administrations of brain disorders including epileptic seizures, panic attack, posttraumatic stress disorder, bipolar disorders, migraine, anorexia nervosa, anxiety disorder, and psychiatric conditions (Loscher, 2002). Although, VPA has various therapeutic properties (Gelder *et al*., 2006), many side effects have also been documented such as congenital malformations (Jentink *et al*., 2010; Witczak *et al*., 2010) and increased fibrosarcomas and adenocarcinomas of the uterus and cervix (Watkins *et al*., 1992). Particularly, VPA causes adverse effects of male reproductive parameter in human and animals. Previous studies have shown that the testosterone levels and semen qualities of epileptic men being treated with VPA significantly decreased (Herzog, 2008; Bauer *et al*., 2004; Isojärvi *et al*., 2004; Roste *et al*.

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2003). Bauer *et al*. have suggested that such adverse effects involved male infertility. In experimental animals, VPA not only significantly decreases FSH, LH, and testosterone levels but also damages testicular tissue (Khan *et al*., 2011; Bairy *et al*., 2010; Krogenase *et al*., 2008; Hamza & Amin, 2007; Svenberg Roste *et al*., 2002; Nishimura *et al*., 2000). Currently, many medicinal plants have been used in searching for alternative treatments to prevent such side effects of VPA administration (Sakr *et al*., 2014; Hamza & Amin). Although, *Momordica cochinchinesis* (MC) has been demonstrated to have antioxidant activities and various phyto-therapeutic properties, the preventive effects on adverse male reproductive parameters and testicular damage have never been reported. Therefore, this study aimed to demonstrate the protective effect of MC aril extract on male reproductive organs damaged in VPA-induced.

MATERIAL AND METHOD

Plant collection and extraction: The trees of *Momordica cochinchinensis* (Lour.) Spreng were planted at a farm in Kuchinarai district, Kalasin province, Thailand. All parts of M. cochinchinesis (MC) were identified for its actual species by Prof. Dr. Pranom Chantaranothai, a professional plant taxonomist, Department of Biology, Khon Kaen University. Thailand. The vouchers of dried plant samples were kept in the KKU plant herbarium (No. Apichakarn Sampannang 01 [KKU]). MC-ripe fruits were cultivated on May, 2014. Each fruit was cut through at middle line to collect arils (red color) covering seeds. The aril parts were separated from the seeds by gentle squeezing. After that, MC aril were mixed with distilled water (1:1 ratio), blended by stainless electric spinner (trademark, Tafal, BL116), and finally filtered by nylon cloth (2 layers) for 2 times. Then, MC juice was dried to produce powder extract by Spray dryer (Nitro A/S-Gladsaxevej 350-DK-2860 Soeborg, Denmark) at the Faculty of Phamacentical Sciences, Khon Kaen University, Thailand. The percentage yield and antioxidant activities were determined and approved by Assist. Prof. Somsak Nualkaew, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Mahasarakham University, Mahasarakham, Thailand.

Animals and treatment regime: Male Wistar rats (180-200 g) were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. They were acclimatized for 7 days before using in experiment at Northeast laboratory animal center (NELAC), Khon Kaen University, Thailand. Animals were maintained with commercially standard pellet diet and tap water ad libitum and housed in polycarbonate cages containing wood chip bedding under the controlled condition of a 12 h light/dark cycle. After acclimatization, 48 male rats were divided into six groups (n= 8 in each group) and were treated as shown in Table I. The study was approved by the Animal Ethics Committee of NELAC, KKU, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (ref. No. 0514.1.12.2/12 with record No. AEKKU-NELAC 17/2557).

Sperm concentration analysis: The cauda epididymal sperm concentration was performed as previously described in Iamsaard *et al*. (2014). After animal euthanasia, the sperm fluid was squeezed from both left cauda epididymis and vas deferens. Milky-like sperm fluid was dipped and re-suspended in 1 mL of phosphate buffer saline (37 °C, pH 7.4). Then, sperm suspension was centrifuged (3000 rpm, 37 °C, 2 min) to wash and separate the mature sperm pellet from epididymal fluid. To examine the cauda epididymal sperm concentration, such sperm pellets were re-suspended again with 1 mL fresh PBS (37 °C, pH 7.4) before dilution. The diluted sperm (1:20-50 dilution) were counted for three times of each animal by using a Neubauer's counting chamber and calculated for its concentration (represented as 106 sperm cell/mL).

Table I. Treatments of control and experimental groups

Groups	Treatments	
	Days 1–23 (via a gastric tube)	Days 24–33 (intraperitoneal injection)
Control	Distilled water, 1 mL	Saline, 0.5 mL
VPA	Distilled water, 1 mL	VPA (500 mg/kg BW), 0.5 mL
MC200	MC aril extracts (200 mg/kg BW), 1 mL	Saline, 0.5 mL
MC50+VPA	MC aril extracts (50 mg/kg BW), 1 mL	VPA (500 mg/kg BW), 0.5 mL
MC100+VPA	MC aril extracts (100 mg/kg BW), 1 mL	VPA (500 mg/kg BW), 0.5 mL
MC200+VPA	MC aril extracts (200 mg/kg BW), 1 mL	VPA (500 mg/kg BW), 0.5 mL

BW= body weight; VPA= valproic acid (saline-dissolved). The VPA group (Negative control) is designed based on Hamza & Amin (2007) (n= 8).
The preventive period (Days 1–23) and VPA-induction period (Days 24–33).

Histopathological examinations of the testes: At the end of the treatment period, all rats of control, MC, VPA and MC-VPA co-administrative groups were first weighed and euthanized by cervical dislocation to carefully collect testis and epididymis plus vas deferens. Immediately, the testis were cleaned of fats and weighed. Then, right testes were fixed in 10 % (v/v) formalin in phosphate buffered saline (PBS) (pH 7.4), dehydrated, embedded in paraffin, sectioned at 5–7 mm thickness, and stained with routine hematoxylin-eosin to make the permanent glass slides (Iamsaard *et al*.). All sections of testes were examined for histopathology under a Nikon light ECLIPSE E200 microscope equipped with a DXM1200 digital camera, Department of Anatomy, Faculty of Medicine, Khon Kaen Universty, Thailand. Approximate average diameters of seminiferous tubules in four different axes (50 tubules per animal) (10x) were measured and calculated by using ImageJ program (Iamsaard *et al*.).

Statistical analysis: To determine the significance of differences among experimental groups, the one-way analysis of variance (ANOVA) and t-test were applied by using Sigma Stat program (Version 3.1.1). All quantitative data were expressed as Mean ± Standard Deviation (SD).

RESULTS

The results showed that the body weight of VPA- and MC-VPA treated rats were significantly decreased although that of MC200+VPA group tended to be improved (Table II). In addition, both absolute and relative weights of the testes in all experimental groups were corroborated with those body weights (Table II). In contrast, these results demonstrated that VPA could significantly increase the absolute except relative weight of epididymis plus vas deferens as compared to the control. In co-administrative groups (Table II), this result showed that the low and high doses of MC extract significantly increased the absolute weight of epididymis plus vas deferens in rats induced with VPA, compared to the VPA group. However, all MC extract (50, 100, and 200 mg/kg BW) significantly improved the relative weights of weight of epididymis plus vas deferens in VPA rats ($P < 0.05$; Table II). Interestingly, MC extracts could significantly ameliorate both absolute and relative weight of seminal vesicles compared with only VPA-induced group ($P < 0.05$; Table II). Moreover, this study showed that MC extract in all doses could significantly prevent the reduction of sperm concentration in male rats induced with VPA induced ($P < 0.05$; Table II). Although MC extract tended to prevent the decrease of seminiferous tubule diameters in testis of VPA rats, there was no significant difference (Table II).

In histopathological examination of the testis, the results showed that MC extract (200 mg/kg BW) did not affect testicular tissues compared with the control demonstrating normal arrangement of seminiferous epithelium and interstitial tissues (Figs. 1 A and B). In contrast, the testicular damages presenting various seminiferous tubule degeneration and atrophy, multinucleated giant cells, dilated blood vessels, and spacious interstitial tissue were observed in the testis of rats induced

Table II. Values of analyzed parameters on the reproductive system of control and experimental male rats.

Parameters		Groups					
		Control	MC200	VPA	MC50+VPA	MC100+VPA	MC200+VPA
Body weight (g)		417.46±13.06	422.57±12.36	365.75±14.07*	362.73±9.10*	362.98±14.07*	381.09±6.33*
Testicular weight		1.9035±0.01	1.8663±0.04	1.3941±0.10*	1.2175±0.08*	1.3068±0.09*	1.2806±0.05*
	Absolute (g)	0.4576±0.01	0.4444±0.02	---	---	0.3636±0.03*	0.3361±0.01*
	Relative (g/100 g)	---	---	0.3586±0.03*	0.3353±0.02*	---	---
Epididymis + Vas deferens weight	Absolute (g)	0.6801±0.01	0.7150±0.01	0.6179±0.01*	0.6546±0.01#	0.6260±0.02	0.6555±0.03#
	Relative (g/100 g)	0.1632±0.003	0.1702±0.004	0.1697±0.006	0.1780±0.003#	0.1736±0.009#	0.1707±0.008#
Seminal vesicle weight	Absolute (g)	1.5563±0.16	1.5898±0.05	0.8163±0.07*	0.9438±0.11*	0.9644±0.08*	0.9970±0.03*
	Relative (g/100 g)	0.2292±0.05	0.3807±0.02*	0.2292±0.01	0.2477±0.02#	0.2698±0.03#	0.2707±0.02#
Sperm concentrations (106 cells/mL)		111.80±4.52	109.07±8.27	58.31±6.74*	96.50±19.71#	100.31±24.07#	105.13±14.11#
Seminiferous tubular diameters (µm)		321.113±3.63	322.182±6.05	239.754±0.15*	251.056±0.31*	254.067±7.50*	255.441±0.26*

*= Significant differences ($P<0.05$) as compared with the control group.
#= Significant differences ($P<0.05$) as compared with the VPA-induced group.
Data are expressed as mean ± SD (n= 8).

with VPA (Fig. 1C). It seems that these testicular damages were dose-dependent, improved by MC preventing treatments (Figs. D, E and F). In MC 50+VPA group, there was the decrease of seminiferous tubule atrophy and epithelial degeneration but did not improve the degenerative interstitial tissues as compared with the control (Fig. 1D). In MC100+VPA group, no atrophy and slight degeneration were found whereas some nucleated giant cells in seminiferous tubule and broad interstitial spaces were still present (Fig. 1E). In MC 100+VPA group, the results showed the improved histology of testis tissue as compared to the control and VPA groups although some nucleated giant cells were still found, located within seminiferous epithelium (Fig. 1F).

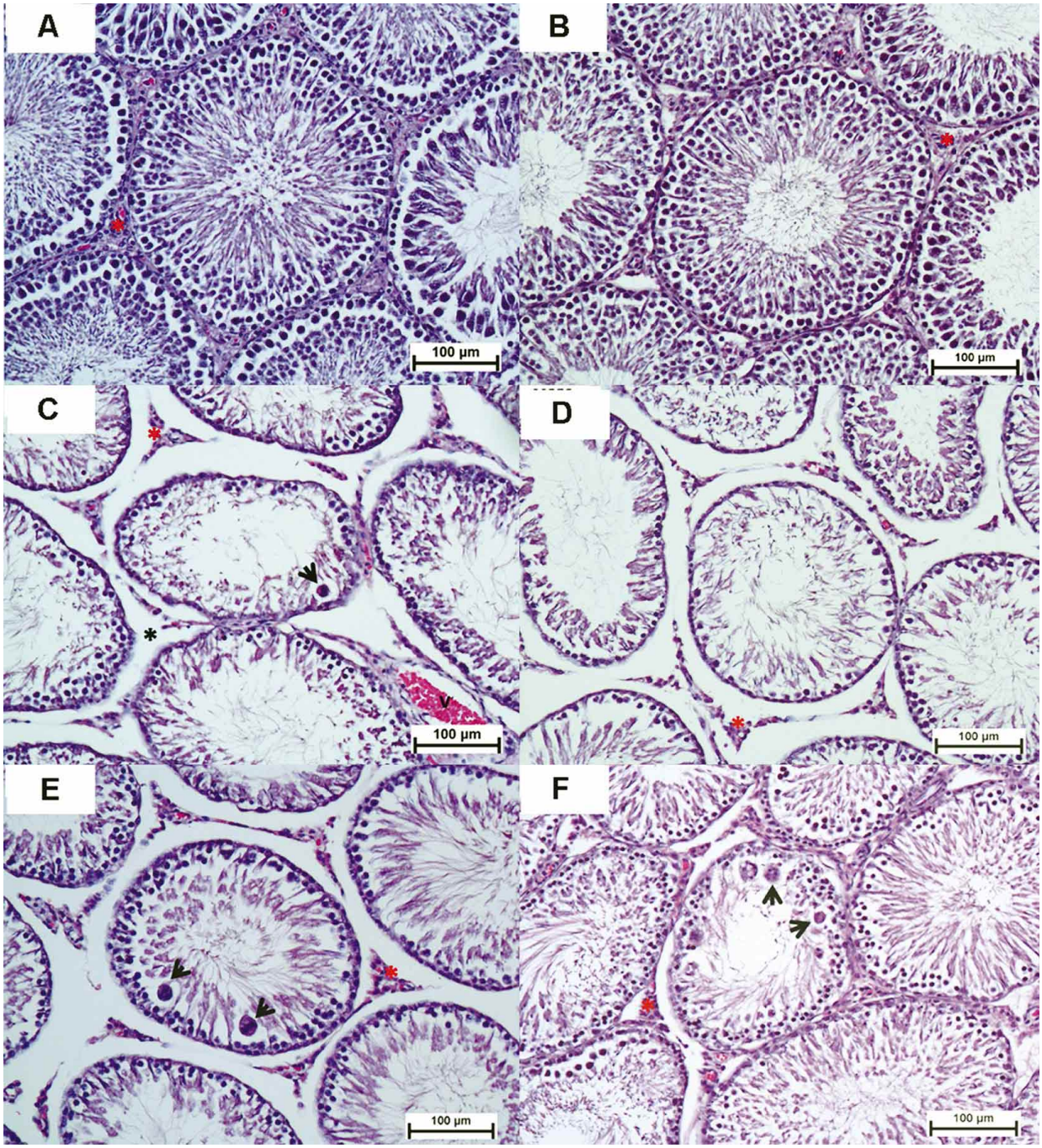

Fig. 1. Showing testicular histology (H&E) of rats from a representative sections (A) Control; (B) MC200; (C) VPA; (D) MC50+VPA treated; (E) MC100+VPA treated; (F) MC200+VPA treated groups, respectively. Short arrows indicate multinucleated giant cells and asterisks indicate interstitial tissue space.

DISCUSSION

In consistency to other reports (Sakr *et al*., 2014; Hamza & Amin, 2007), our results also demonstrated that VPA treatments affect the weighs of body, testis, epididymis plus vas deferens and seminal vesicle, respectively (Table II). These adverse effects of VPA treatment include reductions of sperm concentration and seminiferous tubule diameters (Khan *et al*., 2011; Bairy *et al*.; Krogenase *et al*., 2008; Svenberg Roste *et al*., 2002; Nishimura *et al*. 2000). Recently, this study revealed that MC aril extract significantly prevented the adverse reproductive parameters including testicular damage in male rats induced with VPA (Table II and Fig. 1). However, MC extract did not significantly prevent the animal body and testicular weights but it tended to protect such parameters (Table I). It is possible that significant preventive effects might be promising if the higher dose of MC extract was performed. The increase of epididymis weight in MC-VPA treated groups was associated with that of epididymal sperm concentration (Table II). Obviously, MC extract prevented the damages of testicular tissue and seminiferous tubule diameters although some slight multinucleated giant cells were found in all MC-VPA treated groups (Fig. 1). Taken all results together, this study showed for the first time that MC extract has indeed protective effects on testicular damage induced by VPA. Many medicinal plants have been documented to have protective effects for adverse reproductive parameters and testicular damages in VPA animal experimental models (Sakr *et al*., 2014; Hamza & Amin). Since MC extract has been proved for its high antioxidant activities (Kubola & Siriamornpun, 2011), we assumed that MC study might have abilities in decreasing of malondialdehyde and increasing of catalase and glutathione peroxidase in the testicular tissue as previously reported (Sakr *et al*.; Hamza & Amin). In further studies, the biochemical and molecular analyses will be performed to explain additional mechanisms of how the MC could prevent such adverse effects. The protective tissue damage effect demonstrated in this study is a phytotherapeutic property of MC extract besides other pharmacological properties shown in previous reports (Zheng *et al*.; Jung *et al*., 2013a, 2013b; Liu *et al*.; Chuethong *et al*.; Tien *et al*.; Kang *et al*.; Tsoi *et al*.; Xiao *et al*., 2007, 2009; Rajput *et al*., 2007, 2010). Moreover, MC extract indeed did not affect any male reproductive parameters including testicular histology as compared to the control. In conclusion, this study indicates that MC aril extract is safe for use and can protect adverse male reproductive parameters in treatment with VPA.

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SUKHORUM, W.; SAMPANNANG, A. & SRIPANIDKULCHAI, B. & IAMSAARD, S. El extracto del arilo *Momordica cochinchinensis* (L.) Spreng. previene los parámetros reproductivos adversos inducidos con ácido valproico en ratas macho. *Int. J. Morphol., 34(3)*:870-876, 2016.

RESUMEN: El objetivo fue investigar el efecto protector del extracto de arilo de *Momordica cochinchinensis* (MC) sobre los parámetros reproductivos adversos de la rata macho inducida con ácido valproico (AV) que se utiliza comúnmente en el tratamiento de enfermedades epilépticas. Las ratas se dividieron en 6 grupos (control, AV, 200 mg/kg por peso corporal de PE solamente, y 50, 100, 200 mg/kg por peso corporal MC+AV, respectivamente). Los animales fueron tratados previamente con extracto acuoso MC durante 23 días, antes de la administración de AV durante 10 días. Al término del experimento, se examinaron todos los parámetros reproductivos masculinos y la histología testicular. Los resultados indicaron que todas las dosis de PE protegen de manera significativa la disminución de los pesos de epidídimo y vesículas seminales, pero no de peso corporal y testicular. El extracto de MC también aumentó la concentración de espermatozoides y los diámetros de los túbulos seminíferos en los grupos de administración con MC+AV. Por otra parte, la histología testicular de los grupos MC+AV mostró una disminución significativa de histopatologías testiculares en comparación con el grupo AV. En conclusión, el extracto de arilo *M. cochinchinensis* puede prevenir la aparición de parámetros reproductivos masculinos negativos y los daños testiculares inducidos con AV.

PALABRAS CLAVE: *Momordica cochinchinensis*; Ácido valproico; Parámetros reproductivos masculinos; Daño testicular; Ratas.

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