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ORIGINAL RESEARCH

Effect of white tea consumption on serum leptin, TNF-α and UCP1 gene expression in ovariectomized rats

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ABSTRACT. ***Background:*** **Obesity and dyslipidemia due to estrogen deficiency are among the important health problems in menopausal women. Increasing evidence reports the anti-obesity and anti-hyperlipidemic properties of tea polyphenols. However, the effect of white tea (WT) with high polyphenol content on overweight and lipid profile is uncertain. Here, we aimed to examine the effects of long-term WT consumption on serum leptin, tumor necrosis factor- alpha (TNF-α) and uncoupling protein 1 (UCP1) mRNA gene expression in ovariectomized (OVX) rats.** ***Methods:*** **Adult rats were divided into four groups (n = 8): (i) sham, (ii) OVX, (iii) WT and (iv) OVX + WT. WT was given at a dose of 0.5% w/v for 12 weeks. In the study, body weight, serum leptin, TNF, estradiol (E2) levels, lipid profile and UCP1 mRNA gene expression in brown adipose tissue (BAT) were evaluated.** ***Results:*** **There was a significant increase in body weight of OVX rats, which was decreased following WT consumption. While leptin and E2 levels decreased in the OVX group, TNF levels increased. There was no difference between the NF-kB levels of the groups. In addition, BAT UCP1 mRNA expression was significantly decreased in OVX groups, while WT treatment stimulated UCP1 activity.** ***Conclusion:*** **We explain the stimulatory effect of WT on weight loss mainly by the induction of UCP1 gene-mediated thermogenesis and suppression of inflammation. Therefore, we suggest that prolonged WT consumption may have beneficial effects in limiting excess weight gain caused by estrogen deficiency.**

Key words: Ovariectomized, white tea, body weight, UCP1, TNF-α

INTRODUCTION

Menopause is a physiological process in which fertility ends due to estrogen deficiency in women. It has been shown that menopause causes various diseases, such as obesity, cardiovascular disease and metabolic syndrome [1]. Overweight and obesity occurring in the menopausal period are among the major health problems [2]. Moreover, obesity has been reported to be associated with menstrual cycle imbalance, changes in reproductive hormone releases and decrease in fertility in pre-menopausal women and rodents [3]. However, the causes of weight gain arising during the menopause have still not been clearly established.

Gonadal steroids have a key role in regulating body weight and energy metabolism. Menopause or experimental ovariectomy (OVX) is associated with low estradiol (E2) concentration due to ovarian failure [4, 5]. E2, a sex hormone, plays an important role in the regulation of body weight and energy metabolism as well as reproductive activities. It was reported that the decrease in E2 resulted in an increase in food intake and a decrease in energy expenditures in the long term [6]. Estrogen is effective in fat oxidation and thermogenesis-mediated energy expenditure. Irregularity of thermogenesis is linked to obesity in humans [7]. Mitochondrial uncoupling protein 1 (UCP1) is the protein responsible for thermogenesis in brown adipose tissue (BAT), which plays an important role in energy expenditure mechanisms [8]. UCP1, an important protein of BAT, mediates the release of energy as heat. UCP1 activity is controlled by the sympathetic nervous system. Sympathetic system plays a very important role in the regulation of lipolysis and energy expenditure. It has been reported that the level of UCP1 gene expression in BAT of rats with estrogen deficiency decreased compared to the control group [9]. In another study, food components that stimulate UCP1 activity in BAT and white adipose tissue (WAT) have been shown to reduce weight gain in obese rodents [10, 11].

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Tumor necrosis factor-alpha (TNF-α) is an important proinflammatory cytokine that increases weight gain and obesity [12]. It has also been reported that TNF-α levels increase in rats with OVX [13]. However, an in vitro study has shown that TNF-α suppresses UCP1 gene expression in brown adipocytes [14]. These results show that weight gain due to estrogen deficiency triggers inflammation while inhibiting thermogenesis-induced energy expenditure mechanisms. Tea types exhibit many beneficial functions, including anti-inflammatory and anti-obesitic effects, by means of important components such as catechins, caffeine and flavones [15, 16].

Tea (*Camellia sinensis*) contains catechin compounds including epicatechin (EC), epigallocatechin gallate (EGCG), epigallocatechin (EGC) and epicatechin gallate (ECG). Some components in tea were shown to have a thermogenic effect and increase fatty tissue oxidation by activating sympathetic nervous system [17, 18]. EGCG, the major catechin, was shown to induce weight loss in obese mice in a previous study [19]. Stimulation of UCP1 mRNA activity is one of the most important mechanisms for inducing weight loss by tea catechins. Similarly, tea catechins have been reported to exhibit the anti-obesitic effect by increasing UCP1 mRNA expression in rat BAT [20].

White tea (WT) is an unfermented tea obtained by removing young shoots and buds of *C. sinensis*. The presence of WT in the bud area protects it from sunlight, thus preventing the degradation of polyphenols. Despite these results, the role of WT, known for its lipolytic effect and high bioactive components, in preventing excess weight gain due to estrogen deficiency remains uncertain. For this purpose, this study aimed to investigate the role of WT treatment in body weight regulation for 12 weeks after OVX in adult female rats.

MATERIALS AND METHODS

Experimental animals and groups

A total of 32 Sprague Dawley female adult rats (283 ± 1.89 g) were obtained from the Laboratory Animals Research Center of Recep Tayyip Erdogan University (RTEU). The study was approved by the Local Ethics Committee for Laboratory Animals of RTEU (Protocol 2017/28). The rats were kept in transparent polyethylene cages and fed ad libitum. For adaptation, the rats were transferred to artificially controlled rooms with temperature 23 ± 2 °C, light at 12:12 h photoperiod and humidity 55 ± 5% for 1 week prior to the experiment. The animals were divided into four groups, eight rats in each group, namely, sham (abdomen opened and closed, tap water administered), ovariectomy (OVX), WT (abdomen opened and closed + WT 0.5% w/v administered) and OVX + WT 0.5% w/v.

OVARIECTOMY OPERATION

For ovariectomy operation, rats were administered intraperitoneal ketamine + xylazine (60 and 20 mg/kg, respectively). Subsequently, the abdominal area of the rats were cleaned with an antiseptic and shaved. Afterward, the ovaries were reached with a 2 cm incision in the abdomen. Both ovaries were removed surgically. Then, the abdomen was closed by suturing the muscle and skin layer. Subsequently, the rats were allowed to recover for 1 week. Similar surgical procedures were performed in sham group animals, but ovaries were not removed.

PREPARATION OF WHITE TEA INFUSION

WT was purchased from the supplier (General Director of Tea Enterprises, Rize, TURKEY). High-performance liquid chromatography (HPLC, ISO 14502 1: 2005, HPLC, ISO 14502 2: 2005) method was used for the analysis of active ingredients within the WT. Caffeine, gallic acid, catechin (C), EC, ECG, EGC and EGCG concentrations were determined as % values in dry matter of the samples (*table 1*). Sample preparation and dose selection were performed based on previously reported studies [21, 22]. Briefly, the solution (0.5% w/v) was prepared by adding 0.5 g of WT to 100 ml of boiled water at 100 °C and then left to infuse for 3 minutes. The infusion was filtered through a 0.2 μm cellulose acetate filter. For maintaining the final volume, pre-boiled tap water was added to substitute evaporated water. The infusions were prepared daily and cooled down to room temperature before administration.

COLLECTION OF BLOOD AND TISSUE SAMPLES

Twelve weeks after the experiment started, the animals were fasted for 12 hours and subsequently placed under general anesthesia with ketamine-xylazine

Table 1
Analysis of white tea samples with HPLC method.

Type of content	Result	Unit
Epigallocatechin gallate (EGCG)	6.98 (dry matter)	%
Epicatechin (EC)	0.14 (dry matter)	%
Epigallocatechin (EGC)	0.33 (dry matter)	%
Caffeine	4.38 (dry matter)	%
Catechin (C)	0.00 (dry matter)	%
Total polyphenol analysis	14.98 (dry matter)	%

HPLC: high-performance liquid chromatography

(60 and 20 mg/kg, respectively). The rats were dissected by opening abdominal and thoracic cavities. Tissue samples were quickly removed and stored in the cold chain. Then, blood samples (8-10 ml) were taken from the left ventricle with an injector and the rats were sacrificed in this way. Blood samples were taken into tubes without anticoagulants and centrifuged at 3500 × g for 5 minutes at +4 °C. The serum samples obtained were stored at −80 °C until analysis for glucose, high density lipoproptein (HDL), low density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC), leptin, TNF-α and E2 concentration. BAT samples were removed and rinsed three times with cold 0.9% sodium chloride and then dried in filter paper and stored at–80 °C for gene expression analysis.

MEASUREMENT OF BODY AND UTERUS WEIGHTS

Body weights of the animals were measured in grams at the beginning of the experiment and weekly for 12 weeks. In addition, the initial and final body weight change of the rats was calculated in %. After sacrification, the uterus was removed and cleaned of adipose tissues and its weight was measured in grams. The ratios of uterine weight to body weight (g/100 g BW) were calculated.

RNA ISOLATION

BAT samples obtained from rats after sacrification were frozen in nuclease-free microcentrifuge tubes using liquid nitrogen and stored at–80 °C until the RNA isolation protocol was performed. PureLink RNA Mini Kit (Life Technologies Corp., CA, USA, Cat. No. 12183018A) was used for RNA isolation. Before starting RNA isolation, the samples stored at–80 °C were taken out and then homogenized using TissueLyser II (QIAGEN GmbH, Hilden, Germany) with the lysis solution contained in the PureLink RNA Mini Kit. Tissue samples were placed into 2 ml microcentrifuge tubes containing steel balls of 3 mm diameter and 600 µl of fresh lysis solution and then vibrated at 30 Hz for 5 minutes in TissueLyser II. Then, RNA was isolated from the homogenized tissues in accordance with the protocol of the manufacturer. Concentration and purity of the isolated RNA samples were measured by using the Thermo Scientific™ Multiskan™ GO (Thermo Fisher Scientific Inc., Finland) device. RNA samples were diluted by nuclease-free sterile water such that the concentration of each sample was equivalent to the RNA sample with the lowest concentration. These RNA samples with equalized RNA concentrations were stored at–80 °C until the real-time PCR protocol was performed.

QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION (QRT-PCR) DETECTION OF UCP1 MRNA EXPRESSION

LightCycler EvoScript RNA Probes Master (Roche Diagnostics GmbH, Mannheim, Germany) kit containing the enzymes and components necessary for reverse transcription and qRT-PCR was used to quantitatively determine the amount of UCP1 and beta-actin (ACTB) mRNAs. In addition, the RealTime ready Catalog Assay (Roche Diagnostics GmbH, Mannheim, Germany) containing a primer pair and fluorescent dye-labeled probe mixes was used for the amplification of UCP1 and ACTB mRNAs. Real-time PCR reaction protocol was programmed on the Roche Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics GmbH, Mannheim, Germany) as follows: reverse transcription step at 60 °C for 15 min, denaturation step at 95 °C for 10 min, 45 cycles of amplification step at 95 °C for 15 sec, 58 °C for 15 sec, 72 °C for 1 sec and the cooling step at 40 °C for 30 sec. The cycle threshold (CT) value of each sample was obtained after the real-time PCR was performed on the instrument. The reaction was repeated three times independently to obtain the most accurate results for each sample. Relative expression levels of UCP1 mRNA were calculated by use of the $2^{-\Delta\Delta CT}$ method as previously described [23]. The data were presented as the fold change in UCP1 mRNA levels normalized to the ACTB used as the reference gene.

BIOCHEMICAL ANALYSIS

The serum leptin, E2 and TNF-α concentrations were measured by the enzyme-linked immunosorbent assay (ELISA) technique using Rat ELISA kit (SunRed Biological Technology, Co., Ltd. Shanghai) following the manufacturer's procedure. Concentrations of leptin, E2 and TNF-α were expressed as pg/mL. In addition, serum TG, TC, HDL and LDL concentrations were measured using a commercial kit on Abbott Architect c16000 autoanalyzer (Abbot Diagnostics, Inc., Lake Forest, IL, USA). Serum glucose concentration was measured by a glucometer (Bayer Healthcare AG, Leverkusen, Germany) in accordance with the manufacturer's procedure.

STATISTICAL ANALYSIS

Statistical evaluation of the data was performed with IBM SPSS for Windows 20.0 (IBM Corporation, NY, USA). The one-way analysis of variance test (ANOVA) was used for comparisons between the groups. Post hoc Tukey test was used for statistical values of the mean difference between the groups. Data represent mean ± SEM. Values of UCP1 mRNA expression were presented as mean ± standard deviation (SD) obtained from three independent experiments. The unpaired Student's *t*-test was used to analyze the differences of gene expression data between two groups. $p < 0.05$ was considered statistically significant.

RESULTS

Body and uterine weights

After 12 weeks, there was a significant increase in body weight in OVX (343.6 ± 4.36 g) and OVX + WT (331 ± 2.58 g) groups compared to sham (295.2 ± 1.47 g). The body weight of OVX + WT group was significantly lower compared to OVX group (*figure 1*A,

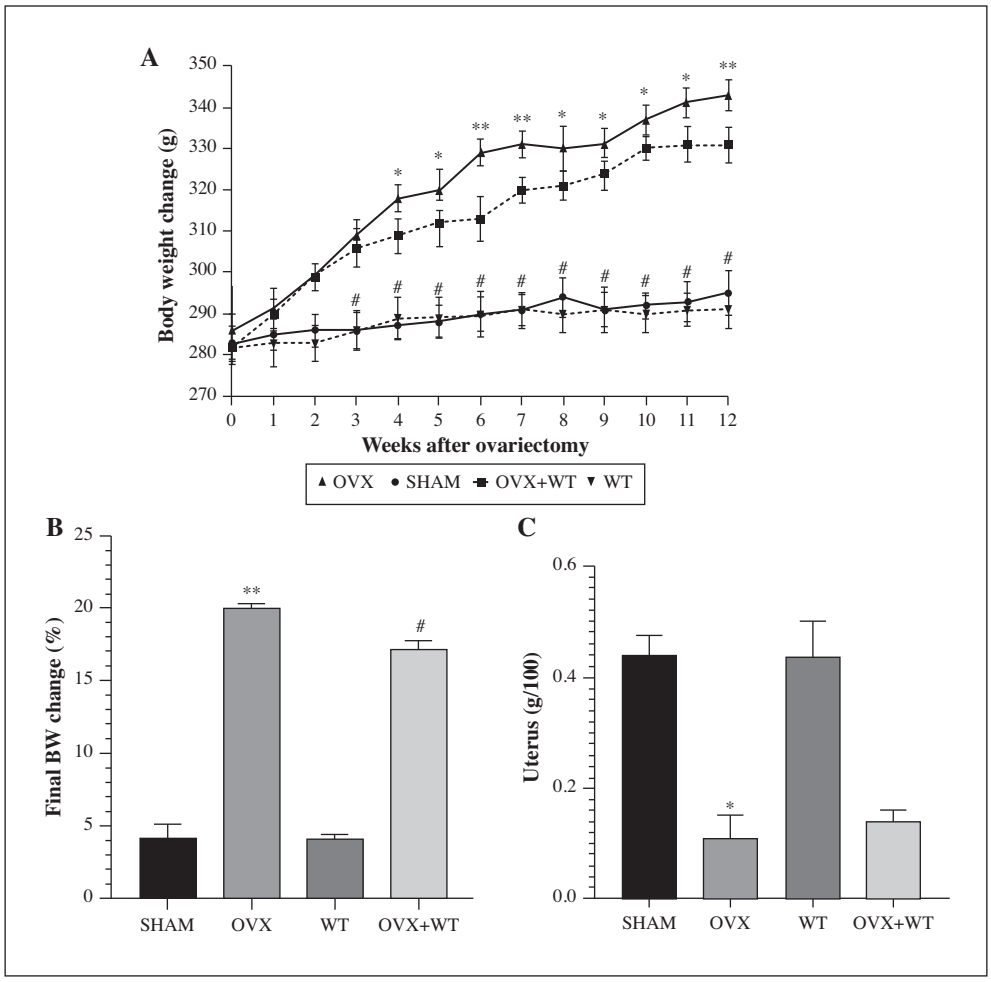

Figure 1

Effect of long term WT treatment (12 week) on (A) body weight change and (B) the ratio of uterus weight to body weight in rats. Data represents mean ± SEM (n=8). *P <0.05, **P <0.01 versus the OVX group, $^{\#}P$ <0.01 versus the sham group. Abbreviations: WT-white tea; OVX- ovariectomy.

$p < 0.001$). The difference between these two groups appeared after the fourth week of the study. There was no significant difference between sham and WT groups ($p > 0.05$). The results of the change in body weight of the rats after 12 weeks are also shown in *figure 1*B. In addition, uterine weight was significantly reduced in the OVX group compared to the sham group. However, there was no significant difference between OVX and OVX + WT groups (*figure 1*C).

UCP1 mRNA gene expression in brown adipose tissue

Data obtained by quantitative real-time PCR method were calculated as the fold change of UCP1 mRNA level in BAT. As shown in *figure 2*, the mRNA expression of UCP1 in BAT was found to be significantly lower in OVX rats in comparison to sham ($p < 0.01$). The expression level of UCP1 mRNA in WT group was higher than that in sham group. On the other hand, UCP1 mRNA expression was determined to be significantly higher in OVX + WT group when compared to OVX group ($p < 0.05$).

Biochemical results

Serum leptin levels in the OVX and WT group were significantly decreased in comparison to the sham

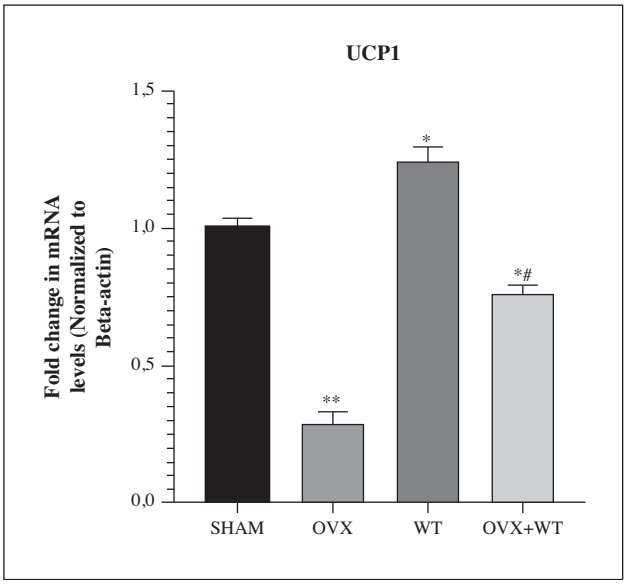

Figure 2

Effect of long term WT on UCP1 mRNA expression in interscapular BAT in rats. Data represents mean ± SD (n=8). *P <0.05, **P <0.01 compared with the Sham group, $^{\#}P$ <0.01 compared with the OVX group. Abbreviations: BAT-Brown adipose tissue; WT-White tea; OVX- Ovariectomy.

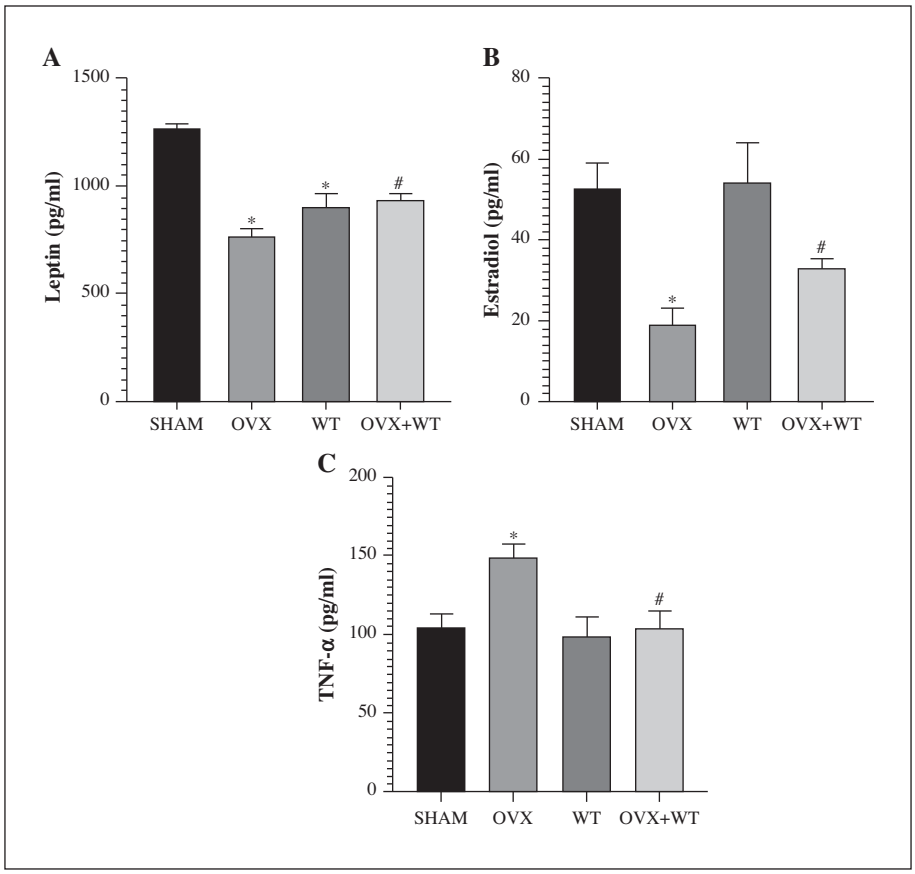

Figure 3
Effect of long-term (12 weeks) consumption of WT on serum A) Leptin, B) E2 and C) TNF-α levels in rats. Data represent mean ± SEM (n=8). *P <0.05 compared with the sham group, $^{\#}P$ <0.05 compared with the OVX group. Abbreviations: E2- Estradiol, TNF-α-Tumor necrosis factor-α, WT-white tea; OVX- ovariectomy.

group (*figure 3*A, $p < 0.05$). In addition, the leptin levels in the OVX + WT group were significantly higher than the OVX group ($p < 0.05$). When the OVX and sham groups were compared in terms of serum E2 levels, low concentration was observed in the OVX group (*figure* B, $p < 0.05$). Moreover, E2 levels in the OVX + WT group were found to be higher compared to the OVX group ($p < 0.05$). There was no significant difference in E2 levels between sham and WT groups ($p > 0.05$). Serum TNF-α concentration in the OVX group was higher than in the sham group. However, it was observed that TNF-α concentration of OVX + WT group decreased significantly when compared with OVX group (*figure 3*A, $p < 0.01$).

SERUM LIPID PROFILE AND GLUCOSE LEVELS

The concentration levels of serum TC, TG, LDL, HDL and glucose obtained from the experimental groups are presented in *table 2* table 2. The serum TC, TG and LDL levels of the rats in the OVX group were statistically significantly higher than in the sham group ($p < 0.05$, for each parameter). However, there was no significant difference in the serum glucose and HDL concentration between all the groups. Moreover, only the WT group had a significantly lower LDL level compared to the sham group. On the other hand, there was no statistically significant difference between TC,

Table 2
Effect of long-term WT treatment (12 wk) on clinical biochemical parameters in rats

	Groups			
	SHAM	**OVX**	**WT**	**OVX + WT**
HDL (mg/dl)	56,9 ± 4,26	54,8 ± 7,19	56,7 ± 4,26	54,9 ± 6,11
LDL (mg/dl)	104,6 ± 3,52	114,9 ± 3,68[*a]	99,3 ± 4,91[*b]	112,1 ± 3,01
Total cholesterol (mg/dl)	173,6 ± 6,16	191 ± 12,17[*a]	175 ± 6,16	177,1 ± 7,23
Triglycerides (mg/dl)	74,37 ± 43,35	115 ± 27,15[*a]	76,5 ± 25,57	113,6 ± 40,78
Glucose (mg/dl)	170,1 ± 39,75	163 ± 35,429,659,65	164,7 ± 16,89	158,3 ± 35,77

All data are presented as the mean ± SEM (n = 8). *$P < 0.05$. Abbreviations: HDL- High-density lipoprotein; LDL- Low-density lipoprotein; WT-white tea; OVX- ovariectomy
[a] Versus the sham group,
[b] Versus the OVX group.

TG and LDL levels of OVX + WT group compared to OVX group ($p > 0.05$).

DISCUSSION

The present study showed that long-term consumption of WT reduced body weight in OVX rats. Morover, estrogen deficiency increased inflammation while reducing UCP1 mRNA expression in OVX rats. OVX rats are considered to be an important attempt to model postmenopausal conditions. The decrease in estrogen secretion was thought to be responsible for increasing body weight and obesity during menopause [24]. In addition, OVX-induced estrogen deprivation increases food intake and reduces motor activity [25, 26], a phenomenon in rodents. It was shown that estrogens can prevent body fat accumulation by the lipolytic effect in different regions of the body [27]. Such effects of estrogens can occur by increasing direct expression of UCP1 primarily *via* BAT estrogen receptor alpha (ERα)-mediated AMP-activated protein kinase [5]. Activity of UCP1 gene expression is an indicator of thermogenesis in fat tissue. It is known that UCP1 can trigger weight loss by activating the pathways of the sympathetic nervous system. These results support that reduced E2 concentration in the rats of experimental OVX model may lead to a decrease in the UCP1 activity. In our group of rats undergoing ovariectomy, the level of estrogen was significantly lower compared to the groups that did not. Decrease in both E2 concentration and UCP1 activity in OVX groups may induce weight gain by suppressing lipolytic activity. Moreover, there was a significant increase in UCP1 gene expression in the OVX + WT group compared to OVX. This finding indicates that WT treatment may increase UCP1 activity. Although the mechanism is not clear enough, it is thought that EGCG may be responsible for the increase in UCP1 mRNA expression. The dominant catechin type in WT content was shown to be EGCG in the studies [28]. In a previous study, EGCG has been shown to increase BAT UCP1 activity and stimulate thermogenesis in dietary-induced obese mice [29]. Leptin is known to be an appetite suppressant hormone. Increased serum leptin concentration and body weight have been reported in rats after OVX operation [30, 31]. On the other hand, Chu et al. demonstrated significantly decreased serum leptin concentration and accompanying increased body weight in rats during the first 7 weeks following OVX [32]. In another study, it was shown that leptin mRNA expression and serum leptin concentration decreased while body weight increased in OVX rats [33]. Similarly, serum leptin concentration decreased significantly in our OVX group compared to sham group. In addition, it was found that body weight decreased while leptin concentration increased in OVX + WT group compared to OVX group. Our findings show that a decrease in the leptin concentration of the OVX group can stimulate the effect of appetite hormones, resulting in weight gain.

It has been reported that a decrease in the ovarian function during menopause correlates with increased interleukin-1, interleukin-6 (IL-6) and TNF-α concentration [34]. Morover, systemic inflammation markers were reported to be elevated in women with ovariectomy as it is in natural menopause [35, 36]. In a recent study, TNF-α and IL-6 concentrations were increased in different tissues of OVX rats [13]. It is also found in the literature that TNF-α concentration increases with obesity and decreases with weight loss.[12] It has been proposed that TNF-α is responsible for the BAT apoptosis as well as the decrease in the expression of UCP-1 and β3-adrenergic receptors in ob/ob rats. A previous study showed that TNF-α activates extracellular-regulated kinases (ERKs) and p38 mitogen-activated protein kinase (p38MAPK) in rat fetal brown adipocytes, while it inhibits UCP1 gene expression [14]. Similarly, no BAT anomalies are observed in rats lacking TNF receptor [37]. In our study, TNF-α concentration of the OVX group was significantly increased compared to the sham group, and the WT treatment was observed to reduce this concentration in the OVX + WT group. These results indicate that the increase in the TNF level in OVX suppresses the expression of UCP1 mRNA in BAT. On the other hand, our results have shown that long-term consumption of WT in OVX rats can stimulate weight loss by both suppressing TNF-α level and stimulating UCP1 activity.

Regarding the effect of WT on serum lipid profile, Těixěira et al. [22] reported that 0.5% WT supplementation to the diet for 8 weeks did not change LDL concentration in obese mice. Serum LDL concentration were noted to decrease in postmenopausal women supplemented with 400 and 800 mg EGCG to the diet [38]. In another study by Jin *et al.* [39], tea polyphenols were reported to reduce LDL concentration in diet-induced obese rats. In our study, we found that long-term consumption (12 weeks) of WT, which contains high levels of polyphenols, may have an effect of lowering LDL and TC concentrations in rats undergoing an ovariectomy operation. Despite these results, further studies are needed to explain the effects of WT on lipid metabolism in rats.

CONCLUSION

Our study indicates that weight gain due to estrogen deficiency may decrease with long-term consumption of WT. The cellular pathways underlying this effect were tried to be elucidated by evaluating inflammation markers, lipid profile, hormonal status and especially UCP1 activation showing the lipolytic effect. Based on the fact that increasing the activity of UCP1 in brown fat tissue and suppressing inflammation trigger weight loss in OVX rats, we have shown that WT can have these effects. However, further studies are needed to investigate the effect of WT on other possible cellular pathways and clinic outcomes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.Author Contrubution

S.S designed the study and took part in data interpretation and article writing; F.S contributed to the determination of gene expression and interpretation of results; E.D contributed to article writing; L.T

contributed to animal experiments; M.A and HA. U contributed to biochemical analysis and interpretation. All authors read and approved the final manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Ethics committee approval of the present study was obtained from the Experimental Animals Local Ethics Committee of RTE University (Protocol No: 2017/28).

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