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Inhibitory effects of thymoquinone on hypothalamic aromatase and ghrelin gene expression in intact and diabetic rats

Tahereh Jahani[1], Fariba Mahmoudi[1]*, Homayoun Khazali[2], Asadollah Asadi[1], Abolfazl Bayrami[1]

1. Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran
2. Department of Animal Science and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

Abstract

Background and Objective: Thymoquinone stimulates the activity of hypothalamic-pituitary-gonadal axis (HPG). Diabetes, ghrelin and aromatase are associated with decreased function of HPG axis. This study aimed to investigate the influence of thymoquinone on aromatase and ghrelin gene expression in intact and diabetic rats.

Materials and Methods: Twenty male Wistar rats weighing 190-220 g were used. Diabetes type 1 was induced by alloxan. Saline or thymoquinone (10 mg/kg) was injected into intact or diabetic rats intraperitoneally for two weeks. One day after last injection, the hypothalamic samples were removed. Relative gene expression of aromatase and ghrelin was determined by RT-PCR method.

Results: Thymoquinone did not alter the aromatase expressions in the healthy rats. However, it caused a marked decrease in ghrelin expression in healthy rats. The aromatase and ghrelin expression significantly reduced in the diabetic rats receiving thymoquinone in comparison with diabetic group.

Conclusion: Thymoquinone may be a drug to improve decreased HPG axis activity of diabetic rats due to its inhibitory effects on aromatase and ghrelin upstream GnRH neurons.

Keywords: Thymoquinone, Diabetes, Alloxan, Aromatase, Ghrelin

1. Introduction

Diabetes is an important endocrine disease which occurs as a result of insulin deficiency, inadequate insulin secretion or insensitivity of target cells to insulin. Diabetes results in hyperglycemia and oxidative stress, and dysfunction of liver and kidney (1,2) In addition, hyperglycemia exerts a negative influence on hypothalamic-pituitary-gonadal axis activity and patients with diabetes suffer from hypogonadism and infertility (3).

Aromatase is an enzyme which convert androgens to 17β- estradiol in central nervous system and peripheral organs such as gonads, liver and so on. The Cyp19 gene codes the aromatase. In addition to testosterone, the estradiol produced by aromatization of hypothalamic androgens partly participates in the regulation of the negative feedback mechanism HPG axis in males. They established that mutation of aromatase gene or dysfunction of hypothalamic aromatase induces infertility in males (4,5). The changes of aromatase gene expression have been established in the pathogenesis of disorders related to reproductive process. For example, the reduced

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***Corresponding Author:** Fariba Mahmoudi **E-mail:** f.mahmoudi@uma.ac.ir

hypothalamic or ovarian aromatase gene expression was shown in polycystic ovarian syndrome (PCOS) (6). Decreased level of aromatase synthesis was shown in the ovary, testis or adipose tissue of diabetic rats (7-9). However, there is no information about the central nervous system aromatase level in diabetic patients.

Ghrelin is a peptide with 28-amino acids. It is synthesized in digestive tract, hypothalamus, gonads and other tissues. It binds to growth hormone secretagogue receptor (GHSR) (10). Ghrelin participates in increasing appetite, growth hormone secretion and down- regulation of HPG axis activity predominantly via inhibiting GnRH/LH release. In addition to indirect neural pathways, ghrelin can inhibit GnRH neurons directly due to GHS-R expression on GnRH neurons (11). Mutations of ghrelin or GHS R is associated with the development of diabetes (10). Blocking ghrelin signaling has been suggested as a beneficial treatment for diabetes due to ghrelin-induced inhibition of insulin secretion.

Thymoquinone is an important derivatives of Nigella sativa. Several previous studies have shown the antioxidant, hypoglycemic, neuroprotective effects of thymoquinone or Nigella sativa extract. Also, it has been established that they stimulate insulin, protecting liver and kidney against damage. Thymoquinone increases the testicular and serum concentration of testosterone in diabetic rats and it exerts protective influences on spermatogenesis and diabetes-induced testicular damage (12-15). Given the importance of thymoquinone, hypothalamic aromatase and ghrelin in the controlling of the HPG axis. This study tried to investigate the effects of thymoquinone on the hypothalamic aromatase and ghrelin expression levels in intact and diabetic rats.

2. Materials and Methods

2.1. Animals

In this experimental study, twenty adult male Wistar rats (190-220 g) were kept at 22 ± 2°C in a 12 h light/12 h dark cycle. Animals had free access to water and food all the time except during induction of diabetes and sampling. All procedures for the maintenance and the use of experimental animals were approved by the research ethics committees of University of Mohaghegh Ardabili (code: IR.UMA.REC.1400.034).

2.2. Induction of diabetes

Induction of diabetes was performed as described in a previous study (16). Diabetes type 1 was induced by intraperitoneal injection of 150 mg/kg alloxan following 16 h fasting. Rats with blood glucose above 300 mg/dl were considered as diabetic rats.

2.3. Injection of drugs and real-time polymerase chain reaction (RT- PCR)

Ten intact rats in groups 1 and 2 received saline or 10 mg/kg thymoquinone. Ten diabetic rats in groups 3 and 4 received saline or 10 mg/kg thymoquinone. The drugs were injected intraperitoneally every morning at 9:00-9:30 for two weeks. One day following the last injection, animals were anesthetized using intraperitoneal injection of ketamine and xylazine mixture. They were decapitated. Hypothalamic samples were removed and stored at -80°C.

Acid guanidinium thiocyanate-phenol-chloroform method was used to extract total RNA based on the PureZol kit instructions (Bio Rad Co., USA). The cDNA synthesis was done using 1 µg of RNA according to the instructions of a cDNA synthesis kit (Thermo Scientific Co., USA). Corbett rotor gene 6000 (Qiagen Co, Germany) and SYBR Green I kit (Takara Bio Inc., Japan). were used to determine the alteration of gene expression levels. The first denaturation 95ºC for 2 min, followed by 40 cycles of denaturation at 95ºC for 5 sec, annealing at 60ºC for 20 sec and extension at 60ºC for 25 sec was used for PCR cycling. The sequences used for forward and reverse primers are as follows: ghrelin forward: 5'-AATGCTCCCTTCGATGTT GG -3' and reverse 5'-CAGTGGTTACTTGTTAGCTGG -3', aromatase forward: 5'- CGTCATGTTGCTTCTCATCG -3' and reverse: 5'- TACCGCAGGCTCTCGTTAAT -3', and GAPDH forward: 5'-AAGTTCAACGGCACAGTAAG-3' and reverse: 5'-CATACTCAGCACCAGCATAC-3' (6). The amplification products of ghrelin, aromatase, and GAPDH are 132 bp, 149 bp, and 120 bp, respectively. The relative gene expression was determined based on the formula $2^{-(\Delta\Delta Ct)}$.

2.4. Statistical analysis

Data were analyzed by SPSS software using the one-way analysis of variance (ANOVA) and Tukey's post hoc tests. The results were presented as mean ± standard deviation of means (± SEM). Significance was defined by $P < 0.05$.

3. Results

Injection of thymoquinone to healthy rats did not cause a significant reduction in mean relative aromatase gene expression as compared to intact control rats (Fig. 1). Induction of diabetes increased aromatase expression levels as compared to intact control rats but this increase was not statistically significant (Fig. 1). Aromatase gene expression in diabetic rats receiving thymoquinone declined significantly in comparison with diabetic group ($P<0.05$, Fig. 1).

Injection of thymoquinone to healthy rats significantly reduced ghrelin expression level as compared to intact control rats (Fig. 2). Induction of diabetes caused a marked increase in ghrelin expression level as compared to intact control rats (P<0.05, Fig. 2). Ghrelin gene expression in diabetic rats receiving thymoquinone significantly reduced in comparison with diabetic group (P<0.05, Fig. 2).

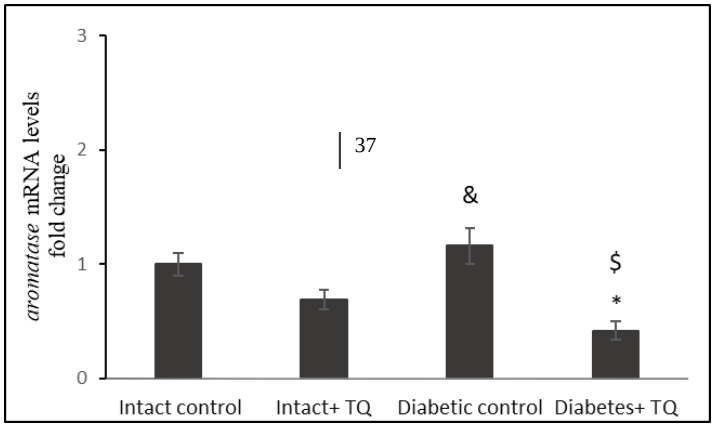

Fig 1. Effects of thymoquinone (TQ) on aromatase gene expression in intact or diabetic rats. *: compared to intact control; &: compared intact + TQ group $: compared to diabetic control group.

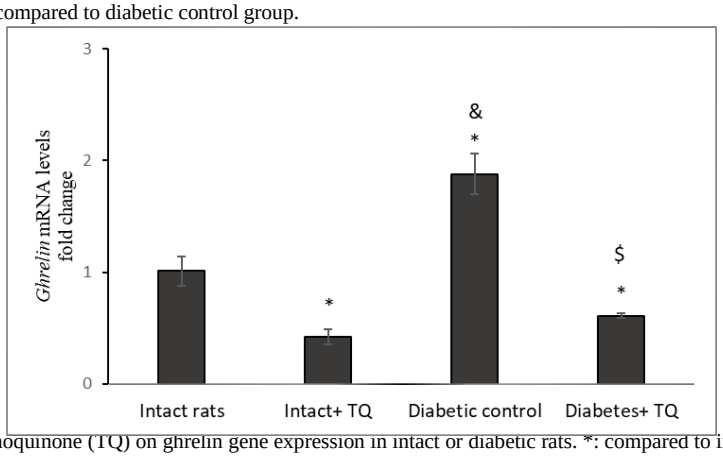

Fig 2. Effects of thymoquinone (TQ) on ghrelin gene expression in intact or diabetic rats. *: compared to intact control; &: compared intact + TQ group $: compared to diabetic control group.

4. Discussion

Based on present results, aromatase expression level did not significantly alter in the hypothalamus of diabetic rats. In this study, the effect of alloxan induced-diabetes was investigated on hypothalamic aromatase gene expression for the first time. However, previous studies reported the inhibitory effects of diabetes on aromatase expression in peripheral organs. In streptozotocin-induced diabetic rats, the aromatase gene expression decreased significantly in the ovary and testis but its expression did not change in the uterus and vas deferens (9). The gene expression of aromatase significantly declined in the adipose tissue and skeletal muscles of hypogonadal men with type 2 diabetes (7). Injection of thymoquinone to diabetic rats inhibited the hypothalamic aromatase in comparison with diabetic control rats. It has been reported that both Nigella sativa and thymoquinone exert antioxidant and hypoglycemic effects and they act as a positive factor to improve reproductive dysfunctions (8,12). The present results are in contradiction with the effect of thymoquinone on aromatase expression in peripheral organs. Atta and his collogues demonstrated that thymoquinone causes a significant increase in the aromatase expression levels in testicles of diabetic rats (8). Several previous studies have reported the fertility improvement of normal or hypogonadism rats following Nigella sativa or thymoquinone treatments. These studies concluded that Nigella sativa and thymoquinone promote spermatogenesis, protect testis against damage and augment testosterone concentration (12-15). Also, it has been stablished that aromatase take parts in different functions in each organ. Its central roles differ from its peripheral ones. In thalamus, it modulates the transmission of sensory information, and in amygdala, it participates in processing social behavior, motivation, aggression. In hippocampus, the prefrontal cortex, and the temporal cortex, it is involved in processing of learning and memory (17). In hypothalamus, 17β- estradiol produced by aromatase enzyme action, partly take parts in controlling of hypothalamic negative feedback mechanism of HPG axis or other hormones. In liver, adipose tissue, aromatase takes part in regulation of metabolic functions such as glucose and lipid

homeostasis, inhibition of lipogenesis and hepatic fat accumulation, gluconeogenesis, increasing lipolysis, or metabolizing some drug (18-20). Taking into account the findings from the several roles of aromatase in different organs, the inhibitory effects of thymoquinone on hypothalamic aromatase expression may be due to its role in controlling hypothalamic negative feedback mechanism of HPG axis.

The present results showed the increased expression level of hypothalamic ghrelin in diabetic rats in comparison with the control group. This finding is in agreement with previous studies which reported a positive correlation between ghrelin concentration and diabetes. They concluded that diabetic hyperphagia and decreased plasma LH and testosterone levels of diabetic animals are closely related to increased ghrelin and ghrelin induced neuropeptide Y (NPY) synthesis (21-24).

Also, the present data demonstrated that thymoquinone suppresses ghrelin gene expression in intact and diabetic rats. In this study for the first time, the effects of thymoquinone were investigated on hypothalamic ghrelin expression level. However, some probable mechanisms may be involved in mediating the inhibitory effects of thymoquinone on hypothalamic ghrelin. Previous studies indicated that diabetes is associated with marked increase of norepinephrine concentration (25). Elevation of norepinephrine impairs insulin sensitivity in healthy individuals and it suppresses insulin production in diabetic patients (26). Norepinephrine exerts stimulatory effects on ghrelin secretion by rising the cAMP and protein kinase A activity (27). Also, it has been established that Nigella sativa or thymoquinone treatment reduce significantly norepinephrine concentration in diabetic rats (25). So, it is possible that thymoquinone take parts in reduction of hypothalamic ghrelin in part via decreasing norepinephrine release.

Another mechanism for thymoquinone to inhibit hypothalamic ghrelin expression level may be due its stimulatory effects on insulin secretion. Injections of ghrelin reduces insulin secretion. However, elevation of ghrelin levels is associated with a marked decline of insulin secretion (28). Intracerebroventricular (ICV) injection of insulin significantly declines blood ghrelin levels via PI3-K pathway. It has been established that inhibitory effects of insulin on food intake and reduction of circulating ghrelin is related to its central neural action in the arcuate nucleus of hypothalamus (29). So, stimulatory influence of thymoquinone on insulin secretion may be involved in decreasing ghrelin gene expression.

Conclusion

The present results indicated that thymoquinone significantly reduces relative gene expression of hypothalamic aromatase and ghrelin as compared to diabetic rats. Thymoquinone may be a drug to improve decreased hypothalamic-pituitary-gonadal axis activity of diabetic rats due to its inhibitory effects on aromatase and ghrelin upstream GnRH neurons.

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Conflict of interest

The authors declare that they have no competing interest.

References

1. Shpakov AO. The role of disturbances in hormonal signaling systems in etiology and pathogenesis of diabetes mellitus. Journal of Evolutionary Biochemistry and Physiology 2014;50(6):552-6.
2. Gargouri M, Magné C, El Feki A. Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation. Nutrition research 2016;36(11):1255-68.
3. Schoeller EL, Schon S, Moley KH. The effects of type 1 diabetes on the hypothalamic, pituitary and testes axis. Cell and Tissue Research 2012;349(3):839-47.
4. Roselli CF. Brain aromatase: roles in reproduction and neuroprotection. The Journal of Steroid Biochemistry and Molecular Biology 2007;106(1-5):143-50.
5. Carreau S, Wolczynski S, Galeraud-Denis I. Aromatase, oestrogens and human male reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 2010 27;365(1546):1571-9.
6. Haghighat gollo Kh, Mahmoudi F, Bayrami A, Zahri S. Influences of l-dopa and blocking dopamine receptors on aromatase gene expression and serum concentration of lh in rat model of polycystic ovary syndrome. Journal of Fasa University of Medical Sciences 2020;10(3):2448-55.
7. Ghanim H, Dhindsa S, Abuaysheh S, Batra M, Kuhadiya ND, Makdissi A, Chaudhuri A, Dandona P. Diminished androgen and estrogen receptors and aromatase levels in hypogonadal diabetic men: reversal with testosterone. European Journal of Endocrinology 2018 ;178(3):277-83.
8. Atta MS, Almadaly EA, El-Far AH, Saleh RM, Assar DH, Al Jaouni SK, Mousa SA.

Thymoquinone defeats diabetes-induced testicular damage in rats targeting antioxidant, inflammatory and aromatase expression. International Journal of Molecular Sciences 2017; 18(5):919.

9. Burul-Bozkurt N, Pekiner C, Kelicen P. Diabetes alters aromatase enzyme levels in gonadal tissues of rats. Naunyn-Schmiedeberg's Archives of Pharmacology 2010;382(1):33-41.
10. Banks KA, Murphy KG. Role of ghrelin in glucose homeostasis and diabetes. Diabetes Management 2013;3(2):171.
11. Farkas I, Vastagh C, Sárvári M, Liposits Z. Ghrelin decreases firing activity of gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid signaling dependent manner. PLoS One 2013;8(10):e78178.
12. Parandin R, Yousofvand N, Ghorbani R. The enhancing effects of alcoholic extract of Nigella sativa seed on fertility potential, plasma gonadotropins and testosterone in male rats. Iranian Journal of Reproductive Medicine 2012;10(4):355.
13. Kanter M. Thymoquinone reestablishes spermatogenesis after testicular injury caused by chronic toluene exposure in rats. Toxicology and Industrial Health 2011;27(2):155-66.
14. Attari SS, Mohammadi S, Ebrahimzadeh A, Hosseinzadeh H, Soukhtanloo M, Rajabzadeh A. Effects of thymoquinone on sperm parameters, apoptosis, testosterone level, and oxidative stress in a mouse model of D-galactose-induced aging. Pharmaceutical Sciences 2018;24(3):180-6.
15. Salahshoor MR, Haghjoo M, Roshankhah S, Makalani F, Jalili C. Effect of thymoquinone on reproductive parameter in morphine-treated male mice. Advanced Biomedical Research 2018;7:18.
16. Mahmoudi F, Mahmoudi F, Gollo KH, Amini MM. Biosynthesis of novel silver nanoparticles using Eryngium thyrsoideum Boiss extract and comparison of their antidiabetic activity with chemical synthesized silver nanoparticles in diabetic rats. Biological Trace Element Research 2021;199(5):1967-78.
17. Azcoitia I, Mendez P, Garcia-Segura LM. Aromatase in the Human Brain. Androgens: Clinical Research and Therapeutics 2021;2(1):189-202.
18. Aloisi AM, Ceccarelli I, Fiorenzani P, Maddalena M, Rossi A, Tomei V, Sorda G, Danielli B, Rovini M, Cappelli A, Anzini M. Aromatase and 5-alpha reductase gene expression: modulation by pain and morphine treatment in male rats. Molecular Pain 2010;6:1744-8069.
19. Khazali H, Mahmoudi F. Morphine and kisspeptin influences on 5-α reductase and aromatase gene expression in adult male rats. Iranian Journal of Basic Medical Sciences2019;22(12):1462.
20. Shen M, Shi H. Sex hormones and their receptors regulate liver energy homeostasis. International Journal of Endocrinology 2015;2015.
21. Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H, Oikawa S. Role of ghrelin in streptozotocin-induced diabetic hyperphagia. Endocrinology 2002;143(12):4934-7.
22. Dong J, Peeters TL, De Smet B, Moechars D, Delporte C, Vanden Berghe P, Coulie B, Tang M, Depoortere I. Role of endogenous ghrelin in the hyperphagia of mice with streptozotocin-induced diabetes. Endocrinology 2006;147(6):2634-42.
23. Gelling RW, Overduin J, Morrison CD, Morton GJ, Frayo RS, Cummings DE, Schwartz MW. Effect of uncontrolled diabetes on plasma ghrelin concentrations and ghrelin-induced feeding. Endocrinology 2004;145(10):4575-82.
24. Sönmez MF, Karabulut D, Kilic E, Akalin H, Sakalar C, Gunduz Y, Kara A, Dundar M. The effects of streptozotocin-induced diabetes on ghrelin expression in rat testis: biochemical and immunohistochemical study. Folia Histochemica et Cytobiologica 2015;53(1):26-34.
25. Hamdy NM, Taha RA. Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. Pharmacology 2009;84(3):127-34.
26. Walters JM, Ward GM, Barton J, Arackal R, Boston RC, Best JD, Alford FP. The effect of norepinephrine on insulin secretion and glucose effectiveness in non—insulin-dependent diabetes. Metabolism 1997;46(12):1448-53.
27. Gagnon J, Anini Y. Insulin and norepinephrine regulate ghrelin secretion from a rat primary stomach cell culture. Endocrinology 2012;153(8):3646-56.
28. Chabot F, Caron A, Laplante M, St-Pierre DH. Interrelationships between ghrelin, insulin and glucose homeostasis: Physiological relevance. World Journal of Diabetes 2014;5(3):328.
29. Ueno M, Carvalheira JB, Oliveira RL, Velloso LA, Saad MJ. Circulating ghrelin concentrations are lowered by intracerebroventricular insulin. Diabetologia 2006; 49(10):2449-52.