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Full Length Research Paper

The effects of the edible bird's nest on sexual function of male castrated rats

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In this study, the effects of edible bird's nest on sexual function of male castrated rats were investigated for the first time. The testosterone (T), luteinizing hormone (LH) and estradiol (E_2) levels, the penis and prostate and seminal vesicle indexes, and the protein expression of endothelial nitric oxide synthase (eNOS) were estimated. The prostate and seminal vesicle index and the expression of eNOS increased significantly in groups treated with edible bird's nest in comparison to the control group. The results demonstrated that the edible bird's nest may promote the sexual function of male castrated rats and T was supposed to be responsible for this function. Edible bird's nest may serve as an effective medicine for erectile dysfunction (ED) treatment.

Key words: Edible bird's nest, sexual function, castrated rats, erectile dysfunction, hormones, nitric oxide synthase.

INTRODUCTION

Erectile dysfunction (ED) is a common male sexual disorder. It is defined as the persistent inability to achieve or maintain penile erection sufficient for satisfactory sexual performance (NIH Consensus Conference, 1993). ED results from a continuous spectrum of clinical factors, including physical illness, reaction to stress and relationship difficulties (Corona and Maggi, 2010). Testosterone (T) supplementation and phosphodiesterase-5 inhibitors are mainly used to treat ED, but the results are not always satisfactory (Rajfer et al., 2002; Tsertsvadze et al., 2009).

Edible bird's nest is the nest made from saliva of *Collocalia* swiftlets during the breeding and nesting season. The white edible bird's nest is built by *Aerodramus fuciphagus* (Valli and Summers, 1990). Edible bird's nest is highly esteemed for their nutritional and medicinal value. It has been used for a long time in traditional Chinese medicine. It was used in consumption disease, stomach ulcers, haematemesis, general debility and asthenia. It is claimed that consuming edible bird's nest regularly can give a person exuberant physical and mental strength as well as restore the fine and fair complexion of one's youthfulness (Leh, 2001).

In this experiment study, the effects of the edible bird's nest on the sexual function of male castrated rats were studied, intending to find a new possible solution for ED treatment. No literature has reported on this before.

MATERIALS AND METHODS

Animals

Thirty-six adult male Wistar rats weighing 250 to 300 g were randomly divided into six groups with six rats each. The six groups were: one sham operated group (A) and five castrated groups (B, C, D, E, and F). The castrated rats were obtained by carrying out bilateral orchiectomy with both testes removed under anesthesia. Rats from group A were sham operated. All the rats were injected intramuscularly with penicillin potassium (30000 IU/rat/day) for three days just after the operation. One week after the surgery, rats from

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groups A and B were intragastrically administered normal saline (6 ml/kg/day) for ten days, whereas rats from groups C, D, and E were intragastrically administered the edible bird's nest (1 mg/kg/day, 3 mg/kg/day, and 9 mg/kg/day, respectively) for ten days. At the same time, rats from group F were subjected to testosterone propionate intramuscular injection (2 mg/kg/day) for ten days.

Drugs

Unprocessed white edible bird's nest was obtained from CINRA Food Industries SDN., BHD (Malaysia). It was processed as described by Guo et al. (2006). The grounded nest was dissolved in water to make turbid solutions of proper concentration.

Serum hormones assay

After treatment with the drugs, the blood sample was obtained and centrifuged to obtain serum. The serum was stored at -26°C before analysis. T, luteinizing hormone (LH) and estradiol (E_2) analyses were carried out by full-automated microparticle chemiluminescent immunoassay at Key Laboratory for Improving Birth Outcome Technique of Shandong Province.

Penis and prostate and seminal vesicle indexes assay

The rats were dissected to obtain the penis, prostate, and seminal vesicle. The organs were washed with normal saline before weighing and the organs indexes (mg/g) were calculated. Corpus cavernosum was isolated from the penis and stored at -26°C as samples for Western-blot.

Western-blot

Tissues of the corpus cavernosum were grounded in liquid nitrogen and centrifuged at 10000 rpm for 2 min at 4°C after mixing with the extract buffer. The supernatant was collected. Protein concentration was determined by Lowry method (Waterborg and Matthews, 1994). Twenty milligrams of the total protein were used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Goat polyclonal immunoglobin G (IgG) (NOS (c-12): sc-49055, Santa Cruz, CA, USA) was used as primary antibody for endothelial nitric oxide synthase (eNOS) and diluted at 1:500 in Tris-buffered saline (TBS). Horse-radish peroxidase (HRP)-labeled rabbit anti-goat IgG (SB 300, Jingmei Biotech, Biotech, China) was used as secondary antibody for eNOS and diluted at 1:700 in TBS. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The EZ-ECL Chemiluminescence Detection Kit for HRP was used to react with the membrane. The membrane was exposed onto X-ray films. The density of the band was measured. The results were presented as the mean density of the protein band in question relative to that of the GAPDH band in the sample.

Statistical analysis

Statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) 13.0 for Windows (SPSS, InC., Chicago, IL, USA). Differences among samples of groups B, C, D, and E were determined by Duncan's multiple range test. Comparison of samples of groups A and B as well as comparison of samples of groups A and F were made with independent samples t-test. Differences reaching a confidence level of 95% were considered as statistically significant.

RESULTS

Effects of the edible bird's nest on serum hormones level

The results are shown in Table 1. For the levels of all of the three kinds of hormones (T, LH, and E_2), there was no significant difference between the sham operated group (group A) and the testosterone propionate injected group (group F) ($p > 0.05$) and hormone levels in the castrated group (group B) were significantly lower than that in group A ($p < 0.05$). The edible bird's nest treated group (group E, 9 mg/kg/day) exhibited significantly higher T and LH levels when compared with that of group B ($p < 0.05$), while the other two groups (group C, 1 mg/kg/day and group D, 3 mg/kg/day) showed no significant difference when compared with group B ($p > 0.05$). For E_2, the three edible bird's nest treated group (groups C, D, and E) did not show any significant difference when compared with group B ($p > 0.05$), but there was a trend that E_2 level increases with the dose of the edible bird's nest administered.

Effects of the edible bird's nest on penis and prostate and seminal vesicle indexes

As is shown in Table 1, the penis index and prostate and seminal vesicle index in the castrated group (group B) were significantly lower than that of the sham operated group (group A) ($p < 0.05$), while no significant difference was observed between group A and the testosterone propionate injected group (group F) ($p > 0.05$). The penis index of the edible bird's nest treated group (group E, 9 mg/kg/day) was significantly higher than that of group B ($p < 0.05$), while the penis indexes in groups C (1 mg/kg/day) and D (3 mg/kg/day) showed no significant difference when compared with that of group B ($p > 0.05$). For prostate and seminal vesicle index, significant increases were observed in groups D and E when compared with that in group B ($p < 0.05$).

Effects of the edible bird's nest on protein expression of eNOS

The Western blot results are shown in Figure 1. Significant decrease was observed when comparing the protein expression level of eNOS in the castrated group (group B) with that in the sham operated group (group A) ($p < 0.05$), while significant increases were observed in the edible bird's nest treated groups (group C, 1 mg/kg/day; group D, 3 mg/kg/day; and group E, 9 mg/kg/day) when compared with that of group B ($p < 0.05$). No significant difference was observed between

Table 1. Serum hormones level and the penis and prostate and seminal vesicle indexes.

Group	T (ng/ml)	LH (mIU/ml)	E2 (pg/ml)	Penis index (mg/g)	Prostate and seminal vesicle index (mg/g)
F	2.09 ± 0.06	0.91 ± 0.08	27.25 ± 2.22	1.21 ± 0.13	5.80 ± 1.60
A	2.24 ± 0.03	0.92 ± 0.07	26.34 ± 2.35	1.22 ± 0.13	5.85 ± 1.12
B	0.58 ± 0.01$^{\#}$	0.25 ± 0.07$^{\#}$	18.60 ± 1.67$^{\#}$	0.87 ± 0.15$^{\#}$	0.80 ± 0.16$^{\#}$
C	0.58 ± 0.04	0.24 ± 0.06	16.33 ± 3.14	0.83 ± 0.15	0.79 ± 0.10
D	0.69 ± 0.01	0.30 ± 0.10	20.17 ± 3.97	0.88 ± 0.11	1.02 ± 0.09*
E	0.85 ± 0.02*	0.36 ± 0.08*	22.17 ± 3.66	1.03 ± 0.06*	1.44 ± 0.27*

Values represent means ± standard deviation (SD) (n = 6); $^{\#}p < 0.05$ vs. group A; *p < 0.05 vs. group B.

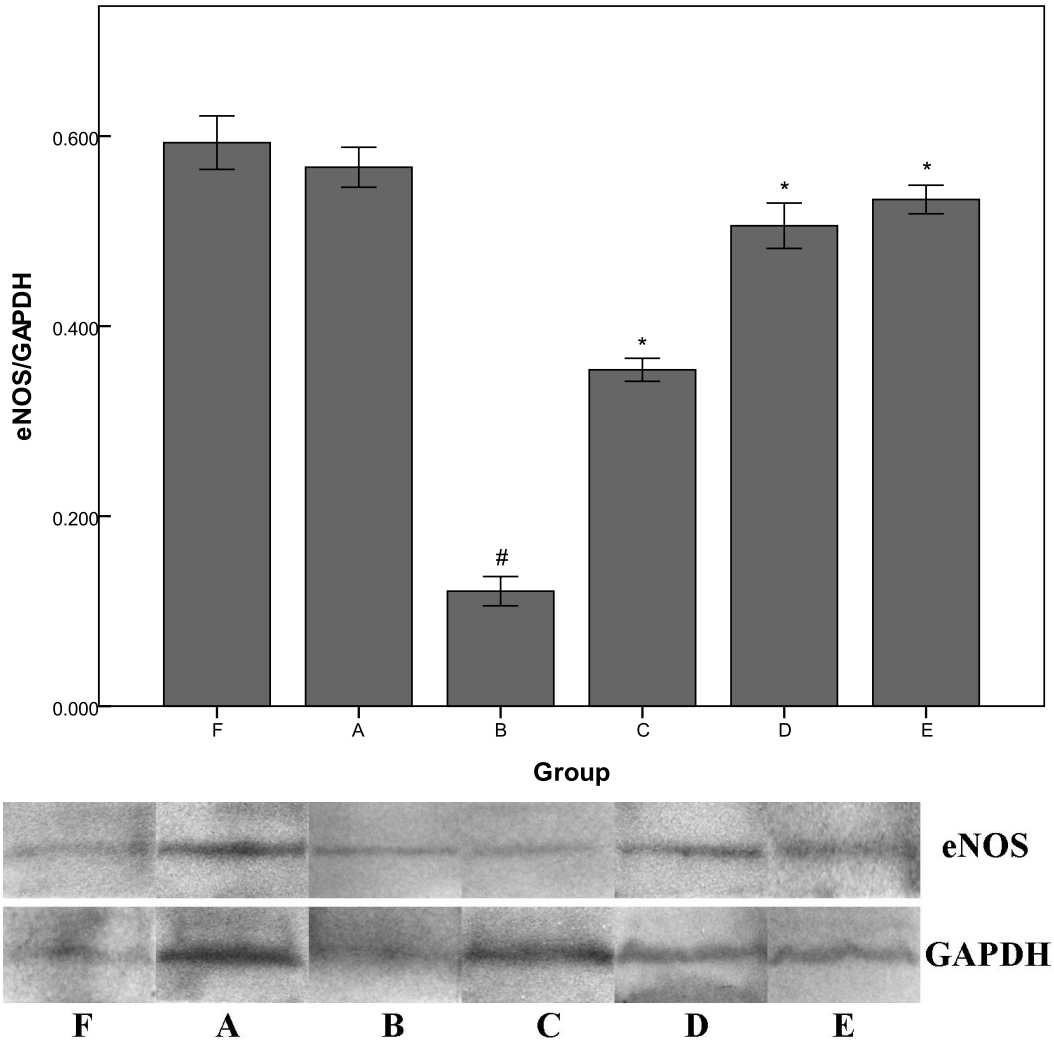

Figure 1. Results of Western-blot. Densitometry and statistical analysis of eNOS (ratio to GAPDH) in the corpus cavernosum.
Values are presented as means ± standard deviation (SD) (n = 6). $^{\#}p < 0.05$ vs. group A; *p < 0.05 vs. group B.

group A and the testosterone propionate injected group (group F) and between group C (1 mg/kg/day) and group B ($p > 0.05$).

DISCUSSION

As show in the results, the administration of edible bird's

nest promotes the development of penis and prostate and seminal vesicle, secretion of sex hormones (T and LH) and the expression of eNOS in male castrated rats.

We chose castrated rats as our ED animal model. Castration results in impaired erectile response to central and peripheral stimulation decrease in penile tissue concentration of nitric oxide synthase-nerves, and apoptosis in the rat penis (Shabsigh, 1997). In addition, castration causes vascular smooth muscle cell atrophy, venous leakage, adipocytes in the subtunical space, loss of elastic fibers, and increase in collagen deposition (Jones, 2009). In our experiment, the comparison of the outcome measures between the sham operated group (group A) and the castrated group (group B) confirmed the success of our animal model.

The characterization of the edible bird's nest has been researched in several studies (Marcone, 2005). The bioactive component for enhancing the sexual function is supposed to be T. Androgens especially T plays important roles in male sexual function. Animal data show that androgens support erectile function through a direct effect on the erectile tissue (Shabsigh, 1997) and they are essential in the maintenance of nitric oxide (NO)-mediated erectile activity in the rat (Reilly et al., 1997). Animal studies have demonstrated that T plays critical physiological (activity of NOS and phosphodiesterases), biochemical (through an endothelial-independent pathway and adrenergic tonicity), and structural (change of fibro-elasticity and hollow cell accumulation) roles in the erectile function (Hwang and Lin, 2008). T increases the expression of NOS which involved in the erectile process (Jones, 2009) and increases the amount of NO produced by corpus cavernous and penile arteries during erection (Lugg et al., 1995; Schirar et al., 1997). In animal models, it has been shown that T may regulate erectile function locally via actions on the NO-guanylate cyclase-cGMP pathway. Animal experiments show that T can regulate the corporeal smooth muscle and penile arterial tone (Penson et al., 1996; Bivalacqua et al., 1998; Sato et al., 1998; Alcorn et al., 1999; Mills et al., 1999). In this experiment, rats in group F were intramuscularly injected with testosterone propionate, and the sexual function-promoting effect was very significant. So, we guess it was the T that increased after administration of the edible bird's nest (9 mg/kg/day) that contributed to the increase of the penis and prostate and seminal vesicle indexes and the protein expression of eNOS in the male castrated rats.

The NOS has three isoforms: neuronal nitric oxide synthase (nNOS) is originally discovered in neurons, eNOS is from endothelial cells and inducible nitric oxide synthase (iNOS) is an inducible isoform (Bischoff et al., 2003; Hung et al., 1995). NOS is highly concentrated within the pelvic plexus, the cavernosal nerve and adventitia of the deep cavernosal arteries and the sinusoids in the penis (Burnett and Lowenstein, 1992; Rajfer et al., 1992). NO is synthesized through the oxidation of L-arginine by NOS (Aoki et al., 1995). NO is required for the maintenance of vascular tone (Calver et al., 1993) and it mediates blood vessel relaxation and regulate sexual and aggressive behavior (Dawson and Dawson, 1996). NO is involved in reproductive functions and behaviors, including E_2-synthesis (Olson et al., 1996), penile erection (Mani et al., 1994) and luteinizing hormone releasing hormone (LHRH) releasing control (Rettori et al., 1993). In this study, the increase of LH and the trend of increase of E_2 may be attributed to the NO synthesis. T and E_2 have effects on Sertoli cells which play important roles in the development of a functional testis (Colenbrander et al., 1993). LH promotes Leydig's cells proliferation and stimulates the synthesis and secretion of T by Leydig's cells to provide for spermatogenesis (Lui et al., 2010). So, the increase of serum E_2 and LH levels in this experiment may enhance the sexual function of the male castrated rats further. But since there is also evidence that the risk of ED was higher as the levels of LH increased (Kupelian et al., 2006), further studies are needed to define whether excessive intake of the edible bird's nest can lead to excessively-high LH level and determine the optimal intake dose of the edible bird's nest.

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