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Full Length Research Paper

Effect of rosmarinic acid on estrogen, FSH and LH in female diabetic rats

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Rosmarinic acid from the plants quenched superoxide radicals from xanthine oxidase and inhibited cyclooxygenase I and II enzymes. Antioxidants have essential effect on diabetes. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. Wistar male rat (n=40) were allocated into three groups, control group (n=10), rosmarinic acid (Ro) group that received 5 mg/rat (gavage) (n=10) and diabetic group that received 55 mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided into two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus 5 mg/rat Ro daily for 4 weeks, respectively. However, the control group just received an equal volume of distilled water daily (gavage). Diabetes was induced by a single (IP) injection of streptozotocin (55 mg/kg). Animals were kept in standard condition. In 28 days after inducing, diabetic 5 cc of blood were collected for sex hormones, TAC and MDA levels from the whole groups analysis. Level of MDA significantly decreased in group that has received 5 mg/rat of Ro (P<0.05) in comparison to experimental groups and sex hormones, and TAC significantly increased in groups that received Ro (P<0.05). Since in our study, 5 mg/rat of Ro, have significantly preventive effect on diabetic disorder in female sex hormone, so it seems that using it can be effective for treatment in diabetic Rat

Key words: Rosmarinic acid, diabetic, female sex hormone, streptozotocin.

INTRODUCTION

Rosmarinic acid was identified as a major anti-oxidant compound (0.22 to 0.97%) in all the seven herbs, confirmed by nuclear magnetic resonance. Rosmarinic acid from the plants quenched superoxide radicals from xanthine oxidase and inhibited cyclooxygenase I and II enzymes (Park, 2011). Diabetes is associated with gender-specific changes in sex steroid hormones. How-ever, the mechanisms responsible for these associations as well as the link to sexual dysfunction are not well understood. Imbalances in sex steroid hormone levels are strongly associated with diabetes and this may negatively impact upon sexual function. Although, numerous factors are likely to contribute to the development of diabetes and its complications, the role of sex steroid hormones must be acknowledged (Kim, 2009). Antioxidants secreted by the reproductive tract protect spermatozoa against the toxic effects of reactive oxygen species (ROS) after ejaculation (Koziorowska-Gilun et al., 2011). Vitamin E can regulate apoptosis-related protein Bcl-2, Bax expression and confront free radical damage which contributes to a protective effect for ovarian grandiose cells. Lu et al. (2009) evaluated that the effect of rosmarinic acid (RosA) on the proliferation and apoptosis in activated hepatic stellate cells (HSC-T6), which is useful to decrease this cell population (Zhang et al., 2011). Rosemary (*Rosmarinus*

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officinalis), a culinary spice and medicinal herb, has been widely used in European folk medicine to treat numerous ailments. Many studies have shown that rosemary extracts play important roles in anti- inflammation, anti-tumor and anti-proliferation in various *in vitro* and *in vivo* settings (Cheng et al., 2011). Rosemary (*R. officinalis*) leaves possess a variety of bioactivities. Previous studies have shown that the extract of rosemary leaves from supercritical fluid extraction inhibits the expression of inflammatory mediators with apparent dose-dependent responses (Kuo et al., 2011). The aim of this study was to see antioxidant effect of rosmarinic acid on hormonal change in diabetic female rats.

MATERIALS AND METHODS

Animals

Forty adult Wistar albino female rats were 8 weeks old and weighed 250 ± 10 g, they were obtained from animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40 to 70%) and 12 h/12 h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance to the principles of laboratory animal care. 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 Tabriz Medical University. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly selected and divided into control (n=10), and rosmarinic acid (Ro) group that received 5 mg/rat (gavage) (n=10), and diabetic group that received 55 mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus 5 mg/rat of Ro (gavage). The control group just received an equal volume of 1 cc distilled water daily (gavage). Diabetes was induced by a single intra peritoneal (IP) injection of streptozotocin (STZ, Sigma- U.S.A.) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight. Rosmarinic acid (Ro) inducement was continued to the end of the study (for 4 weeks) (Khaki et al., 2010).

Induction of experimental type 1 diabetes

Experimental type 1 diabetes was induced in rats by intra peritoneal (IP) injection of 55 mg/kg streptozotocin (STZ) in distilled water. Control rats received distilled water, only

Blood glucose determination

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL). Sample collections were then made 48 h after STZ injection and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dl were declared diabetic and were used in the experimental group. One week after the induction of experimental diabetes, protocol was started

Surgical procedure

On the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether, blood samples in control and experimental groups were immediately obtained.

Measurement of serum total antioxidant capacity (TAS)

TAS was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di- (3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L) (Khaki et al., 2010).

Measurement of serum MDA

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard.

Measurement of LH, FSH and estrogen hormones

Serum concentrations of FSH, luteinizing hormone (LH) and progesterone were measured with a two-site chemiluminescence (sandwich) immunoassay using two antibodies specific for the intact FSH molecule. Serum LH concentrations were measured with a two-site chemiluminescent immunoassay by Bayer diagnostics. The serum progesterone assay is also a competitive chemiluminescent immunoassay.

Statistical analysis

Statistical analysis was done using the ANOVA to test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

RESULTS

Total blood anti-oxidant capacity

Amount of the total blood anti-oxidant capacity in control group was 0.67 ± 0.55 mmol/ml and extract STZ. Extracts with STZ groups were 1.5 ± 0.01, 0.50 ± 0.55 and 0.75 ± 0.5 mmol/L, respectively. Statistical analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group ($P<0.05$) (Table 1).

MDA (malondialdehyde) level in blood

MDA level in control group was 3.70 ± 0.55 mmol/L and extract STZ. Extracts with STZ groups were 1.11 ± 0.10,

Table 1. The effect of the rosmarinic acid on estrogen, FSH and LH, TAC, SOD, MDA and blood glucose of control and experimental groups in the rats.

Extract with STZ	STZ	Extracts	Control	Groups
5.6 ± 0.05*	0.5 ± 0.05	7.2 ± 0.05*	0.7 ± 0.05	LH (ng/ml)
1 ± 0.05	0.7 ± 0.5	2.2 ± 0.05*	1 ± 0.11	FSH (ng/ml)
61 ± 0.05*	57 ± 0.05*	80 ± 0.05*	70 ± 0.01	Estrogen (ng/l)
2.20 ± 0.55	5.5 ± 0.55*	1.11 ± 0.10*	3.70 ± 0.55	MDA (mmol/ml)
0.75 ± 0.5	0.50 ± 0.55*	1.5 ± 0.01*	0.67 ± 0.55	TAC (mmol/ml)

Data are presented as mean ± SE. *Significant different at P< 0.05 level (compared with the control group).

5.5 ± 0.55 and 2.20 ± 0.55 mmol/L, respectively. Statistical analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05) (Table 1).

LH, FSH and estrogen hormones

LH level in control group was 0.7 ±0.05 mmol/L and extract STZ. Extracts with STZ groups were 7.2 ± 0.05, 0.5 ± 0.05 and 5.6 ± 0.05 mmol/L, respectively.

FSH level in control group was 1 ± 0.11 mmol/L and extract STZ. Extracts with STZ groups were 0.30 ± 0.212, 4.1 ± 0.06 and 1.1 ± 0.08 mmol/L, respectively.

Estrogen level in control group was 70 ± 0.01 mmol/L and extract STZ. Extracts with STZ groups were 80 ± 0.05, 57 ± 0.05 and 61±0.05 mmol/L, respectively. Statistical analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05) (Table 1).

DISCUSSION

Eostasis dates back to 1960s when it was reported that some women taking high-estrogen oral contraceptives developed insulin resistance (Wynn and Doar, 1966). On the other hand, women with low serum levels of estrogens, as for example during menopause, are at greater risk of developing type 2 diabetes (Livingstone and Collison, 2002)

An increase in the oxidative stress and a decrease in the antioxidant levels have been described in diabetic patients, that have been related with the etiopathogenesis of diabetes and its chronic compli-cations (Cuerda et al., 2011). The intervention studies including different antioxidants have not demonstrated any beneficial effect on cardiovascular and global morbimortality in different populations, including diabetic patients. Neither of these studies has demonstrated a beneficial effect of antioxidant supplementation on the prevention of diabetes. According to these studies, these substances can decrease lipid peroxidation, LDL-cholesterol particles oxidation and improve endothelial function and endothelial-dependent vasodilatation, without significant improvement in the metabolic control of these patients (Cuerda et al., 2011). Quercetin potentiated glucose and glibenclamide-induced insulin secretion and protected -cells against oxidative damage, suggested that ERK1/2 played a major role in those effects. The potential of quercetin in preventing -cell dysfunction associated with diabetes deserves further investigation (Youl et al., 2010). In women, diabetes is reported to slightly increase the risk of decreased sexual arousal, inadequate lubrication and pain on sexual intercourse, while erectile dysfunction is the most common presentation of sexual dysfunction in men (Adeniyi and Adeleye, 2010). The role of diabetic complications is controversial. The comorbidity with depression plays a major role (Bitzer and Alder, 2009). Most trials on the effect of exercise on patients with diabetes mellitus focused on their glycemic control, only a few focused on sexual dysfunction. Sexual dysfunction in general, links between diabetes and sexual dysfunction and management options for sexual dysfunction including therapeutic exercises were reviewed (Adeniyi et al., 2010). Traditionally the role of sexual steroid hormones was focused on primarily reproductive organs: the breast, female reproductive tract (uterus, mammary gland and ovary) and male reproductive tract (testes, epididymis and prostate), the endocrine pancreas has a pivotal role on carbohydrate homeostasis and deterioration in function produces diabetes. It has been shown that steroid hormones have an important role in susceptibility and development of diabetes in animal models, in humans, its role is less clear, however the most evident effect is on the perimenopausal women. In this stage the decrease in gonadal steroids produces an increase on susceptibility to develop diabetes mellitus; in men, hipoandrogenism is associated with an increased prevalence of insulin resistance (Morimoto et al., 2011). Evidence is provided demonstrating that women are able to increase their HDL-cholesterol levels on nutrition high in saturated and monounsaturated fat. This could be explained by a concurrent dietary fat related increase in female sex hormone levels (e.g. estradiol and estrone),

hormones which are known to increase HDL-cholesterol levels (Kesteloot and Sasaki, 1993). High-density lipoprotein (HDL) cholesterol was associated with estradiol and prolactin. The inverse associations of caffeinated coffee and caffeine intake with type 2 diabetes risk observed (Goto et al., 2011). Several possible explanations have been put forth to explain the protective effect of coffee consumption on type 2 diabetes risk, including effects on insulin sensitivity and -cell function by varying coffee components such as magnesium, potassium, chlorogenic acid and caffeine (van Dam and Hu, 2005). Experimental data indicate the important roles of sex hormones in the development of type 2 diabetes (Ding et al., 2007) . SHBG is synthesized primarily in the liver, and binds androgens with high affinity and estrogens with low affinity, thereby regulating the biologically active fraction of sex hormones (Anderson, 1974). Caffeine may increase the level of plasma SHBG without directly altering sex hormones levels (Goto et al., 2011; Huber et al., 2002). Because SHBG is synthesized and metabolized primarily in the liver (Anderson, 1974), coffee intake may affect SHBG metabolism in the liver and influence the plasma levels of SHBG (Reynders et al., 2005).

However, oxidative stress occurs from an imbalance between ROS and antioxidant actions. During chronic oxidative stress caused by environmental factors (that is, UV light, ionizing radiation and toxic substances), infections, diabetes or lack of dietary antioxidants, an inequity of cellular reducing equivalents capable of detoxifying the increased burden of ROS has marked effects on normal cellular processes. However, in times of oxidative stress, normal cellular respiration is still functioning, resulting in dysregulated mitochondrial free radical production and disparity between ROS generation and antioxidant defenses (Schriner et al., 2005; Limón and Gonsebatt, 2009). Current data indicate that estradiol (*E2*) is more than a sex hormone, as it has been demonstrated for years that *E2* plays an important role in the function of the cardiovascular, musculoskeletal, immune and central nervous systems (Heldring et al., 2007).

Moreover, recent studies have shown the importance of *E2* for energy balance and glucose homeostasis (Barros et al., 2006; Nadal et al., 2009). However, whether *E2* has a positive or a negative effect on glucose, homeostasis is still a matter of debate. Many authors consider that *E2* at physiological levels is involved in the maintenances of normal insulin sensitivity, but outside the physiological range, *E2* may promote insulin resistance and diabetes (Godsland, 2005). The notion that high *E2* concentration is detrimental to blood glucose homeostasis dates back to 1960s when it was reported that some women taking high-estrogen oral contraceptives developed insulin resistance (Wynn et al., 1966). On the other hand, women with low serum levels of estrogens, as for example during menopause, are at of developing type 2 diabetes (Livingstone and Collison, 2002). Estrogen replacement in postmenopausal depending women, on dose and duration of treatment, is associated with an improvement of insulin sensitivity and a reduction of blood glucose, lipid, cholesterol levels and body fat (Bryzgalova et al., 2008).

Our results revealed that rosmarinic acid (RO), had the ability to increase total antioxidant capacity and decrease malondialdehyde in whole groups ($P<0.05$), in other hand level of estradiol in extract groups were significantly increased and level of estradiol in diabetic group was returned as compared to STZ group. In other hand Ro have no significant effects on serum LH and FSH levels.

In conclusion rosmarinic acid via increasing TAC levels caused antioxidant protective effects in diabetics group that are treated with Ro as compared to diabetic group without using any extracts. It will be suggestion that using rosmarinic acid has beneficial effect in diabetic patients.

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