

·Original Article·

Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats

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Abstract

Aim: To investigate effects of quercetin on weight and histology of testis and accessory sex organs and on sperm quality in adult male rats. **Methods:** Male Sprague-Dawley rats were injected s.c. with quercetin at the dose of 0, 30, 90, or 270 mg/kg body weight/day (hereafter abbreviated Q0, Q30, Q90 and Q270, respectively), and each dose was administered for treatment durations of 3, 7 and 14 days. **Results:** From our study, it was found that the effects of quercetin on reproductive organs and sperm quality depended on the dose and duration of treatment. After Q270 treatment for 14 days, the weights of testes, epididymis and vas deferens were significantly increased, whereas the weights of seminal vesicle and prostate gland were significantly decreased, compared with those of Q0. The histological alteration of those organs was observed after Q270 treatment for 7 days as well as 14 days. The sperm motility, viability and concentration were significantly increased after Q90 and Q270 injections after both of 7 and 14 days. Changes in sperm quality were earlier and greater than those in sex organ histology and weight, respectively. **Conclusion:** Overall results indicate that quercetin might indirectly affect sperm quality through the stimulation of the sex organs, both at the cellular and organ levels, depending on the dose and the duration of treatment. Therefore, the use of quercetin as an alternative drug for treatment of male infertility should be considered. (*Asian J Androl* 2008 Mar; 10: 249–258)

Keywords: epididymis; prostate; quercetin; seminal vesicle; sperm quality

1 Introduction

The *Mucuna macrocarpa* Wallich (synonym: *Mucuna colletti* Lace), locally called the black Kwao Krua, is one

of the leguminous herbs that has been traditionally consumed by Thai men for the purposes of rejuvenation as well as maintenance of sexual performance or prevention of erectile dysfunction [1]. Several studies regarding the influence of black Kwao Krua on reproductive organs and its anti-estrogenic (or androgenic) effects have been carried out recently [2–4]. Thansa [2] reported that the black Kwao Krua has no effect on the weight or histopathology of reproductive organs in male rats. Srijunggam *et al.* [4] examined the subchronic effects of crude extract of the black Kwao Krua on gonadal develop-

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ment in male tilapias (*Oreochromis niloticus*) and found no indication of enhanced reproductive function. The black Kwao Krua exhibits an anti-proliferative effect on the growth of MCF-7 cells (i.e. estrogen receptor positive [ER⁺] human mammary adenocarcinoma), which might suggest an anti-estrogenic function [3]. So far, the effects of the black Kwao Krua on male reproductive organs are still inconclusive. As the black Kwao Krua contains many substances that are considered to be bioactive on the reproductive organs [5], its effects should not be evaluated using its crude extract or powder. It is necessary to evaluate the reproductive effects of each substance of this plant.

Through analyses of the extracts from the stem of the black Kwao Krua, three substances were found to be bioactive: quercetin, kaempferol and hopeaphenol. These three substances function as cAMP phosphodiesterase inhibitors [5]. Of the three isolated substances, quercetin appears to be a major one for research on male fertility and reproductive functions. In an *in vitro* study, quercetin inhibited the collective motility of ejaculated ram spermatozoa in the first 2 h of incubation and stimulated it for the next 3–4 h of incubation [6]. The incubation of human semen with quercetin induced an irreversible and dose-dependent fall in sperm motility and sperm viability [7]. Quercetin did not affect cortisol production in human adrenal H295R cells stimulated with di-buthyl cAMP [8]. An *in vivo* study in male rats revealed that i.p. injection of 200 mg/kg body weight of quercetin for twice had no effects on fertility [9], but a daily gavage of quercetin at doses of 50–150 mg/kg body weight/day slightly increased prostate gland weights and dilated prostate lumens, which were full of secretory materials [10]. Quercetin can increase serum testosterone levels and decrease serum dihydrotestosterone levels in male rats [10]. The reproductive effects of quercetin in male animals are still controversial, perhaps because most of the previous studies have been carried out *in vitro* using cell lines. We therefore studied the *in vivo* effects of quercetin on reproductive organs and sperm quality in adult male rats.

2 Materials and methods

2.1 Animals

Male 7-week-old Sprague-Dawley rats, weighing 250–300 g, were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom,

Thailand. They were allowed to acclimatize to the laboratory environment for 1 week prior to the experiment and were used for the present study at 8 weeks old. Stainless steel cages containing sawdust housed 5 animals in a room under temperature ($25 \pm 1^\circ\text{C}$) and photoperiod (12h:12 h Light:Dark cycle) control at the Primate Research Unit, Chulalongkorn University, Bangkok, Thailand. They were given free access to water and fed rat chow (SWT, Bangkok, Thailand). All experiments were performed between 8:00 am and 11:00 am. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Chulalongkorn University.

2.2 Experimental protocol

The LD₅₀ of quercetin given p.o. in the study by Sullivan *et al.* [11]. was 160 mg/kg body weight in mice and 161 mg/kg body weight in rats. Therefore, the initial dose of quercetin chosen for the present study is 30 mg/kg body weight/day (approximately one-fifth of LD₅₀). Besides, Aravindakshan *et al.* [9] report that i.p. injections of 200 mg/kg body weight/day and 300 mg/kg body weight of quercetin given to male rats did not impair fertility. Therefore, the other two doses of quercetin treatment in the present study were raised to 90 mg/kg body weight/day and 270 mg/kg body weight/day.

One hundred and twenty male rats were randomly divided into four groups (30 rats/group) and injected s.c. with quercetin at doses of 0, 30, 90 or 270 mg/kg body weight/day (hereafter abbreviated Q0, Q30, Q90 and Q270, respectively) and each dose group was further divided into three groups (10 rats/group) that were treated with quercetin for 3, 7 and 14 days. At the end of the treatment period, the rats were killed with diethyl ether, and the testes, epididymis-vas deferens, prostate glands and seminal vesicles were dissected, weighed and kept in Bouin's fixative for histopathological examination. Fresh epididymis and vas deferens were kept for sperm quality analysis. Rats were also weighed on the first and last day of the study period.

2.3 Quercetin preparation

Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved and diluted with 20% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. The quercetin solution was freshly prepared each week.

2.4 Histological examination

After the overnight fixation of reproductive organs in Bouin's fixative, tissues were dehydrated in a series of ethanol gradients and cleared in xylene. Tissues were then embedded in paraffin, microtomed into 6- μ m sections and stained with hematoxylin and eosin. Permanent preparations of all tissues were histologically examined and photographed using a digital camera (Canon Virginia Inc., Newport News, VA, USA) mounted on a light microscope (Carl Zeiss Inc., Jena, Germany).

Digital images of seminiferous tubules and epididymides were examined with a digital image analysis program (Image Pro Express; Media Cybernetics, Silver Spring, MD, USA). Seminiferous tubular area was measured and averaged from 200 tubules per rat (10 rats/group). The tubular area, luminal area and tubular thickness of the epididymis were measured and averaged from 100 tubes per rat (10 rats/group).

2.5 Sperm quality analysis

After rats were killed, epididymis and vas deferens were removed. The caudal epididymis and vas deferens were squeezed with pairs of fine forceps and the contents of these structures were subjected to sperm quality analysis.

Sperm quality was determined by three parameters: sperm concentration, motility and viability. Sperm concentration was analyzed using the haemocytometer method [12]. Sperm suspensions from the caudal epididymis and vas deferens were diluted 1:20 with Baker's solution and transferred into microcentrifuge tubes. The diluted samples were put into the counting chamber and the number of sperm was counted using a haemocytometer with improved double Neubauer ruling under a light microscope. The sperm concentration was expressed as $\times 10^6/\text{mL}$. Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa and expressed as the percent motility. Sperm viability was analyzed by the Trypan blue staining method [12]. The nonviable spermatozoa, which were stained blue, and the viable ones, which were unstained, were counted under the light microscope. The viability of sperm was expressed as the percent of viable spermatozoa.

2.6 Statistical analyses

The results were expressed as means \pm SEM. The relative organ weights (%) were obtained by the division

of the organ weights by the body weight $\times 100$. Statistical analyses were performed using SPSS version 11.0 (SPSS, Chicago, IL, USA). A test for homogeneity of variance was also performed. Dose responses and time responses were analyzed by one-way analyses of variance (ANOVA) for factorial and repeated measure design with post-hoc testing using the least significant difference (LSD) test. The correlation between times and treatment groups was analyzed by two-way ANOVA. $P < 0.05$ were considered statistically significant.

3 Results

3.1 Effect of quercetin on body weight

There were no significant differences of rat body weights between the Q0, Q30, Q90 and Q270 groups, at 3, 7 and 14 days of quercetin treatment (Figure 1). With increasing age, however, the body weights in all four groups (Q0, Q30, Q90 and Q270) of rats were significantly increased and seemed to reach a plateau at 9–10 weeks of age.

3.2 Effect of quercetin on organs weights

3.2.1 Weight gains of testis and accessory sex organs with advancing age of rats

There was a positive, linear relationship between weights of accessory sex organs (epididymis-vas deferens, prostate glands and seminal vesicles) and age ($R^2 = 0.915\text{--}0.985$) in control rats (Q0) (Figure 2A). In contrast, the testis weights of control rats did not in-

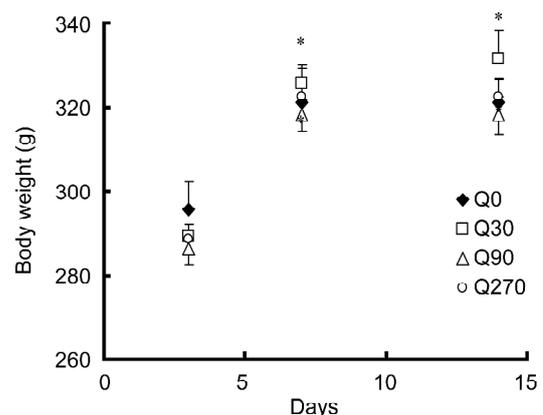


Figure 1. Body weights of male rats after s.c. injection of 0, 30, 90 and 270 mg/kg body weight/day of quercetin (Q0, Q30, Q90 and Q270, respectively) for 3, 7 and 14 days. * $P < 0.05$, compared with 3 days in all the four quercetin treatment groups.

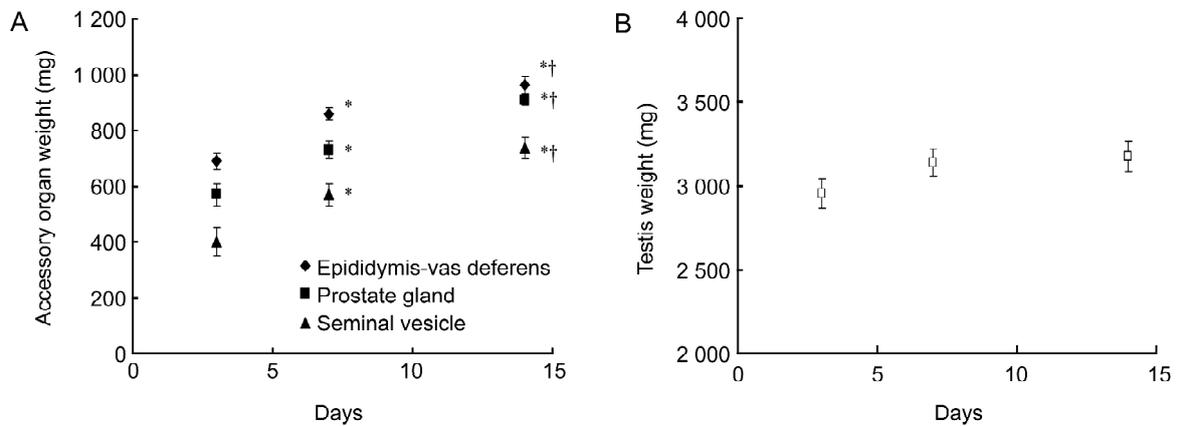


Figure 2. Weight gains of accessory sex organs (A) and testes (B) of normal male rats (Q0 group) from 8 to 10 weeks old. **P* < 0.05, compared with 3 days; †*P* < 0.05, compared with 7 days.

Table 1. Relative weights of testis, epididymis-vas deferens, prostate gland and seminal vesicle of male rats after s.c. injections of 0, 30, 90 and 270 mg/kg body/day of quercetin (Q0, Q30, Q90 and Q270) for 3, 7 and 14 days. For Q270, ^a*P* < 0.05, ^b*P* < 0.05, ^c*P* < 0.05, compared with Q0, Q30 and Q90, respectively; At 3 days, **P* < 0.05, ***P* < 0.05, compared with 7 and 14 days, respectively; At 7 days, †*P* < 0.05, compared with 14 days.

Quercetin treatment	Q0	Q30	Q90	Q270
Testis				
3 days	0.99 ± 0.06	1.01 ± 0.08	1.03 ± 0.07	1.05 ± 0.06
7 days	0.98 ± 0.11	1.02 ± 0.07	1.03 ± 0.06	1.06 ± 0.07 ^a
14 days	0.99 ± 0.05	1.00 ± 0.03	1.01 ± 0.06	1.07 ± 0.07 ^a
Epididymis-vas deferens				
3 days	0.23 ± 0.03 ^{***}	0.24 ± 0.02 ^{***}	0.24 ± 0.03 ^{**}	0.25 ± 0.02 ^{***}
7 days	0.27 ± 0.03 [†]	0.28 ± 0.02	0.26 ± 0.03	0.29 ± 0.02 ^{†a,c}
14 days	0.30 ± 0.02	0.30 ± 0.02	0.30 ± 0.01	0.33 ± 0.03 ^{a,b,c}
Prostate gland				
3 days	0.19 ± 0.03 ^{***}	0.19 ± 0.03 ^{***}	0.21 ± 0.03 ^{**}	0.19 ± 0.02 ^{***}
7 days	0.23 ± 0.04 [†]	0.22 ± 0.03	0.20 ± 0.03 [†]	0.23 ± 0.03
14 days	0.28 ± 0.02	0.24 ± 0.02	0.26 ± 0.05	0.23 ± 0.04 ^{a,c}
Seminal vesicle				
3 days	0.13 ± 0.05 [†]	0.15 ± 0.04 ^{***}	0.14 ± 0.06 ^{**}	0.14 ± 0.02 ^{***}
7 days	0.18 ± 0.04 [†]	0.17 ± 0.03	0.16 ± 0.03 [†]	0.16 ± 0.02
14 days	0.23 ± 0.04	0.20 ± 0.05	0.22 ± 0.05	0.15 ± 0.03 ^{a,b,c}

crease throughout the 2-week period of the study (Figure 2B). Because both the body and organ weights increased with advancing age, the relative organ weights (%) were used for the next step of analysis.

3.2.2 Effect of quercetin on relative organ weights

Changes of relative organ weights were examined in two ways: dose dependence and time dependence

(Table 1).

The relative testis weights increased with a dose dependence. Only those of the Q270 group were significantly higher than those of the control group, at day 7 and 14 of treatment. Even after 14 days of treatment, no significant increases were detected in the relative testis weights of Q30 and Q90 groups.

With regard to the quercetin dose, only the Q270

Table 2. Seminiferous tubular area of male rats after s.c. injections of 0, 30, 90 and 270 mg/kg body weight/day of quercetin for 3, 7 and 14 days. ^b*P* < 0.05, compared with Q270; ^c*P* < 0.01, compared with 14 days.

Quercetin treatment	Seminiferous tubular area ($\times 10^3 \mu\text{m}^2$)		
	3 days	7 days	14 days
Q0	62.46 \pm 1.89	61.49 \pm 1.43	60.82 \pm 1.31 ^b
Q30	61.34 \pm 1.31	63.50 \pm 1.67	61.65 \pm 1.06 ^b
Q90	60.67 \pm 1.56	63.61 \pm 1.45	63.33 \pm 1.32 ^b
Q270	62.80 \pm 1.78 ^c	63.54 \pm 1.34	66.61 \pm 1.25

group exhibited an increase in relative epididymis-vas deferens weights (at day 7 and 14) and a decrease of relative prostate gland and seminal vesicle weights (at day 14), compared to those in the Q0 group. No alterations in accessory sex organ weights were observed for the other two doses of quercetin treatment (Q30 and Q90).

With regard to the duration of treatment (3–14 days), all four groups of quercetin-treated rats (Q0, Q30, Q90 and Q270) showed increases in relative weights of the epididymis-vas deferens, prostate gland and seminal vesicle. The increases of relative sex organ weights were observed even in the Q0 group, which means that the weight gains of accessory sex organs were faster than the body weight gain.

3.3 Effects of quercetin on histological alterations of sex organs

3.3.1 Testis

The stratified epithelium of the seminiferous tubules contained different stages of developing sperm cells in the control group (Q0 group) (Figure 3, A1). There was no difference in the structure of the seminiferous tubules in any treatment groups between days 3 and 7. However, at day 14 of quercetin treatment, in Q90 and Q270 groups the retention of spermatozoa in seminiferous tubules was higher than those in Q0 and Q30 (Figure 3, A1–4). The tubular area of seminiferous tubules increased with a time and dose dependence. That is, only the Q270 group showed significantly higher values than the control and lower dosage groups (Q30 and Q90) and only at day 14 (Table 2). Histological examination of the testis did not reveal any evidence of degeneration of germ cells in all dose groups.

3.3.2 Accessory sex organs

Accessory sex organs (epididymis-vas deferens, prostate gland and seminal vesicle) did not show any difference between control and treatment groups (Q0, Q30, Q90 and Q270) at day 3.

The epididymis of the control group (Q0 group) consisted of numerous tubes filled with spermatozoa and fluid (Figure 3, B1). The epididymal tubes were lined by a very tall pseudostratified stereociliated columnar epithelium. Most epithelial cells (or principal cells) have long stereocilia. In Q90 and Q270 groups at day 14, tubes containing spermatozoa and fluid were slightly more numerous than in the Q0 group, and the epithelia were lined with pseudostratified cuboidal cells (Figure 3, B1–4). Principal cells of the epithelium in Q90 group were vacuolarized and had more stereocilia than in the Q0 group (Figure 3, B3). Compared with the Q0 group, changes of the tubular and luminal areas of epididymis depended on dosage and time (Table 3). The Q270 group showed a significant increase of tubular area at days 7 and 14, and the Q90 group showed a significant increase of tubular area only at 14 days, compared with the lower doses. The Q90 and Q270 groups showed a significant increase in luminal areas both at days 7 and 14. The increase in tubular thickness of the epididymis depended on an increased ratio of tubular area to lumen area, because tubular thickness showed no change in the Q30 and Q270 groups, whereas it increased in the Q90 group at days 7 and 14.

The mucosa of the vas deferens of the control group formed longitudinal folds and was lined with pseudostratified columnar epithelial cells with long stereocilia (Figure 3, C1–4). There were no differences in the vas deferens between control and treatment groups, regardless of either the quercetin dose or the duration of treatment.

The prostate gland of the control group (Q0 group) contained many tubuloalveolar glands or secretory alveoli (Figure 3, D1–4). The secretory alveoli were lined with a layer of tall columnar epithelial cells with a high cytoplasm/nuclear ratio. The epithelial cells also had irregular shapes because the mucosa had papillary projections into the lumen of the gland. The lumen was filled with secretory fluid. The prostate gland observed in the Q30 group was similar to that in the Q0 group. The prostate lumen in the Q90 and Q270 groups at day 14 were highly dilated and the number of tubes observed also decreased (Figure 3, D1–4). The luminal epithelial cells in the Q90 and Q270 groups showed a marked re-

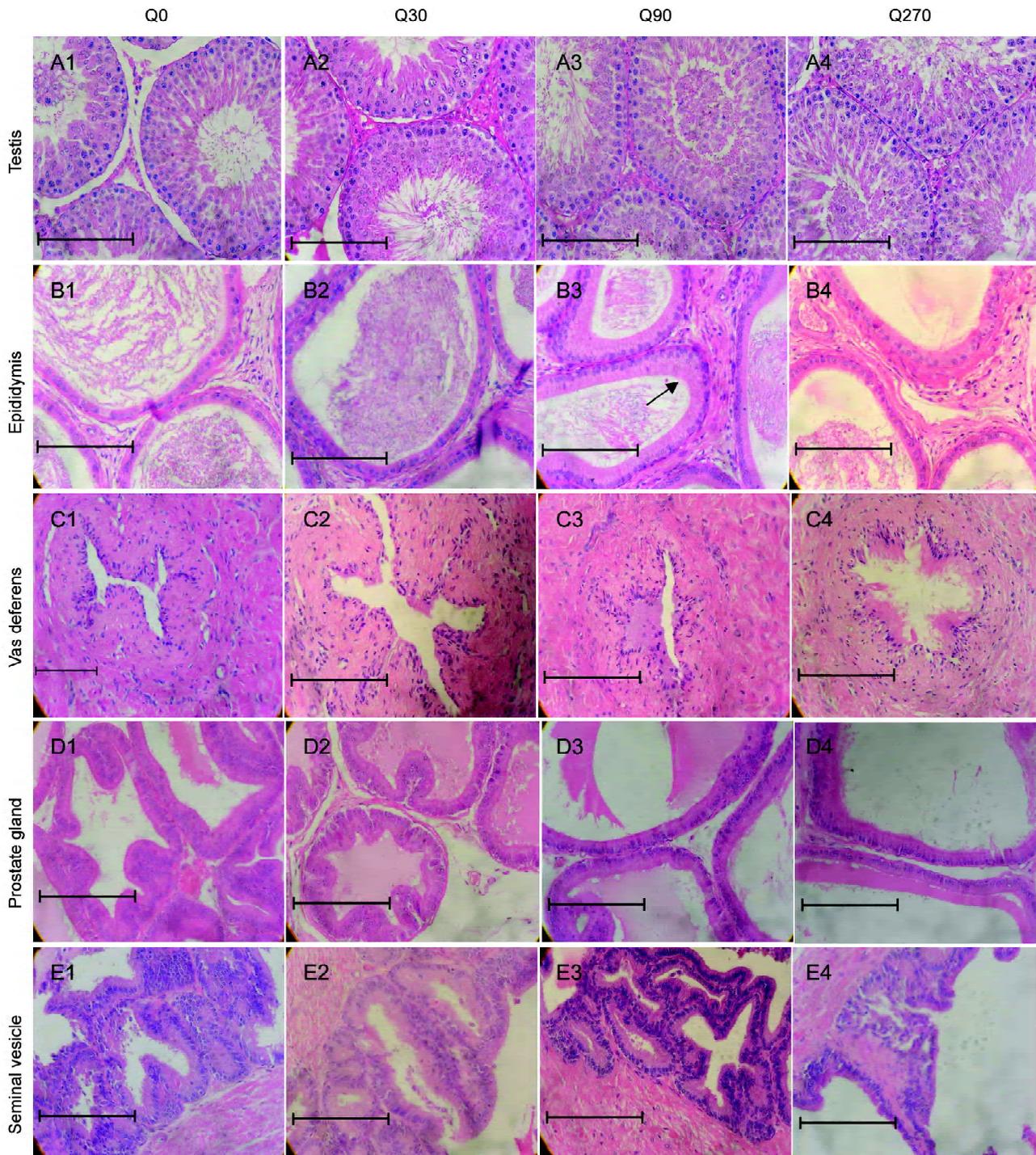


Figure 3. Histological alterations of testis (A), epididymis (B), vas deferens (C), prostate gland (D) and seminal vesicle (E) of male rats after s.c. injections of 0, 30, 90 and 270 mg/kg body weight/day of quercetin (Q0, Q30, Q90 and Q270, respectively) for 14 days. Scale bars = 50 μ m. Black arrow indicates long stereocilia of epithelium cells in the Q90 group.

Table 3. Tubular area, lumen area and tubular thickness of epididymis after s.c. injection of 0, 30, 90 and 270 mg/kg body weight/day of quercetin (Q0, Q30, Q90 and Q270) for 3, 7 and 14 days. Tubular thickness (μm^2) = Tubular area (μm^2) – lumen area (μm^2). For Q270, ^a $P < 0.05$, ^b $P < 0.05$, ^c $P < 0.05$, compared with Q0, Q30 and Q90, respectively. For Q90, ^d $P < 0.05$, ^e $P < 0.05$, compared with Q0 and Q30, respectively. At 3 days, ^{*} $P < 0.05$, ^{**} $P < 0.05$, compared with 7 days and 14 days, respectively. At 7 days, [†] $P < 0.05$, compared with 14 days.

Quercetin treatment	Q0	Q30	Q90	Q270
Tubular area ($\times 10^3 \mu\text{m}^2$)				
3 days	105.32 \pm 7.33	109.65 \pm 5.32	120.20 \pm 10.12	124.30 \pm 13.70 ^{**}
7 days	117.80 \pm 10.20	121.81 \pm 7.56	130.12 \pm 8.02	178.35 \pm 17.10 ^{a,b,c}
14 days	113.66 \pm 8.60	115.78 \pm 3.70	131.27 \pm 5.91 ^{d,e}	182.56 \pm 20.80 ^{a,b,c}
Lumen area ($\times 10^3 \mu\text{m}^2$)				
3 days	70.46 \pm 4.17	71.53 \pm 5.92 ^{**}	80.25 \pm 8.38	83.35 \pm 10.20 ^{**}
7 days	73.26 \pm 3.54	82.44 \pm 8.41	88.15 \pm 8.21 ^d	90.77 \pm 6.82 ^{†,a}
14 days	72.91 \pm 7.06	86.14 \pm 6.27	94.54 \pm 6.52 ^d	103.68 \pm 5.21 ^{a,b}
Tubular thickness (μm^2)				
3 days	33.80 \pm 1.85 [*]	33.19 \pm 1.32	39.27 \pm 3.67	36.85 \pm 1.88 ^{**}
7 days	29.36 \pm 1.90 [†]	32.35 \pm 1.57	45.14 \pm 3.85 ^d	43.42 \pm 3.22 ^{†,a,b}
14 days	32.10 \pm 1.43	30.75 \pm 1.95	32.27 \pm 1.28	31.09 \pm 1.14

duction in cytoplasm and thickness of mucosa compared with the control group after quercetin treatment. A dramatic dilation of the prostate lumen was induced by treatment of Q90 and Q270.

The seminal vesicle of the control group (Q0 group) was complex and glandular, and the lumen was highly irregular and recessed with honeycomb-like features (Figure 3, E1). The mucosa of the seminal vesicle exhibited thin, branched and anastomosing folds. The epithelia had a variable appearance columnar or pseudostratified columnar. In agreement with the decrease of seminal vesicle weights, the lumens were markedly dilated and the branching of the mucosa of seminal vesicles of rats treated with Q270 for 14 days was markedly reduced in comparison with that after lower doses of quercetin treatment (Q0, Q30 and Q90) (Figure 3, E1–4). In addition, the mucosal epithelia of Q270 rats had either cuboid or squamous cells.

3.4 Effects of quercetin on sperm quality

There was no difference in sperm quality (sperm motility, sperm viability and sperm concentration) after all three doses of quercetin treatment (Q30, Q90 and Q270) for 3 days, compared with the control group (Q0) (Figure 4). However, the increases in sperm quality depended on both the dose and duration of quercetin treatment. With regard to the dose, the motility, viability and concentration of sperm in the Q90 and Q270 groups

were higher than those in the control group when the duration of treatment was prolonged to 7 and 14 days. With regard to the treatment duration, the sperm quality was increased after 14 days of treatment for Q30, Q90 and Q270 and increased after 7 days of treatment for either Q90 (only sperm motility and concentration) or Q270. Only the sperm concentration increased with the advancing age of Q0 rats ($R^2 = 0.991$, $P < 0.05$), whereas the sperm motility and sperm viability did not increase significantly ($R^2 = 0.280$ and 0.564 , respectively, $P > 0.05$).

4 Discussion

Traditionally, the black Kwao Krua (*Mucuna macrocarpa* or *M. colletti*) has been used by Asian men for maintenance or improvement of reproductive functions [1]. Quercetin, one of the bioactive constituents isolated from the black Kwao Krua, was expected in the present analysis to have androgenic effects; however, its reproductive effects have not been clarified. From our study, it was found that the effects of quercetin on reproductive organs and sperm quality depend on the dose and duration of treatment. The shortest time of quercetin treatment (3 days, s.c. injection) at any dosage (30, 90 and 270 mg/kg body weight/day) had no effects either on reproductive organ weights or on sperm quality. However, these effects could be observed for the longer quercetin treatments (7 and 14 days) and the higher doses

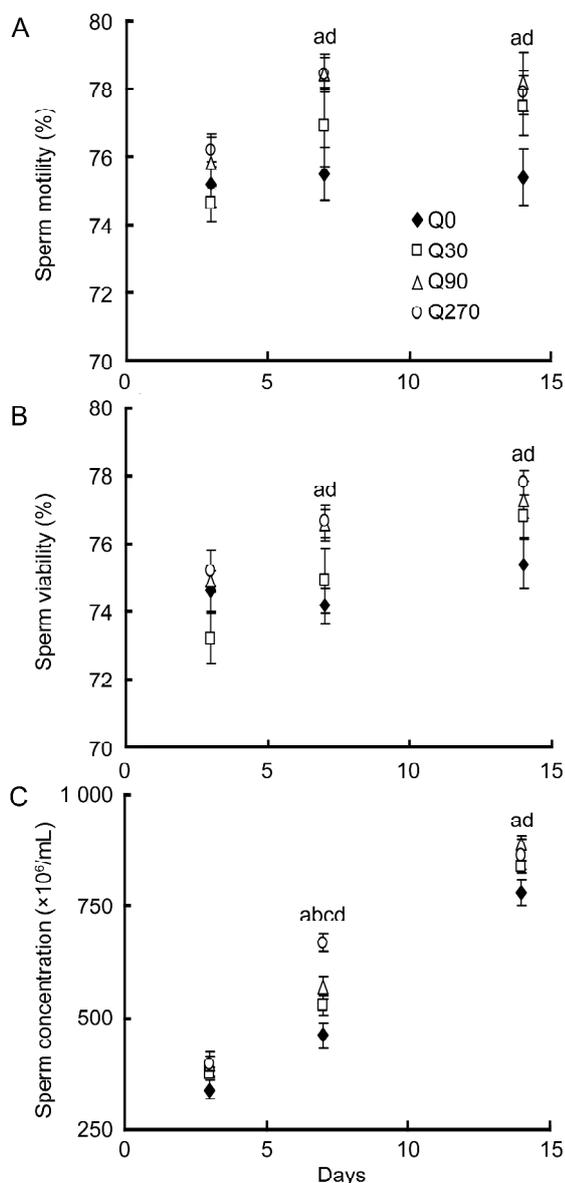


Figure 4. Sperm motility (A), viability (B) and concentration (C) of male rats after s.c. injections of 0, 30, 90 and 270 mg/kg body weight/day of quercetin for 3, 7 and 14 days. For Q270, ^a $P < 0.05$, ^b $P < 0.05$, ^c $P < 0.05$, compared with Q0, Q30 and Q90, respectively. For Q90, ^d $P < 0.05$, compared with Q0.

(Q90 and Q270). The significant increase of testis and epididymis-vas deferens weights and decrease of prostate gland and seminal vesicle weights were found only after Q270 treatment for 14 days. The histological alteration of those organs was observed after Q270 treatment for 7 days as well as 14 days. The response of sperm quality (motility, viability and concentration) to

quercetin was faster and greater than those in weights and histology of sex organs. Furthermore, quercetin did not disturb the body weight gain in male rats compared with that in control rats (Q0). Rats of 8 weeks of age gained weight by approximately 1.1–1.2 times during the 2 weeks of our study period. In a study by Mukerjee and Rajan [13], male Wistar rats gained weight by approximately 1.45 times from 8 to 10 weeks of age.

During the study period, the weights of the epididymis-vas deferens, prostate gland and seminal vesicle of the control group (Q0) significantly increased with advancing age, whereas the testis weight was not changed. As the age of rats used in the present study was 8–10 weeks old (8 weeks old at the starting day and 10 weeks old after the 14 days of treatment), this represents the time course of growth in those organs (Figure 2). It was reported that the prostate gland [13] and seminal vesicle [14] of Wistar rats progressively increased approximately two times from 8 to 10 weeks of age, and the testis of male Sprague-Dawley rats reached a plateau at around 8 to 14 weeks of age [15]. It appears that the weight of the seminal vesicle increases greatly when the rats achieve sexual maturity. Therefore, the induction of the increased testis weights by Q270 treatment for 14 days was caused only by the quercetin treatment, whereas the increase of epididymis-vas deferens weights was caused by both quercetin and growth.

Increase in testis weight after Q270 treatment for 7 and 14 days is probably caused by both the retention of spermatozoa in seminiferous tubules (Figure 3, A4) and the enlargement of seminiferous tubules (Table 2). Therefore, it is postulated that sperm production can be increased by quercetin treatment in male rats. Increase in sperm retention and weight of testes is linked with the increase in epididymis-vas deferens weights. The retention of fluid and spermatozoa in the vas deferens subsequently caused the dilation of the vas deferens tubular lumen and the increased epididymal sperm count.

The improved sperm quality (motility, viability and concentration) after quercetin injection in rats of our *in vivo* study disagree with the results of previous reports that were obtained from an *in vitro* study [7]. In the *in vitro* study, the human semen was incubated with quercetin and it was shown that the sperm motility (at 5–200 mmol/L) and sperm viability (50–100 mmol/L) decreased in agreement with the decrease of Ca^{2+} -ATPase activity, which is a key enzyme involved in the regulation of sperm motility [7]. One possible explanation for

this disagreement is that in our *in vivo* study, the quercetin might act through other sex organs (i.e. stimulating the testis or epididymis in the present study) or through a hypothalamic-pituitary-testis axis (i.e. stimulating testosterone secretion) [10], not directly stimulating to the spermatozoa inside the testis or epididymis. It was previously reported that quercetin can act dose-dependently as either an agonist of endogenous steroids at low doses or an antagonist at high doses [16]. In contrast to our study showing an increase in sperm quality, Aravindashan *et al.* [9] showed that the treatment with a higher dose of quercetin (300 mg/kg body weight, two injections) reduced the fertility rate of male rats during the first two matings with female rats and thereafter the fertility was recovered to be comparable to the control group.

It is possible that the increased epididymal sperm quality might be a result of the antioxidant activity of quercetin on the epididymis [18]. The epididymis serves important functions in the transportation, maturation and storage of sperm cells, during which period the spermatozoa develop motility [17]. The epididymis also protects spermatozoa from oxidative injury by encouraging scavengers of reactive oxygen species [18]. In the present study, such sperm quality, as evaluated by the epididymal sperm count, sperm motility and sperm viability were found to be improved by the quercetin treatment. These changes are considered to be a consequence of fluid and sperm retention in the epididymis, epididymal lumen dilation and increased epididymis weight, which are reflected by the quality of the stored sperm reserve.

The time course and mechanism of quercetin's effect on sperm quality should be examined. It is not possible for quercetin to increase testicular spermatogenesis and subsequent epididymal sperm count within 14 days of treatment. That would require the duration for the development from spermatogonia to spermatozoa. The duration of renewal of spermatogonia type A in rat seminiferous tubules takes 12 days and the progression from spermatogonia to spermatozoa takes 48–52 days [17]. The 3–14-day period of our study covered only one cycle of spermatogonia type A renewal. Therefore, the increase in the epididymal sperm count observed after 14 days of quercetin treatment, compared with the control group, requires further investigations. Ma *et al.* [10] reported that the levels of serum quercetin metabolites reached a plateau on the sixth day of daily quercetin feeding in male rats. In our study, the increase in some

parameters of sperm quality could be detected only after 7 days of treatment at the higher doses (Q90 and Q270) of quercetin. Gozales *et al.* [19] reported that the epididymal sperm count was increased due to black maca (*Lepidium meyenii*), believed to be another androgenic plant, after only 1 day of feeding in male rats and the subsequent sperm count in the vas deferens was increased after feeding for 3 days. They described the increase of the sperm count due to black maca as caused by change in the regulatory mechanism of the distribution of sperm produced in the testis rather than higher production of spermatozoa.

A significant reduction in the weight of the prostate gland and seminal vesicle after Q270 treatment for 14 days was accompanied by changes in their histology, a decrease of tube number and dilation of the lumens. Ma *et al.* [10] report that quercetin slightly increased the wet prostate weight in rats fed with lower doses (50 and 100 mg/kg body weight/day for 10 days), which was accompanied by the dramatic dilation of the prostate lumen and the greater retention of fluid. In agreement with our study, when the dose of quercetin was increased up to 150 mg/kg body weight/day, the wet prostate gland weights became lower than those in rats fed with lower doses. Although the dramatic dilation of the prostate lumen still occurred, the fluid retention was slightly reduced. Because the weight of an organ (i.e. the prostate or seminal vesicle) is the combined weight of the organ itself (dry weights) and the fluid (secretion) contained inside, the effects of test substances on sex organs should not be evaluated only by the weight changes but also by histological changes. Furthermore, in addition to the lumen dilation, the prostatic epithelial height, which is known to be androgen-dependent [20], appeared to be decreased by the Q90 or Q270 treatment. Although Ma *et al.* [10] reported that quercetin stimulates an increase in the serum testosterone level, we could not detect changes in serum testosterone levels even by Q270 injection for 28 days (data not shown). In agreement with this, Gonzales *et al.* [20] found that red maca (*Lepidium meyenii*) reduced the prostatic epithelial height in testosterone-treated rats without affecting serum testosterone or estradiol levels. They concluded that red maca interferes with androgen action at the prostate gland.

Considering all parameters of the doses of quercetin treatment (s.c. injection), it is noteworthy that the positive reproductive effect (improving the sperm quality and sex organ function) was observed for Q270 and partially

for Q90 after 14 days. Overall results indicate that quercetin might indirectly affect the sperm quality through stimulation of the sex organs, both at the cellular and organ levels. Based on our results, the use of quercetin and black kwao krua as alternative drugs for treatment of male infertility should be considered. The mechanisms of action need to be further investigated to determine the effective dose and duration of administration.

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