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Investigation of effects of myricetin on thyroid-gonadal axis of male rats at prepubertal period

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ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form
16 April 2015
Accepted 18 April 2015
Available online 27 April 2015

Keywords:
Phytoestrogens
Myricetin
Flavonoid
Thyroid axis
Prepubertal male rat

ABSTRACT

The present study is to investigate the effects of myricetin on pubertal development and thyroid hormone concentrations in the male rat. The rats were exposed to 25 and 50 mg/kg/day of myricetin by gavage from post natal day (PND) 23 to 53. Preputial separation (PPS), organ weights and biochemical and hormone analysis were investigated. PPS was significantly delayed in low dose myricetin groups. Total serum thyroxine (T4) and, triiodothyronine (T3) levels increased in 25 mg/kg myricetin dose group but thyroid-stimulating hormone (TSH) level increased in 0.7 μg/kg/day ethinyl estradiol dose groups. Myricetin exposure did not significantly change androgen dependent tissue weights; however myricetin exposure caused congestion, germinal cell debris and tubular atrophy in testis colloidal and tubular degeneration in thyroid gland were observed while there was germinal cell debris in epididymis. This study demonstrated that orally gavages myricetin caused adverse effects on male thyroid-gonadal axis during peripubertal period to pubertal period.

1. Introduction

In recent years, there is an increasing concern about the relationship between human male reproductive disorders and environmental factors. It was reported that some compounds have estrogenic and androgenic properties caused male reproductive tract disorders such as increased testicular tumor, reduced sperm count, cryptorchidism and hypospadias. Human are exposed to these environmental chemicals like bisphenol A and phthalate with food packaging, synthetic hormones in pharmaceutics, pesticides in nourishments, solvents in cleaning products, detergents. Also, environmental estrogens include phytoestrogens in fruits and vegetables all day long (Pflieger-Bruss, 2004; Castro et al., 2014). Phytoestrogens' chemical structure is similar to estradiol, an endogenous estrogen. As a result, these biologically active plant compounds can bind to estrogen receptors in various cells (Aguilla et al., 2013). Therefore, they may alter concentrations of endogenous sex hormones and they may alter sex differentiation and increase the risk of reproductive tract tumors or developmental disorders especially during fetal development (Kasum, 2002).

Phytoestrogens are found in several plants and they are daily consumed by people and animals. They are comprised four major classes: isoflavones, flavonoids, coumestans and lignans, are polyphenolic antioxidants (Ganry, 2005). Polyphenolic compounds like flavonoids have come to researchers'

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http://dx.doi.org/10.1016/j.etap.2015.04.015
1382-6689/

attention because of their potent antioxidant features and their ability of preventing from various diseases related to oxidative stress such as cancer, coronary heart disease and aging (Park and Kim, 2011).

Flavonoids also act like a regulator role on hormones such as androgens, estrogens and thyroid hormones (Agrawal, 2011). Studies show that, in mammals, thyroid system plays a critical role in the development of many organs and systems, including the reproductive tract (Mendis-Handagama, 2005). When flavanoids are consumed at large quantities, it is expected that they might be major cause of histological changes in the thyroid such as thyroid gland growth and goiter by increasing TSH levels (Santos et al., 2011).

Opposite of this, Yin (1999) demonstrated that flavonoids exhibit antiproliferative activity in vitro against human thyroid carcinoma cells and they suggested that flavonoids may contribute to new drug developments for the treatment of thyroid cancer. They have antiviral, antiplatelet, anti-allergic, anti-inflammatuar, antitumor and antioxidant activity. Especially, flavonols have strong antioxidant activity (Ferreyra, 2012).

Myricetin belongs to flavonol group present in vegetables such as broccoli (62.5 mg/kg), red pepper (171.5 mg/kg), green bean (47.0 mg/kg), Chinese cabbage (31.0 mg/kg), garlic (693.0 mg/kg) (Miean, 2001) and in fruits such as currant (71 mg/kg), bilberry (14–142 mg/kg), blackberry (23–26 mg/kg) (Hakkinen, 1999) and in black tea (303 mg/kg) (Miean, 2001).

When myricetin is consumed, some part of it is absorbed in gastrointestinal system and the rest of it is metabolized by gastrointestinal microflora. Liver is the main responsible organ for metabolism of myricetin (Ong, 1997). It was showed that among flavonoids (quercetin, kaempferol, catechin and rutin), myricetin had the highest antioxidant capacity. Comparing to others, myricetin has more phenolic hydroxyl groups. It was proved that their antioxidant activity increase depending on a number of phenolic hydroxyl group (Pekkarinen, 1999). These phenolic groups provide binding to estrogen. Thus, myricetin may act like estrogen agonists or antagonists (Makela, 1995). At another study, Tiwari et al. (2009) showed that myricetin has an ability of preventing cardiotoxic effects induced by isoproterenol. Kang (2011) proved that myricetin was a substantial chemo preventive agent in skin carcinogenesis. Knekt (2002) found that men with higher myricetin intakes had a lower prostate cancer risk. Myricetin has also bactericidal properties. It has been reported that myricetin exhibited inhibitory activity against development of *Burkholderia cepacia* multidrug resistant bacteria and vancomysin resistant enterococci (Xu, 2001).

This product is purchased in tablets as dietary supplement due to its benefits on human health. However, there are not enough comprehensive and long term in vivo animal and human experiments about adverse effects of these supplements.

In the present study, we hypothesized that exposure to myricetin in the EDSTAC male pubertal protocol would alter the development of the male rat reproductive system and thyroid function.

2. Materials and methods

2.1. Chemicals

17α-ethinyl estradiol (%98) and myricetin (%96) were purchased from Sigma-Aldrich. Chemicals were dissolved in corn oil (vehicle). ALT, AST, urea, total protein, albumin, creatine, glucose and LDH kits were bought from Audit Diagnostics (Ankara, Turkey). Thyroxin (T_4), triiodothyronine (T_3) and thyroid-stimulating hormone (TSH) were purchased from Calbiotech.

2.2. Animals

Twenty one day male Wistar rats were purchased from the Experimental Animals Production Center, Hacettepe University in Ankara, Turkey. All rats were housed in polycarbonate cages with stainless steel covers in an air-conditioned room (12 h light:12-h dark cycle with a temperature 21 ± 4 °C and a relative humidity of 50 ± 5). During the experimental period they were provided, *ad libitum*, with phytoestrogen free pellet food, and tap water.

2.3. Doses and administration of chemicals

All treatments were administered daily by oral gavage from postnatal day (PND) 23 through 53. Body weights were recorded daily and the dose administered each day and it was adjusted for body weight. The dosing solution was prepared by mixing the compound with corn oil and diluted in series with corn oil to the desired concentration of 25 or 50 mg/kg. Doses were selected on the basis of consumption of daily flavonol intake in literature and the earlier study (Barlas et al., 2014) which examined estrogenic effects of myricetin at juvenile/peripubertal on female rats. The male rats were divided into five groups and each group consisted of six animals. Group of rats were treated with myricetin 25 and 50 mg/kg body weight/day in a suspension of corn oil. Positive control males were gavaged orally with 17α-ethinyl estradiol 0.7 and 7 μg/kg body weight/day and control males received corn oil only.

2.4. Preputial separation

The separation of the foreskin of the penis from the glands penis, preputial separation (PPS), is an early reliable marker of the progression of puberty in male rats (Korenbrot, 1977). In this study, PPS was monitored beginning on PND 30, until all males showed separation. The PPS observations were collected at approximately the same time every day after dosing. For data analyses, the day of complete separation was used (Fig. 1).

2.5. Hormone analysis

At the end of the experiment, the blood was collected in tubes that contained heparin. Plasma was separated after centrifugation at 3000 × *g* for 30 min to pipette serum into silicon micro centrifuge tubes and stored at −80 °C until hormone analysis.