Int J Clin Exp Med 2015;8(5):7599-7605
www.ijcem.com /ISSN:1940-5901/IJCEM0006388

Original Article

Effects of quercetin on intracavernous pressure and expression of nitrogen synthase isoforms in arterial erectile dysfunction rat model

Yueyang Zhang[1*], Changting Huang[2*], Shaoming Liu[5], Jianqi Bai[3], Xiaojing Fan[4], Jun Guo[6], Yingyu Jia[7], Zhijie Zhang[8], Xiaojun Chen[8], Yusen Jia[8], Ping Zhang[3], Bin Wang[9], Xiuju Zhang[10]

[1]Department of Andrology, Wangjing Hospital of China Academy of Traditional Chinese Medicine, Beijing 100102; [2]Department of Pediatrics, Wangjing Hospital of China Academy of Traditional Chinese Medicine, Beijing 100102; [3]Department of Pathology, Wangjing Hospital of China Academy of Traditional Chinese Medicine, Beijing 100102; [4]Department of Acupuncture and Moxibustion, Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300150; [5]Department of Urology, Third Affiliated Hospital of Beijing University of Chinese Medicine, Beijing 100029; [6]Department of Andrology, Xiyuan Hospital of China Academy of Traditional Chinese Medicine, Beijing 100091; [7]Department of Scientific Research, Wangjing Hospital of China Academy of Traditional Chinese Medicine, Beijing 100102; [8]Department of Urology, Oriental Hospital of Beijing University of Chinese Medicine, Beijing 100078; [9]Department of Andrology, Dongzhimen Hospital of Beijing University of Chinese Medicine, Beijing 100700; [10]Department of Traditional Chinese Medicine, Pinggu District Hospital, Beijing 101200. [*]Co-first authors.

Received January 27, 2015; Accepted May 13, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Object: Oxidative stress involved in the regulation of arterial erectile dysfunction (A-ED). Previously report have indicated that quercetin have an antioxidant effect. In the current study, we have established the rats' model for study the therapeutic effect of quercetin on A-ED and further investigated the molecular mechanism of action. Methods: Wistar rats were divided into sham group, A-ED group, A-ED group with low dose of quercetin, and A-ED group with high dose of quercetin. Intracavernous pressure (ICP) and mean arterial pressure (MBp) are two important indicators used for evaluation the A-ED. The changes of ICP and MBp were determined by cavernous nerve electrostimulation after treatment of quercetin at indicated doses. The expression of nitric oxide synthase (NOS) subtypes was detected by RT-PCR and Western blotting. Results: Our results indicated that ICP was significantly reduced in A-ED rats model compared with sham group, and was significantly increased after quercetin treatment ($P < 0.01$), while no significant effect on the MBp. The data also showed that sGC inhibitor ODQ and NOS inhibitor LNNA can significantly inhibited the ICP which induced by quercetin. These results suggest that NO-cGMP signaling pathway plays a crucial role in A-ED. Then, we found that the mRNA and protein levels of eNOS were significantly reduced in A-ED group compared with sham group. After treated with quercetin may cause the eNOS RNA and protein were significantly up-regulated ($P < 0.01$), showing a dose-dependent effect. iNOS expression have a certain degree of increased after quercetin treatment. nNOS expression was not significantly increased before and after treated with quercetin. In a word, quercetin can improved the A-ED by up-regulated ICP, which related to up-regulation of NO-cGMP signaling pathway. Conclusion: Preliminary results of this study suggested that quercetin protected expression and function of eNOS in cavernous endothelial cells, and restored part of normal function of NO-cGMP pathway in the process of penis erection.

Keywords: Quercetin, NOS, ICP, MBp, arterial erectile dysfunction (A-ED)

Introduction

Arterial erectile dysfunction (A-ED) refers to the penis cannot achieve and maintain sufficient erection to complete sexual intercourse of satisfaction, and the course lasts for at least six months [1, 2]. Currently, It is estimated that about one hundred and fifty million men deeply troubled by A-ED in the world [3]. Penile erection is a process of artery supply of blood and cavernous storage of blood which initiated by nerve, and neuro-transmitters play an important role in the regulation of erectile function [4, 5]. Nitric oxide (NO), which was synthesized by

nitric oxide synthase (NOS) and used L-arginine as substrate, is main messenger that induce vasodilation and relaxation of cavernous, involving in the induction and maintain of erection [6, 7]. NO is a small molecule of fat-soluble gaseous, with short half-life usually lasts only 1-2 s, containing unpaired electrons leading to chemically unstable [7]. Moreover, NO can directly enter into cell membrane of smooth muscle cells and bind to ornithine cyclase, which catalytic generate a lot of cGMP and change the level of Ca^{2+} inducing smooth muscle relaxation [8].

The nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling pathway plays a crucial role in regulating the process of penile erection. Nitric oxide synthase (NOS) is an important target for erectile initiate [9, 10]. Up-regulation of NOS is closely related to pathogenesis of A-ED, such as diabetes and senile erectile dysfunction and so on. Inadequate blood supply of penile is one of the most common causes of A-ED [11]. Reduction of penile arterial vascular perfusion can cause chronic ischemia and hypoxia, inducing microstructure and functional impairment of cavernous endothelial cells and smooth muscle cells, eventually leading to arterial erectile dysfunction (A-ED) [12].

Quercetin is a multi-hydroxy flavonoids with a variety of significantly biological activity and obviously medicinal value, which was widely distributed in flowers, leaves and fruit of plants [13]. It has been known that more than 100 kinds of medicinal plants could synthase quercetin, such as Sophora japonica Linn. Quercetin is not only widely distributed in plants, but also have a wide variety of pharmacological effects, containing antioxidant, removing of radical scavenging, anti-cancer, anti-inflammatory, antibacterial, anti-viral, hypoglycemic, hypotensive, immune regulation and heart vascular protective effects [14, 15]. However, the effect of quercetin on erectile dysfunction is still poorly understood.

In the current study, we investigated the therapeutic effect of quercetin on A-ED rat's model and further explore its molecular mechanism. We detected the effect of quercetin on intracavernous pressure (ICP) and peripheral blood pressure (MBp) by cavernous nerve electrostimulation after treatment of quercetin. And ODQ and LNNA were used for determining the mechanism of quercetin effects on A-ED *in vivo*, which were an inhibitor of NO-cGMP and NOS, respectively. In addition, we detected mRNA and protein expression of three NOS isoforms, to further explore the effect of quercetin on penile erection and its mechanism.

Materials and methods

Ethics statements

All experimental procedures and protocols were approved by the local Institutional Animal Care Committee and performed according to the Policies on the Use of Animal and Humans in Neruoscience Research.

Materials

Quercetin (analytical grade with 98% purity) was purchased from Millipore and dissolved in ethanol and stored at -20°C. sGC inhibitor and NOS inhibitor were purchased from Sigma-Aldrich and dissolved in ethanol and stored at -20°C.

Established of A-ED rats model

40 healthy Wistar rats, weighing 300-350 g, provided by Shanghai animal center. Randomly divided into four groups, sham group, A-ED model blank treated group, A-ED model with low-dose of quercetin treatment group and A-ED model with high-dose of quercetin treatment group. Adaptive feeding one week for surgery. According to previous reports to established A-ED rats' model. Each model of A-ED group with 7-0 nylon suture ligation within the iliac artery and its branches under the operating microscope to establish A-ED model; A group as a sham, is not only revealed in the iliac artery ligation. After the model is established and surgical therapy.

Determination of ICP and MBp

Rats using pentobarbital sodium (50 mg/kg) to anesthesia by intraperitoneal injection then isolated, exposure to rat cavernous bank, using 26 G needle penetrates the right side of the penis after connecting with Biopack electrophysiological monitoring ICP instrument. In addition to the use of a 26 G needle penetrates the left root penis, as intracavernous medication liquid channel. Total free right carotid

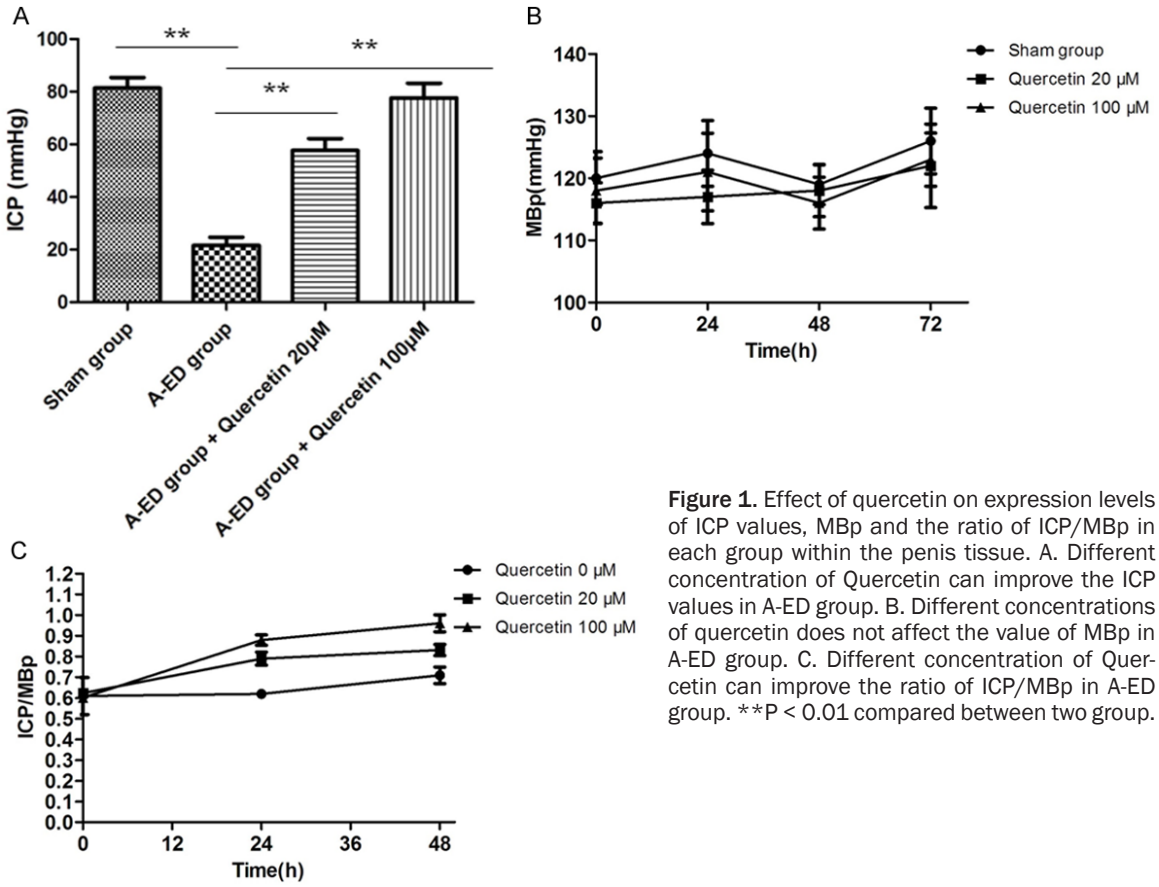

Figure 1. Effect of quercetin on expression levels of ICP values, MBp and the ratio of ICP/MBp in each group within the penis tissue. A. Different concentration of Quercetin can improve the ICP values in A-ED group. B. Different concentrations of quercetin does not affect the value of MBp in A-ED group. C. Different concentration of Quercetin can improve the ratio of ICP/MBp in A-ED group. **P < 0.01 compared between two group.

artery puncture 26 G indwelling trocar and connected with the Biopac more conductive physiological instrument, continuous monitoring the changes of MBp. Intraductal connection with the right amount of heparin to prevent clotting of saline, abdominal incision, the side lobe in rats exposed to the prostate by surgical microscope shown that looking at the pelvic ganglia, and looking for CN along the outside between the prostate and rectum Electrical stimulation of the parameter is set to 3 V, 12 Hz, stimulation time for 60 s, continuous observation the changes of ICP and MBp before treatment and recorded. After quercetin, ODQ, or LNNA treatment, continuously observed the changes of ICP and MBp in different experiment group and recorded. The interval between two electrical stimulation was 30 min.

Western blotting

Total proteins were extracted from the penis tissue by RIPA buffer (Thermo). Proteins were separated by SDS-PAGE on an 10% gel. Primary antibodies are rabbit nNOS, eNOS, iNOS and GAPDH (all primary antibodies were purchased from Abcam). Secondary antibody is HRP-conjugated anti-rabbit (Jackson Labs). The bound antibody was detected with a chemofluorescence detection kit (Amersham, Piscataway, NJ). The representative images were shown.

Reverse transcriptase PCR and quantitative reverse transcriptase PCR

Total RNA were extracted using RNAiso (TaKaRa) according to operating instructions. Reverse transcriptase (RT)-PCR and Real-time PCR (RT-PCR) were used to detect the nNOS, eNOS and iNOS expression. GAPDH was regarded as normalization.

Statistical analyses

ANOVA tests (ex vivo and in vivo) and Pearson correlation analysis were performed the statistical analyses. All experiments were perform in triplicate and data are presented as means ± SEM. If $P < 0.05$ was remarked as *, and $P < 0.01$ was considered as **.

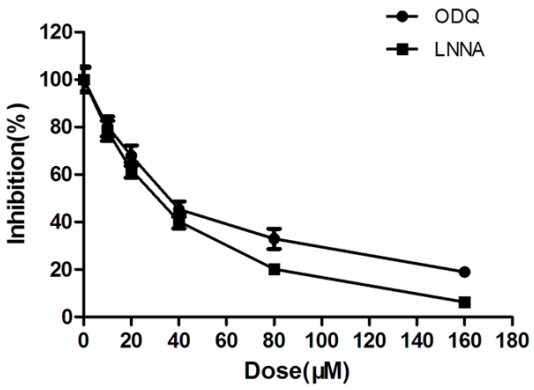

Figure 2. Effect of different concentrations of ODQ and LNNA on ICP changes with quercetin induced.

Results

Different concentration of quercetin can improve the ICP values in A-ED group

For study the therapeutic effect of quercetin in A-ED model and to explore its molecular mechanism, we firstly observed the effect of quercetin on ICP in a rat A-ED model. Our results shown that the ICP value was significantly lower in A-ED group compared with the sham group. Quercetin is a multi-hydroxy flavonoids, with a variety of biological activity and high medicinal value. In the current study, we investigated whether the quercetin has a therapeutic effect in A-ED model. Our data indicated that ICP values was evidently restored with quercetin treatment by intracavernous injection in the penis of rats ($P < 0.01$). And furthermore, we found that the ICP value was gradually increased with increase the doses of quercetin ($P < 0.01$) (**Figure 1A**).

Different concentrations of quercetin does not affect the value of MBp in A-ED group

MBp is an important indicator for measure the impact of quercetin on erectile dysfunction in A-ED rat model. In our study, we further examined the effect of quercetin on erectile function and mean arterial pressure (MBp) changes by evoked the cavernous nerve stimulation in vivo. The data was found that treatment of quercetin have no significant impact on MBp at different time-points or at different doses (**Figure 1B**). This result suggests that quercetin can selectively enhance erectile function, and had no effect on mean arterial pressure, which can be used as an oral medication for erectile dysfunction, to avoid side effects caused due to systemic blood pressure.

Different concentration of quercetin can improve the ratio of ICP/MBp in A-ED group

We observed the changes of the ICP/MBp in A-ED rat model after intracavernous injection with different concentrations of quercetin. In our studies, the data showed that the ratio of ICP/MBp was significantly increased after treatment of quercetin (**Figure 1C**). The ratio of ICP/MBp was increased begin to 24 h after treatment of quercetin with 20 μM. Furthermore, Pearson correlation analysis found that different concentrations of quercetin was enhanced the ratio of ICP/MBp to have a significant dose-dependent manner, and the difference was statistically significant ($P < 0.01$) (**Figure 1C**).

Effect of different concentrations of ODQ and LNNA on ICP changes with quercetin induced

In our study, we further observe the effect of NOS inhibitor ODQ and sGC inhibitor LNNA treated separately on ICP changes which induced by quercetin. The data found that ODQ and LNNA could significantly inhibited the ICP changes with quercetin-induced. And the inhibition of ICP was shown a concentration-dependent manner ($P < 0.01$) (**Figure 2**). These results indicated that the effect of quercetin enhanced ICP was significantly inhibited by NOS inhibitor ODQ and sGC inhibitor LNNA, suggesting that the effect quercetin on erectile function may be related to the release of NO and cGMP synthesis.

Effect of quercetin on expression levels of NOS isoforms in each group within the penis tissue in A-ED rat model

Nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling pathway plays a critical role in regulating the process of penile erectile dysfunction, nitric oxide synthase (NOS) is an important target for erectile start [16]. So, we detected the three different phenotypes expression of NOS in A-ED rat model. RT-PCR results confirmed that expression of NOS three different phenotypes was significantly decreased in the penis tissue of A-ED group. eNOS expression was significantly increased after quercetin treatment, and the expression level of eNOS was higher in the high dose group than

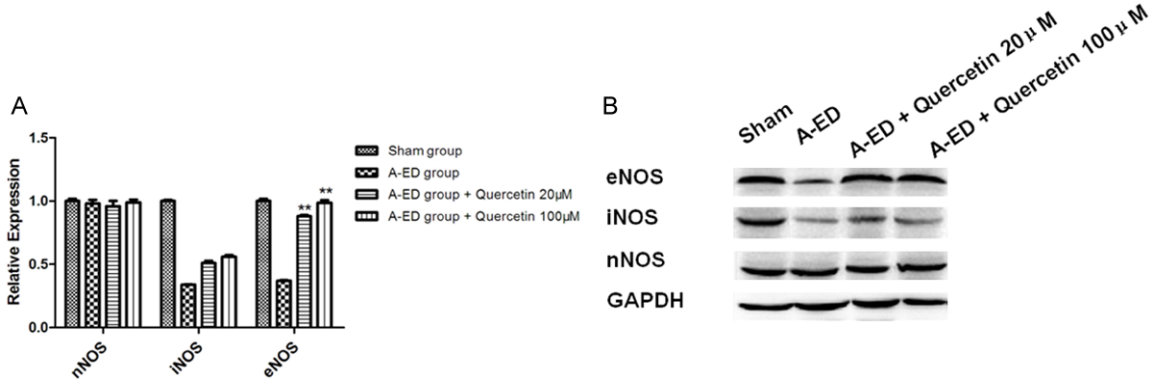

Figure 3. Effect of quercetin on expression levels of NOS isoforms in each group within the penis tissue. A. RT-PCR detected the effect of quercetin on mRNA expression of NOS isoforms; B. Western blot detected the effect of quercetin on protein expression of NOS isoforms. **P < 0.01 compared between high dose and low dose group.

the low dose group. iNOS mRNA expression was increased after quercetin treatment, but no significant difference in the high-dose group and low dose group. Additionally, nNOS was no significant increased expression in each group after treatment of quercetin (**Figure 3A**). WB results found that the protein level of NOS isoforms eNOS and iNOS were significantly reduced in the A-ED model group compared with the sham group. After quercetin treatment, the protein expression of eNOS was significantly recovery, and high-dose group was evidently higher than that of quercetin low-dose group. The protein levels of nNOS in the high-dose group had mild recovery, but quercetin low-dose treatment group had no further improvement. nNOS was no differences among groups before and after treatment (**Figure 3B**). All together, these results suggested that increased eNOS after quercetin may play a critical role in improving the erectile function.

Discussion

Penile erection is a hemodynamic process which penile arteries, penis and penile venous return induced by neuro-endocrine regulation [17]. NOS plays an important role in the pathogenesis of a variety of ED, and expression of NOS is significantly reduced in ED diabetic animals model, ED elder model and ED castrated model [18]. There was significant recovery of erectile function in ED diabetic animal model and ED elder model using eNOS as target for gene therapy. Change of ICP induced by penis cavernous nerve is usually used as an objective and effective assessment index with the function of drugs in erectile function *in vivo* [19, 20].

In this study, we established the A-ED rats model by ligation of bilateral internal iliac artery, to investigate the changes of ICP and MBp induced by quercetin stimulate cavernous nerve of rat penis, and further to observe the effects of quercetin on penis erection and arterial blood pressure. The results showed that quercetin could significantly increased ICP by penis cavernous with a concentration-dependent manner, but had no significant impact on MBp. The results suggested quercetin could selectively enhance erectile function, but had no effect on systemic blood pressure, which could be used as for oral medication for ED and simultaneously avoid side effects caused by systemic blood pressure. Moreover, using quercetin treat A-ED model in our experiments, data implied that ICP had been significantly recovery in low-dose group and high-dose group, and ICP was significantly more effective in high-dose group than low-dose group. Significantly improved of ICP illustrated that it had been established rich blood supply been pelvic vessels and pelvis cavernous, may achieve enough arterial perfusion pressure to penile erection.

Stimulation of penis cavernous nerve could activate NOS activity of non-adrenergic and non-cholinergic nerve endings, promoting production and release of NO, then leading to increase cGMP concentration, which induced relaxation of smooth muscle and finally resulted in erection [21-23]. In the current study, expression of eNOS was significantly up-regulated in the low-dose quercetin group and high-dose quercetin group, which was consistent with change of ICP. Moreover, expression of iNOS also had slightly elevated in low-dose

quercetin group, but with no obvious level compared to eNOS. Expression of iNOS did not apparently increased in high-dose quercetin group, and expression of nNOS was not significantly different between the two groups. All the data suggested that up-regulation of eNOS may play a pivotal role in improving A-ED. In addition, our results showed that the effect of quercetin on enhancement of cavernous ICP could be remarkably inhibited by LNNA that was NOS inhibitor and sGC that was ODQ inhibitor [24, 25], suggesting that quercetin effect on erectile may be related to synthesis and release of both NO and cGMP, therefore affected NO-cGMP signaling pathway and ultimately resulting in A-ED.

This preliminary results suggested that quercetin protected expression and function of eNOS in cavernous endothelial cells, and restored part of normal function of NO-cGMP pathway in the process of penis erection. However, it was still to further studied that quercetin how to restore expression of eNOS in A-ED model, as well as mechanism of erectile in the improved A-ED model.

Disclosure of conflict of interest

None.

Address correspondence to: Shaoming Liu, Department of Urology, Third Affiliated Hospital of Beijing University of Chinese Medicine, No. 51 Anwaixiaoguan Street, Chaoyang District, Beijing 100029. Tel: 18301199188; E-mail: Shaomingdoc@126.com

References

[1] Giuliano F and DS. Erectile dysfunction. Prog Urol 2013; 23: 629-37.
[2] Cirino G, Fusco F, Imbimbo C, Mirone V. Pharmacology of erectile dysfunction in man. Pharmacol Ther 2006; 111: 400-23.
[3] Simopoulos EF and T AC. Male erectile dysfunction: integrating psychopharmacology and psychotherapy. Gen Hosp Psychiatry 2013; 35: 33-8.
[4] Jung J, Jo HW, Kwon H, Jeong NY. Clinical neuroanatomy and neurotransmitter-mediated regulation of penile erection. Int Neurourol J 2014; 18: 58-62.
[5] Argiolas A and Melis MR. Neuropeptides and central control of sexual behaviour from the past to the present: a review. Prog Neurobiol 2013; 108: 80-107.
[6] Courtois F, Carrier S, Charvier K, Guertin PA, Journel NM. The control of male sexual responses. Curr Pharm Des 2013; 19: 4341-56.
[7] Förstermann U and Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012; 33: 829-37.
[8] KE A. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. Pharmacol Rev 2011; 63: 811-59.
[9] Li E, Hou J, Li D, Wang Y, He J, Zhang J. The mechanism of vacuum constriction devices in penile erection: the NO/cGMP signaling pathway? Med Hypotheses 2010; 75: 422-4.
[10] IF G. Nitric oxide-cyclic GMP pathway with some emphasis on cavernosal contractility. Int J Impot Res 2004; 16: 459-69.
[11] Ha US, Koh JS, Kim HS, Woo JC, Kim SJ, Jang H, Yoon BI, Hwang SY, Kim SW. Cyanidin-3-O-β-D-glucopyranoside concentrated materials from mulberry fruit have a potency to protect erectile function by minimizing oxidative stress in a rat model of diabetic erectile dysfunction. Urol Int 2012; 88: 470-6.
[12] Manabe K, Heaton JP, Morales A, Kumon H, Adams MA. Pre-penile arteries are dominant in the regulation of penile vascular resistance in the rat. Int J Impot Res 2000; 12: 183-9.
[13] Sak K. Site-specific anticancer effects of dietary flavonoid quercetin. Nutr Cancer 2014; 66: 177-93.
[14] Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 2008; 11: 733-40.
[15] Formica JV and Regelson W. Review of the biology of Quercetin and related bioflavonoids. Food Chem Toxicol 1995; 33: 1061-80.
[16] Sandner P, Neuser D, and Bischoff E. Erectile dysfunction and lower urinary tract. Handb Exp Pharmacol 2009; 191: 507-31.
[17] Dong HW and wanson LW. Projections from bed nuclei of the stria terminalis, magnocellular nucleus: implications for cerebral hemisphere regulation of micturition, defecation, and penile erection. J Comp Neurol 2006; 494: 108-41.
[18] Lacchini R, Muniz JJ, Nobre YT, Cologna AJ, Martins AC, Tanus-Santos JE. nNOS polymorphisms are associated with responsiveness to sildenafil in clinical and postoperative erectile dysfunction. Pharmacogenomics 2014 15: 775-84.
[19] Armah FA and Gyeabour EK. Health Risks to Children and Adults Residing in Riverine Environments where Surficial Sediments Contain Metals Generated by Active Gold Mining in Ghana. Toxicol Res 2013; 29: 69-79.
[20] Yri HM, Wegener M, Sander B, Jensen R. Idiopathic intracranial hypertension is not benign: a long-term outcome study. J Neurol 2012; 259: 886-94.

[21] Amany S and Heba K. Effect of pregabalin on erectile function and penile NOS expression in rats with streptozotocin-induced diabetes. Exp Clin Endocrinol Diabetes 2013; 121: 230-3.
[22] Yeh KY, Wu CH, and T YF. Noncontact erection is enhanced by Ginkgo biloba treatment in rats: role of neuronal NOS in the paraventricular nucleus and sacral spinal cord. Psychopharmacology (Berl) 2012; 222: 439-46.
[23] Tezer M, Ozluk Y, Sanli O, Asoglu O, Kadioglu A. Nitric oxide may mediate nipple erection. J Androl 2012; 33: 805-10.
[24] El-Yazbi AF, Cho WJ, Cena J, Schulz R, Daniel EE. Smooth muscle NOS, colocalized with caveolin-1, modulates contraction in mouse small intestine. J Cell Mol Med 2008; 12: 1404-15.
[25] Shen F Wang N, Qi C, Li YJ, Cui CL. The NO/sGC/PKG signaling pathway in the NAc shell is necessary for the acquisition of morphine-induced place preference. Behav Neurosci 2014; 128: 446-59.