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Effect of *Momordica charantia* Linn. leaf and fruit juice on fertility in male mice

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Abstract

Fertility effect of *Momordica charantia* Linn. leaf and fruit juice at doses of 200, 400 and 600 mg/ 100 gBW were evaluated by determining blood prolactin (PRL) and testosterone (T) levels, and sperm quality in male mice after 21 days of oral administration. The increase in PRL and decrease in T levels were found to be dose dependent in both treatments. Meanwhile, the sperm quality impairment was found in group receiving fruit juice was more severe than that of groups receiving leaf juice. Interestingly, the potency of testicular dysfunction was related to the degree of alteration of reproductive hormones. In conclusion, *M. charantia* leaf and fruit juice may cause testicular dysfunction, which indirectly affects pituitary PRL synthesis and consequently impaired testicular T synthesis. The use of *M. charatia* products at high dose in traditional medicine should include a caution about this adverse effect, especially in long term treatment.

Keywords: Momordica charantia Linn., fertility, prolactin, testosterone.

Introduction

Momordica charantia Linn. (bitter melon) is a tropical and subtropical vegetable. It is a potential medicinal plant of the family Cucurbitaceae[1]. Its leaves and fruits are widely consumed as a vegetable. Meanwhile, it has also been used in traditional medicine for a long time[2,3] and the most effective use is in type II diabetes mellitus treatment because of its high potential hypoglycemic property[4,5]. Other pharmacological properties of this plant also reported are based on its antihelmintic and antiviral activities[6], anticancerous effect[7] , and cardioprotective effect[8]. Meanwhile, a number of adverse effects of *M. charantia* extracts have also been found, such as antifertility effect in male animals by causing hypogonadal function and decrease of fertility rate[9-12]. It also disturbs the estrous cycle and inhibits ovulation in female animals [13-15]. Furthermore, it causes a teratogenic effect in offspring of pregnant rats after receiving unripe fruit extract of 30 g/animals during days 7-14 of pregnancy[16]. Thus, the use of *M. charantia* products at high dose and for long term treatment should be cautioned.

The purpose of this study was to evaluate the toxicity of *M. charantia* leaf and fruit juice on reproductive function in male mice after 21 days of oral administration.

Materials and Methods

Plant extraction

Fresh *M. charantia* leaves and fruits were purchased from a local market at Khon Kaen province, Thailand. The leaves were cleaned, air dried, then mixed with distilled water (1:1) and minced by electronic blender. The leaf juice was obtained, then filtered through cotton mesh and evaporated in a hot air oven at 45˚C until dry. The mass of leaf extract was minced to a powder and reconstituted with distilled water at concentrations of 400, 800 and 1200 mg/ml before oral administration (0.5 ml/100 gBW). The fruits were cleaned, had seeds removed, were air dried and then processed as in the leaf extraction.

Experimental animals

Adult male mice, ICR strain, 8 week-old and 35-40 g were obtained from the National Laboratory Animal Center, Nakornpathom province, Thailand. They were housed under a 12:12 light-dark cycle and at 25±1 ˚C. The pellet diet (Chareanpogapan Ltd., Thailand) and water were provided *ad libitum*. The experiments were performed after the experimental protocol had been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2/96).

Experiments

The experiments on leaf and fruit juice were done separately by the same protocol. Each experiment was conducted on 5 groups of 6 animals each. The first group served as a negative control, receiving distilled water at 0.5 ml/100gBW, while group 2 served as a positive control, receiving metoclopramide (Pharma Supply Co. Ltd., Thailand, a hyperprolactinemia-inducing agent) at 2.0 mg /100gBW. The groups 3-5 received leaf / fruit juice of *M. charantia* at doses of 200, 400 and 600 mg/100 gBW, respectively, for 21 days. At the end of the treatment, the relative testicular weight /body weight were expressed as the gonadal index, blood reproductive hormones and sperm quality were evaluated.

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Hormonal assay

Blood samples were collected by cardiac puncture and centrifuged at 1,700 rpm for 5 min at room temperature. Plasma samples were obtained and were used for prolactin (PRL) and testosterone (T) assay. Prolactin was measured by radioimmunoassay kit (^{125}I IRMA kit, ICN Biomedicals, Inc. Costa Mesa, CA926261), testosterone was measured by Testosterone Coated-Tube RIA Kits (The DSL-400 ACTIVE$^{(R)}$, Diagnostic System Laboratories, Inc.).

Seminal analysis

After blood sampling, epididymis and vas deferens of all groups were excised and torn with needles (No. 25) in 2 ml of NaCl (0.9%) and then incubated at 35^{o} C for sperm quality investigation including total sperm count and percentage of viable sperms following the method of Yokoi et al. [17]. Motile sperms were evaluated by the method of Sonmez et al. [18] and abnormal sperms were investigated by the method of Atessahin et al. [19].

Statistical analysis

All data were expressed as mean±SD, and each parameter was separately analyzed by one-way ANOVA. Duncan's test was used to compare the different results among groups. A value of *P<0.05* was considered as statistically significant [20].

Results

The results of gonadal index and reproductive hormones are shown in Table 1 and Figure 1. After 21 days of treatment, plasma PRL levels of groups receiving leaf and fruit juice at doses of 200, 400 and 600 mg/ 100 gBW significantly increased as compared to the control group (P<0.05). They increased in a dose dependent manner (Figure 1A) and meanwhile, the decrease in testosterone (T) level of all treated groups was also found to be dose dependent (Figure 1B). However, the disturbance of the reproductive hormones induced by leaf juice was lower than that of fruit juice. In regard to the effect of metoclopramide (a prolactinemia inducing drug), the alteration of the reproductive hormones was also presented in the same manner in both treatments. Meanwhile, no change in gonadal index of all treated groups was found.

The results for sperm quality of groups receiving leaf and fruit juice of *M. charantin* for 21 days are presented in Table 2 and Figure. 2. No significant change in percentage of motile sperm and viable sperm was found in all groups receiving leaf juice. However, a significant increase in the percentage of abnormal morphology sperm was presented in the highest dose treated group (600 mg /100 gBW) only. In contrast, all groups that received fruit juice exhibited impairment of sperm quality with a significant decrease in percentage of viable and motile sperm. Meanwhile, the percentage of abnormal sperm was significantly increased in all groups when compared to the control group (p<0.05). There were many forms of abnormal morphology sperm including medial protoplasmic droplet sperm (Figure. 2B), bent middle piece sperm (Figure. 2C) and detached head sperm (Figure. 2 D). Interestingly, both treatments of leaf and fruit juice did not show any change in the total sperm count. In regard to the group receiving metoclopramide 2 mg/ 100 gBW, it also showed an alteration of sperm quality in the same manner as groups receiving fruit juice.

Table 1 Effect of *Momordica charantia* leaf juice (MCL) and fruit juice (MCF) on gonadal index and hormonal level in male mice

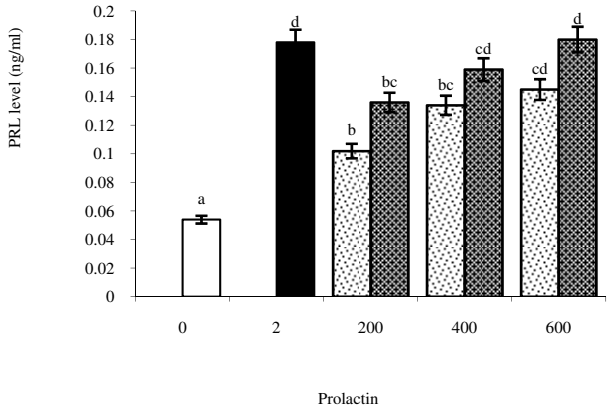
Treatment (mg/100gBW) N=6	Gonadal index (x10^{-2})	Hormonal levels (mean ± SD, ng/ml)	
		Prolactin	Testosterone
Control: 0	0.38 ± 0.01^{a}	0.054 ± 0.02^{a}	0.878 ± 0.07^{a}
Metoclopramide 2	0.35 ± 0.03^{a}	0.178 ± 0.05^{d}	0.547 ± 0.20^{b}
Leaf juice			
MCL 200	0.38 ± 0.01^{a}	0.102 ± 0.05^{b}	0.714 ± 0.20^{a,b}
MCL 400	0.38 ± 0.04^{a}	0.134 ± 0.04^{b,c}	0.610 ± 0.08^{b}
MCL 600	0.36 ± 0.04^{a}	0.145 ± 0.04^{c}	0.590 ± 0.10^{b}
Fruit juice			
MCF 200	0.35 ± 0.03^{a}	0.136 ± 0.06^{b}	0.602 ± 0.06^{b}
MCF 400	0.37 ± 0.07^{a}	0.159 ± 0.06^{b,c}	0.595 ± 0.15^{b}
MCF 600	0.35 ± 0.04^{a}	0.180 ± 0.06^{c}	0.558 ± 0.08^{b}

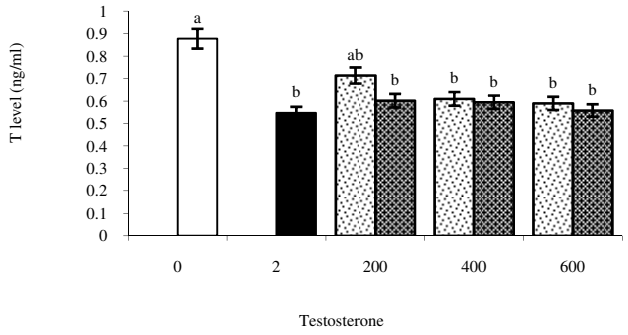
N=number of animals per group
Same letter within column means non significant difference (value *P*>0.05), different letter within column means significant difference (value *P*<0.05)

Table 2 Effect of *Momordica charantia* leaf juice (MCL) and fruit juice (MCF) on sperm quality after 21 days of trearment

Treatment (mg/100gBW) N=6	Sperm quality (mean ± SD)			
	Total sperm counts (x10^6 cell/each)	Motile sperms (%)	Viable sperms (%)	Abnormal morphology sperms (%)
Control: 0	43.21 ± 2.61^a	64.83 ± 4.19^a	77.85 ± 4.00^a	15.45 ± 2.47^a
Metoclopramide 2	41.53 ± 3.72^a	58.95 ± 3.49^{a,b}	67.03 ± 3.02^e	31.26 ± 3.78^c
Leaf juice				
MCL 200	37.39 ± 2.84^a	60.99 ± 2.26^{a,b}	79.28 ± 7.25^a	20.03 ± 4.50^b
MCL 400	36.60 ± 2.85^a	62.95 ± 3.02^{a,b}	76.40 ± 4.84^{a,b,c}	20.13 ± 5.87^b
MCL 600	37.69 ± 5.95^a	60.06 ± 2.72^{a,b}	77.41 ± 7.87^{a,b,c}	23.52 ± 3.93^b
Fruit juice				
MCF 200	38.38 ± 6.15^a	60.16 ± 3.35^{a,b}	74.10 ± 7.62^{b,c,d}	21.35 ± 2.06^b
MCF 400	40.32 ± 6.25^a	57.39 ± 3.79^b	72.80 ± 3.50^{c,d}	27.89 ± 5.47^c
MCF 600	40.38± 6.34^a	57.05 ± 2.91^b	71.36 ± 5.44^{d,e}	29.50 ± 5.04^c

N= number of animals per group
Same letter within column means non – significant difference (value *P*>0.05), different letter within column means significant difference (value P<0.05)





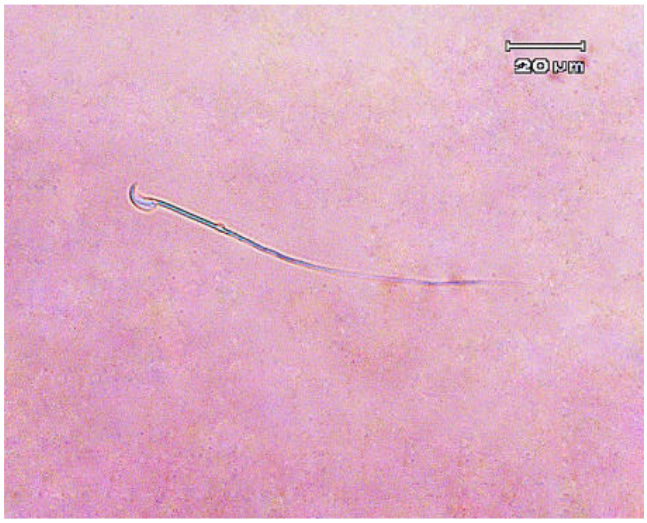


Figure 1 Blood prolactin (A) and testosterone level (B) in groups receiving leaf and fruit juice of *M. charantia* Linn. for 21 days. [Values are mean ± SD of 6 animals per group, same letter in each graph means non - significant difference (value *P>0.05*), different letter in each graph means significant difference (valuae *P< 0.05*)]

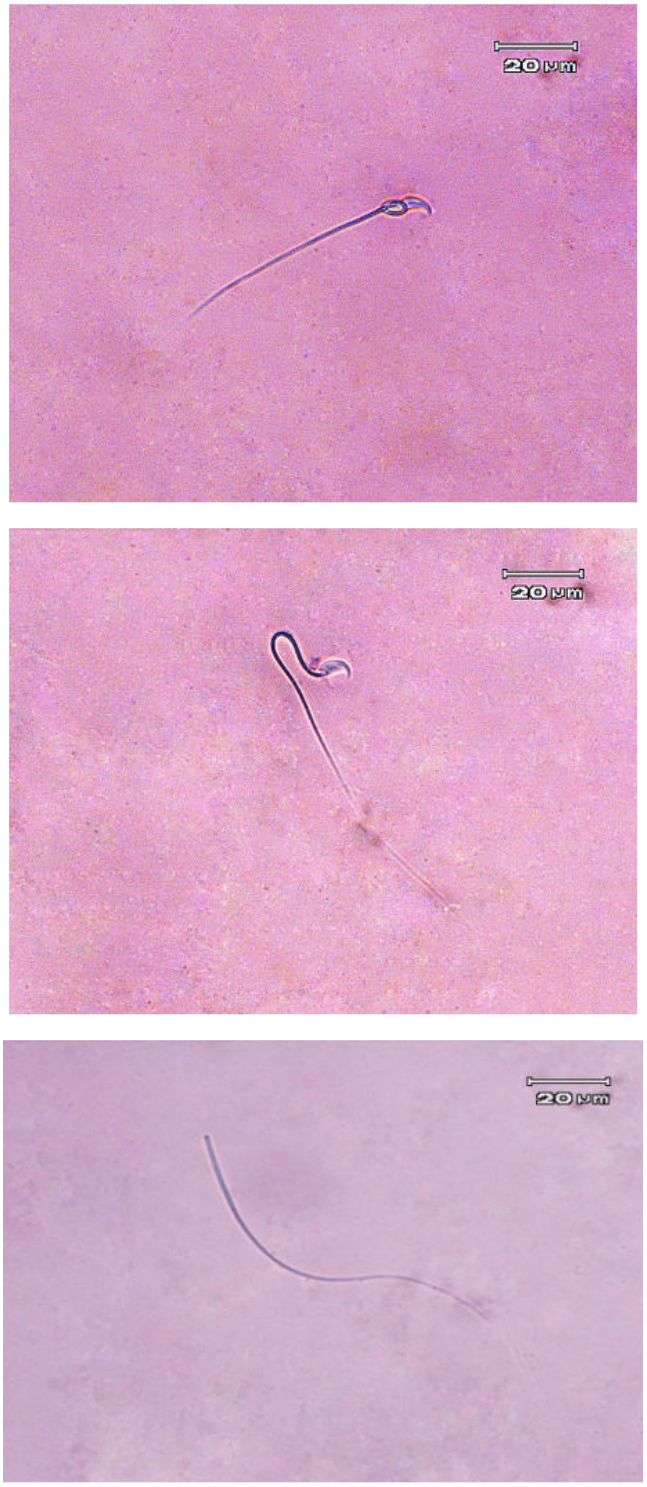


Figure 2 Sperm morphology by smear method, Negrosin-Eosin stained (bar = 20 µm): A, normal sperm; B-D, abnormal sperms: B, medial protoplasmic droplet sperm; C and D, bent middle piece sperm and E, detached head sperm

Discussion

Our results reveal that an increase in PRL level and a decrease in T level were found in both groups receiving leaf and fruit juice of *M. charantia* (200, 400 and 600 mg/ 100 gBW). However, the results of alteration of reproductive hormones of groups receiving leaf juice showed a lower degree than that of groups receiving fruit juice. This study used metoclopramide as reference drug, which is a potent stimulus of PRL secretion by inhibiting dopamine-mediated hypothalamic secretion of PRL inhibiting factor[22]. In regard to the group receiving drug at a dose of 2 mg/ 100 gBW, it also revealed a change in the reproductive hormones in the same manner as the groups receiving leaf and fruit juice. As mentioned above, it is implied that plant juice may affect pituitary PRL secretion and consequently disturb testicular T synthesis. It is well known that a physiological level of PRL is required to support T production, while it also acts as a modulator of the hypothalamic-pituitary function [22]. In the case of high PRL secretion, it has an inhibitory effect on gonadotropin secretion in both humans and animals [23,24]. This occurrence consequently inhibits gonadal function including reduction of T secretion and spermatogenesis[25,26]. However, the gonadal indices of all treated groups were non-significantly different in this study. It is well known that testicular weight is unrelated to testicular function[27]. Meanwhile, blood PRL and T level are the markers in the diagnosis of male fertility [24]. This study also found sperm quality impairment in all groups receiving fruit juice, such as a decrease in the number of viable sperm and motile sperm, as well as an increase in abnormal morphology sperm. Meanwhile, the increase in abnormal morphology sperm was found in groups receiving the high dose of leaf juice only. This implies that the testicular toxicity caused by leaf juice has lower potency than found in groups receiving fruit juice, which concords with the degree of reproductive hormone imbalance. Interestingly, the character of abnormal morphology sperms found in this study were secondary abnormalities, which occur during sperm maturation in the epididymis[28]. The alteration in sperm motility, viability and morphology are an indication of disturbed testicular and epididymal microenvironments[29]. Our results reveal that the total sperm count or sperm concentration found in both groups receiving leaf and fruit juice was unchanged. This implies that these doses of the plant juice did not affect spermatogenesis, unless it affects sperm maturation.

Conclusion

M. charantia leaf and fruit juice administration caused sperm quality impairment in mice after 21 days of treatment, which may indirectly affect pituitary PRL synthesis and consequently disturb testicular T synthesis. The fruit juice exhibited greater testicular toxicity than the leaf juice.

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