Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2018;43(Suppl 1):108-123
DOI: 10.17826/cumj.394855

ARAŞTIRMA / RESEARCH

The protective effect of resveratrol against risperidone-induced brain damage and metabolic side effects

Resveratrolün risperidon kaynaklı beyin hasarına ve metabolik yan etkilere karşı koruyucu etkisi

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Cukurova Medical Journal 2018;43 (Suppl 1):108-123

Abstract

Purpose: The aim of this study was to investigate the protective effect of resveratrol (RSV) against risperidone (RIS) on weight gain/loss via of the expression of some various neurotransmitter receptors.

Materials and Methods: Thirty five female Spraque-Dawley rats were divided into 5 groups (n=7): Control, RIS, RIS+RSV-1, RIS+RSV-2, RIS+RSV-3. Rats were treated orally with RIS (2 mg/kg/day) and RSV (20, 40 and 80 mg/kg/day) for 14 days. On treatment day 15, blood and brain tissues were removed for analysis.

Results: The reduced neuropeptide Y1 expression levels in the RSV groups correlated with the RSV-induced weight loss. The Dopamine D1 receptor and seratonin 5-HT2c expression levels decreased significantly with RSV treatment. In addition, the RSV increased the glucose, triiodothyronine, tetraiodothyronine, and total antioxidant status, and decreased the cholesterol, triglyceride, high density lipoproteins, low density lipoproteins, thyroid-stimulating hormone, oxidative stress index, and total oxidant status levels significantly.

Conclusion: The daily treatment with RSV increased the antioxidant capacity, protected the apoptotic cell injury, and the possibility of death. RSV could be an effective course of therapy to enhance therapeutic efficacy.

Key words: Risperidone, resveratrol, weight gain/loss, dopamine D1 receptor

Öz

Amaç: Bu çalışmanın amacı, risperidon'un (RIS) çeşitli nörotransmitter reseptörlerin ekspresyonu yoluyla kilo alımı/kaybı üzerindeki etkisine karşı resveratrol'ün (RSV) koruyuculuğunu araştırmaktır.

Gereç ve Yöntem: Otuz beş dişi Spraque-Dawley sıçan: kontrol, RIS, RIS+RSV-1, RIS+RSV-2, RIS+RSV-3 olmak üzere 5 gruba ayrıldı. Sıçanlara, 14 gün boyunca RIS (2 mg/kg/gün) ve RSV (20, 40 ve 80 mg/kg/gün) oral yoldan uygulandı. Uygulamanın 15. gününde kan ve beyin dokuları analiz için alındı.

Bulgular: RSV gruplarındaki azalmış nöropeptid Y1 ekspresyon seviyelerinin, RSV ile uyarılan kilo kaybıyla ilişkili olduğu görüldü. Dopamin D1 reseptörü ve seratonin 5-HT2c ekspresyon seviyeleri, RSV uygulanması ile anlamlı olarak azaldı. Ayrıca RSV'nin, glikoz, triiyodotironin, tetraiyodotironin ve total antioksidan kapasitesini arttırdığı ve kolesterol, trigliserit, yüksek yoğunluklu lipoprotein, düşük yoğunluklu lipoprotein, tiroid uyarıcı hormon, oksidatif stres indeksi ve total oksidan kapasitesi düzeylerini anlamlı derecede azalttığı görüldü.

Sonuç: Çalışmamızda; günlük RSV alımının antioksidan kapasitesini arttırdığı, apoptotik hücre hasarına ve ölüm olasılığına karşı koruduğu görüldü. RSV, terapötik etkinliği arttırmak için etkili bir tedavi olabilir.

Anahtar kelimeler: Risperidon, resveratrol, kilo alımı/kaybı, dopamin D1 reseptörü

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Geliş tarihi/Received: 14.2.2018 Kabul tarihi/Accepted: 25.6.2018 Published online: 14.9.2018

INTRODUCTION

Second generation antipsychotics (SGAs) (also known as atypical antipsychotics; AAPs) such as clozapine, olanzapine, RIS, and quetiapine, became an important part of modern psychiatric treatment during the 1990s[1]. When compared with typical antipsychotics, their metabolic side effects, for example weight gain or obesity, hypertension, diabetes mellitus, and changes in the lipid profile, on schizophrenic patients are important[2]. SGAs have binding affinities for various neurotransmitter receptors, such as dopamine D2 receptor (D2R), histamine H1 reseptor (H1R), serotonin 5-HT_{2a} and 5-HT_{2c} receptors[3,4]. RIS is one of the SGAs used for the acute and long-term treatment of patients with schizophrenia[5]. RIS has been associated with moderate weight gain, a sedation effect, an induced significant stunting of the fetal body, and brain weight[6,7]. Also, RIS is associated with substantial reduction in the thickness of neocortical layers and apoptotic neurodegeneration in the fetal brain[8]. It was reported that dopamine D2R and 5-HT_{2a}R played a critical role in the therapeutic effects of SGAs including RIS, while 5-HT_{2c}R and H1R contributing to weight gain/obesity side-effects[4]. SGAs also affect the expression of neuropeptide Y (NPY), associated with the regulation of the body in the brain [9-11] reported that weight gain and obesity associated with SGAs such as olanzapine and clozapine are mediated by the activation of the hypothalamic AMP-activated protein kinase (AMPK) pathway via the blockage of the H1R.

Common side effects include movement problems, sleepiness, trouble seeing, constipation, and increased weight[12]. Serious side effects may include the potentially permanent movement disorder tardive dyskinesia, as well as neuroleptic malignant syndrome, an increased risk of suicide, and high blood sugar levels[13].

Medicinal plants including RSV, It can be useful in the development of drugs nowadays, and have various pharmacological effects. RSV (3,5,4'-trihydroxystilbene), a polyphenol produced in plants in response to stress and activates isoproterenol-induced lipolysis, was discovered in 1939 by Michio Takaoka, and recently found to be a potent inducer of weight loss and longevity in both animals and humans[14,15]. It is present in many plant-based foods such as grapes, peanuts, and berries[16]. The RSV has been studied *in vivo* and *in vitro* and has been shown to possess a wide range of pharmacological effects, including cardioprotective, neuroprotective, nephroprotective, antineoplastic, and antidiabetic effects, as a result of its anti-inflammatory, antioxidant, and cytoprotective properties[17]. In a placebo-controlled study, RSV also decreased the levels of aspartate aminotransferase (AST), glucose, alanine aminotransferase (ALT), total cholesterol (CH), tumor necrosis factor, cytokeratin, and the fibroblast growth factor[18]. Moreover, RSV exerts neuroprotective effects in neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's diseases, and can protect the brain against damage induced by toxins and disease[19].

Many studies have shown that RSV possesses antiplatelet and blood glucose-lowering activities[20,21]. By increasing the production of nitric oxide, the RSV inhibits platelet aggregation and stimulates vasodilation. Recently published data have shown that RSV protects against obesity as well as is being effective in the management of osteoporosis in postmenopausal women without any increased risk of breast cancer[22-25].

In the present study, we aimed to investigate the effect of RSV on weight gain caused by RIS looking biochemical-hormone parameters, the total oxidant/total antioxidant levels (TOS/TAS), the gene expression levels of D1R, dopamine D4 (D4R), 5-HT_{1a}, 5-HT_{2c}, NPY1, H1R and hypothalamic AMPKα receptors, and the histopathological investigation of rat brains for evaluating apoptotic cell death.

MATERIALS AND METHODS

Chemicals

The RIS was purchased from Johnson & Johnson (USA), and the RSV (trans-3,4',5-Trihydroxystilbene, ≥98 %) from Carl-Roth® (Germany).

Study groups and experimental design

Total 35, 3-4 month old female Sprague Dawley-albino rats (weighing 250-300 g) were obtained from the Experimental Research Centre of Fırat Univesity, Elazığ/Turkey. They were housed in polycarbonate cages with wire lids in groups of seven, and given the standard laboratory chow and water throughout the whole experiment. The housing room was maintained at 24 oC with 42 ± 5% of relative humidity, and had a 12-12-h light-

dark cycle (lights on 06:00–18:00 h). Thirty five Sprague Dawley-albino rats were randomly divided into 5 groups as follows:

Group I (Control, n=7) was the control group and was not treated with anything.
Group II (RIS, n=7) received oral daily doses of the RIS solution (2 mg/kg/day) for 14 days 26-27.
Group III (RIS+RSV-1, n=7) received oral daily doses of the RIS solution (2 mg/kg/day) and RSV (20 mg/kg/day) for 14 days 28-29.
Group IV (RIS+RSV-2, n=7) received oral daily doses of the RIS solution (2 mg/kg/day) and RSV (40 mg/kg/day) for 14 days 29.
Group V (RIS+RSV-3, n=7) received oral daily doses of the RIS solution (2 mg/kg/day) and RSV (80 mg/kg/day) for 14 days 29.

The weight of the rats in each group was recorded before and after completing the study. After treatment with the RIS and RSV for 14 days mentioned as above, all the rats were killed ethically, and then the blood and brain were taken. The brains were used for gene expression studies and the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, and blood of the rats for the examination of the biochemical-hormone parameters and the TOS/TAS levels. The experiments were performed according to the protocol approved by the Fırat University Animal Experiments Local Ethics Committee on laboratory animals, Elazığ, Turkey (FUDAM 2015/05-66).

Examination of biochemical-hormone parameters and TOS/TAS levels

The venous bloods (10 ml) were allowed to clot at room temperature in a gel tube, and after centrifugation at 3000 rpm for 5 min, the serums were obtained and stored at -20 °C before using. We examined the glucose, CH, triglyceride (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) (Architect ci16200), thyroid-stimulating hormone (TSH), triiodothyronine (T3), tetraiodothyronine (T4), the insulin levels (Beckman Coulter), and also the TOS/TAS levels (Rel Assay Diagnostics) (Thermo Scientific Varioskan Flash) according to the manufacturer's instructions.

RNA isolation and quantitative real-time PCR (qRT-PCR)

qRT-PCR was used to detect the mRNA expression of the D1R, D4R, 5-HT1a, 5-HT2c, NPY1, H1R, and hypothalamic AMPKα receptors. The RNA isolation of brain tissues was performed. First, an approximate 30 mg tissue was taken from the brain and homogenized in a 1.5 ml-zirconiferous eppendorf tube containing beads with a homogenizer (Bioprep-24, Allsheng). Then the total RNA's were extracted using a ExiPrepTM Tissue Total RNA isolation kit (Bioneer, K-3325) according to the manufacturer's instructions, and quantified by measuring the absorbance at 260/230 nm and 260/280 nm using a NanoDrop spectrophotometer (Denovix DS-11).

RNA must first be reverse transcribed into cDNA in a reverse transcription (RT) reaction. For this purpose, we used the AccuPower® RT PreMix (Bioneer K-2041) according to its instructions. In our study, we used the primer pairs (Bionner S-1001) for qRT-PCR as shown in Table 1.The RT-PCR was conducted following the instructions of the AccuPower GreenStar qPCR PreMix (Bioneer, Cat No: K-6210). The level of the mRNA expression of the D1R, D4R, 5-HT_{1a}, 5-HT_{2c}, NPY1, H1R, and AMPKα genes, was detected using the ExiCyclerTM96 qRT- PCR system (Bioneer). The PCR conditions were: 95 °C for 1 min, followed by 45 cycles at 95 °C for 5 sec, and 55 °C for 40 second. The 2^-ΔΔCt method was used to calculate the results[30-35].

TUNEL assay

Each group of our study population was evaluated using the TUNEL assay to identify the apoptosis rate in their brain. The brains were waxed, and then the sections were taken at 5 mm thickness and transferred onto slides covered with polilizin. We used the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) according to the manufacturer's instructions to determine the apoptotic cells in the brain. The preparations were examined and evaluated under a research microscope (Leica DM500). The nuclei stained blue with the Harris hematoxylin were evaluated as normal and the brown ones indicated apoptotic cells. In the sections, randomly selected areas of 10 were screened, and at least 400 cells were examined. The apoptotic index (AI) was calculated as apoptotic cells/total (normal + apoptotic) cells.

Statistical analysis

The statistical analyses was performed using the SPSS 16.0 (SPSS, Chicago, IL, USA). Data were expressed as mean ± SE (standard error of the

mean). The normality for the variables in the groups was determined by the Shapiro-Wilk test. The comparison of the mean weight of all groups were evaluated with the paired-samples T-test before and after the treatment. The one-way analysis of the variance (ANOVA) followed by the LSD post hoc test were used for the comparison of the biochemical-hormone parameters and gene expression levels. The intergroup differences were evaluated either by the Kruskal-Wallis test or the Mann-Whitney U test. For the histopathological analysis, the data were expressed as mean ± SD (standard deviation). A statistical significant difference was determined by the ANOVA followed by the Tukey's multiple comparison test. The probability (p) less than 0.05 ($p<0.05$) was considered to be statistically significant.

RESULTS

Effects of RIS and RSV on weight gain/loss

The body weight measurements showed that during two weeks animals grew from 238.28 g at day 1 to 252.85 g for the control group, 234.57 g at day 1 to 248.00 g for the RIS group, 225.28 g at day 1 to 233.71 g for the RIS+RSV-1 group, 232.40 g at day 1 to 226.80 g for the RIS+RSV-2 group and 244.80 g at day 1 to 246.80 g for the RIS+RSV-3 group at day 14 (Table 2; and the paired-samples T-test for the body weight at day 14, $p<0.001$, $p<0.005$, $p<0.005$, $p>0.05$, $p>0.05$, respectively).

Changes in the body weight of experimental rats. Values are expressed as mean ± SEM of seven animals. The groups were compared with the paired-samples T-test at initial and final of the treatment. $p\leq0.05$. Abbreviations: RIS: risperidone; RSV: resveratrol; RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV; RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

There was a significantly increased total body weight gain in the control, and the RIS and RIS+RSV-1 treatment groups ($p<0.001$, $p<0.01$, $p<0.01$, respectively). However, the RIS+RSV-2 group was observed to have reduction in weight, and the RIS+RSV-3 group increased weight gain had no significant effect on these measurements ($p>0.05$, $p>0.537$, respectively) (Table 2; Figure 1).

Table 1. Primer sequences used for qRT-PCR

GENE	Primer Sequences	Product sizes (bp)	References
D1R	F: 5'-TGAGCCTTACAGCAGGAGTG-3' R: 5'-AGCCACCACATCAGTCT TTGAGTCTTTG -3'	279 bp	Zhou et al. (2014)30
D4R	F: 5'-TGGTGTTGCCTCTCTTTGTCT-3' R: 5'-GCCATGAGGGTGTCACAGA-3'	76 bp	Kim et al. (2010)31
5-HT1a	F: 5'-TGGCTTTCTCATCTCCATCC-3' R: 5-CTCACTGCCCCATTAGTGC-3'	357 bp	Mohanan et al. (2006)32
5-HT2c	F: 5'-CCAACGAACACCTTCTTTCC-3' R: 5'-GCATTGTGCAGTTTCTTCTCC-3'	362 bp	Mohanan et al. (2006)32
NPY1	F: 5'-CGTTAGGCCATTCACAAGAATGAAG-3' R: 5'-ATGCTCTGATGAGCGATGCAA-3'	174 bp	Xu et al. (2014)33
H1R	F: 5'-TATGTGTCCGGGCTGCACT-3' R: 5'-CGCCATGATAAAACCCAACTG-3'	66 bp	Sato et al.(2009)34
Hypothalamic AMPKα	F: 5'TGTGACAAGCACATTTTCCAA3' R: 5'-CCGATCTCTGTGGAGTAGCAG-3'	134 bp	Al-Qatati et al. (2013)35

Table 2. Body weight (g) of the study population during treatment.

Groups	Weight (mean ± SEM)		
	Before	After	p value
Control	238.29 ± 7.54	252.86 ± 7.47	0.000
RIS	234.57 ± 7.28	248.00 ± 5.86	0.005
RIS +RSV-1	225.29 ± 4.16	233.71 ± 3.69	0.005
RIS +RSV-2	232.40 ± 3.56	226.80 ± 5.26	0.071
RIS +RSV-3	244.80 ± 9.89	246.80 ± 9.42	0.537

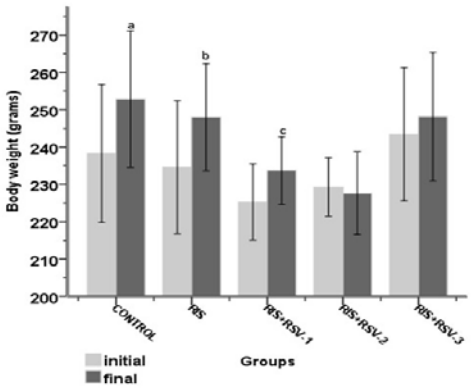

Figure 1. Changes in the body weight of experimental rats.

Values are expressed as mean ± SEM of seven animals. The groups were compared with the paired-samples T-test at the beginning and end of the treatment. a,b,c In each column, different superscript letters mean significant differences at $p<0.05$. Abbreviations: RIS: risperidone; RSV: resveratrol; RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV; RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

Effects of RIS and RSV on biochemical-hormone parameters and TOS/TAS levels and oxidative stress index (OSI)

We measured the levels of the biochemical, hormonal, and oxidative stress parameters in the serum, and results are shown in Table 3. The glucose level significantly increased in the RIS+RSV-1 group compared to the RIS group ($p<0.01$). CH and the LDL levels significantly decreased in the RIS group compared to the RIS+RSV-3 group ($p<0.05$ and $p<0.01$, respectively). The CH level significantly decreased in the RIS+RSV-2 group compared to both the control and RIS+RSV-3 groups ($p<0.05$ and $p<0.01$, respectively). The HDL level significantly decreased in the RIS+RSV-2 group compared to both the control, RIS+RSV-1 and RIS+RSV-3 groups ($p<0.05$). The LDL level significantly increased in the RIS+RSV-3 group compared to the control, the RIS, RIS+RSV-1, and the RIS+RSV-2 groups ($p<0.05$, $p<0.01$, $p<0.05$, and $p<0.01$ respectively). A significant statistical difference in the TG level was not found when the RIS group was compared to the other groups ($p>0.05$, Table 3, Figure 2). The TSH level significantly increased in the RIS group compared to the RIS+RSV-2 group, while the T3 level decreased ($p<0.05$). The T4 level significantly decreased in the RIS group compared to both the control and the RIS+RSV-1 group ($p<0.05$, Table 3, Figure 3).

The ameliorative effects of the RSV treatment against the RIS administration significantly increased the TAS level and decreased the TOS and OSI levels ($p<0.05$). The control group had a significantly higher TAS level when compared to the RIS group ($p<0.05$). The RIS+RSV-1 group had a significantly higher TAS level when compared to both the RIS and RIS+RSV-3 groups ($p<0.001$ and $p<0.001$, respectively). The RIS+RSV-2 group had a significantly higher TAS level when compared to both the RIS and RIS+RSV-3 groups ($p<0.05$ and $p<0.05$, respectively). The TOS level was significantly higher in the RIS group when compared to the control, the RIS+RSV-1, the RIS+RSV-2, and the RIS+RSV-3 groups ($p<0.001$). The RIS+RSV-1 group had a significantly higher TOS level when compared to the RIS+RSV-3 group ($p<0.001$). The OSI level was significantly higher in the RIS group when compared to the control, RIS+RSV-1, RIS+RSV-2 and RIS+RSV-3 groups ($p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) (Table 3, Figure 4). Effect of RIS and RSV on expression of D1R, D4R, 5-HT_{1a}, 5-HT_{2c}, NPY1, H1R, and hypothalamic AMPKα receptors

Table 4 shows the effects of the RIS and RSV treatments on the mRNA expression of the D1R, D4R, 5-HT_{1a}, 5-HT_{2c}, NPY1, H1R and hypothalamic AMPKα receptor genes in all the study groups. The D1R expression increased in the RIS group and decreased in all the RSV treated groups. These decreases were statistically important in the RIS+RSV-1 and RIS+RSV-2 groups when compared to the control and RIS groups ($p<0.05$) (Table 4). The 5-HT_{2c} mRNA expression was seen as relatively low in the RIS+RSV-3 group. This decrease was found statistically important when compared to the control and RIS+RSV-1 groups ($p<0.05$) (Table 4).

There was a relative decrease in the NPY1 expressions in all the RSV treated groups (20, 40 and 80 mg/kg) when compared to the control and RIS groups. The expression seen in the RIS+RSV-1 group was low, whereas in the RIS+RSV-2 and RIS+RSV-3 groups expression levels increased relatively. This increase in the RIS+RSV-2 group was statistically significant when compared to the RIS+RSV-1 group ($p<0.05$) (Table 4).

Effect of RIS and RSV on apoptosis in rat brains

We used the TUNEL assay to determine the apoptotic cells in the brain sections. In the RIS group, the TUNEL-positive cells were significantly higher when compared to the control (32.16 ± 3.31) ($p<0.05$) (Figure 5a and 5b). Despite the fact that in RIS + RSV-1 group it decreased significantly (6.66 ± 1.03) when compared to the RIS group ($p<0.05$), it increased gradually in the RIS+RSV-2 and RIS+RSV-3 groups (Table 5, Figure 5c, 5d and 5e).

Table 3. Comparison of serum biochemical, hormonal and serum oxidative stress parameters

	STUDY GROUPS				
	Control	**RIS**	**RIS+RSV-1**	**RIS+RSV-2**	**RIS+RSV-3**
Biochemical Parameters (Mean ± SEM)					
Glucose (mg/dL)	125.71 ± 5.22	124.43 ± 4.96^{c1}	137.57 ± 5.28^{b1}	128.57 ± 3.45	128.43 ± 2.21
CH (mg/dL)	50.14 ± 3.54^{d1}	43.29 ± 3.35^{e1}	46.43 ± 3.03	40.57 ± 3.08^{a1,e2}	54.86 ± 2.61^{b1,d2}
TG (mg/dL)	72.71 ± 11.39	59.43 ± 11.11	56.29 ± 11.54	50.86 ± 3.69	58.71 ± 9.17
HDL (mg/dL)	21.29 ± 1.36^{d1}	20.71 ± 1.34	21.00 ± 1.09^{d1}	17.71 ± 0.92^{a1,c1,e1}	21.14 ± 0.63^{d1}
LDL (mg/dL)	14.29 ± 2.41^{e1}	10.71 ± 2.20^{e2}	14.14 ± 1.75^{e1}	12.71 ± 2.46^{e2}	22.14 ± 2.65^{a1,b2,c1,d2}
Hormonal Parameters (Mean ± SEM)					
TSH (mIU/L)	0.01 ± 0.00	0.02 ± 0.01^{d1}	0.01 ± 0.00	0.00 ± 0.00^{b1}	0.01 ± 0.01
T3 (pg/dL)	2.93 ± 0.05	2.53 ± 0.17^{d1}	2.92 ± 0.19	2.98 ± 0.16^{b1}	2.79 ± 0.08
T4 (ng/dL)	1.07 ± 0.02^{b1}	0.87 ± 0.07^{a1,c1}	1.05 ± 0.06^{b1}	0.97 ± 0.07	1.02 ± 0.06
Insulin (μU/mL)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Total Oxidant/antioxidant (Mean ± SEM)					
TAS (mmol/L)	1.60 ± 0.26^{b1}	0.90 ± 0.16^{a1,c3}	2.00 ± 0.31^{b3,e3}	1.81 ± 0.15^{b1,e1}	0.95 ± 0.27^{c3,d1}
TOS (μmol/L)	9.31 ± 0.55^{b3}	14.90 ± 0.99^{a3,c3,d3,e3}	10.45 ± 0.75^{b3,e3}	8.77 ± 0.53^{b3}	7.14 ± 0.96^{c3,b3}
OSI (AU)	0.06 ± 1.41^{b3}	0.20 ± 3.54^{a3,c3,d3,e3}	0.05 ± 0.86^{b3}	0.05 ± 0.68^{b3}	0.10 ± 2.20^{b3}

Each group represents the mean ± SEM of seven rats. a: Significant from control; b: Significant from RIS; c: Significant from RIS+RSV-1; d: Significant from RIS+RSV-2; e: Significant from RIS+RSV-3. 1: $p<0.05$, 2: $p<0.01$, 3: $p<0.001$. AU: ArbitraryUnits

Table 4. Effects of RIS and RSV on the expression of D1R,D4R, 5HT1a, 5-HT2c, NPY1, H1R and Hypothalamic AMPKα genes in rat brains.

Groups	**Expression value (Ct value; Mean ± SEM)**						
	D1R	**D4R**	**5-HT1a**	**5-HT2c**	**NPY1**	**H1R**	**Hypothalamic AMPKα**
Control	36.29 ± 0.53^{c2,d1}	30.85 ± 0.74	35.35 ± 1.21	38.17 ± 0.64^{e1}	24.42 ± 0.84^{c1}	24.96 ± 0.78	36.16 ± 0.61
RIS	34.93 ± 0.84^{c1,d1}	30.56 ± 0.47	36.29 ± 0.90	38.93 ± 0.80	25.08 ± 0.52^{c1}	25.85 ± 0.56	35.51 ± 0.72
RIS+RSV-1	38.84 ± 0.63^{a2,b1}	31.52 ± 0.39	35.17 ± 1.12	38.10 ± 0.45^{e1}	26.62 ± 0.24^{a1,b1,d1}	26.89 ± 0.51	34.81 ± 0.47
RIS+RSV-2	38.97 ± 0.97^{a1,b1}	31.42 ± 0.42	35.75 ± 0.48	39.15 ± 0.93	25.66 ± 0.33^{c1}	26.54 ± 0.63	36.50 ± 0.80
RIS+RSV-3	38.26 ± 1.11	31.57 ± 0.42	34.74 ± 0.61	40.76 ± 0.63^{a1,c1}	25.29 ± 0.54	29.30 ± 2.67	36.95 ± 1.80

Each group represents the mean ± SEM of seven rats. a: Significant from control; b: Significant from RIS; c: Significant from RIS+RSV-1; d: Significant from RIS+RSV-2; e: Significant from RIS+RSV-3; 1: $p<0.05$ 2: $p<0.01$ 3: $p<0.001$.

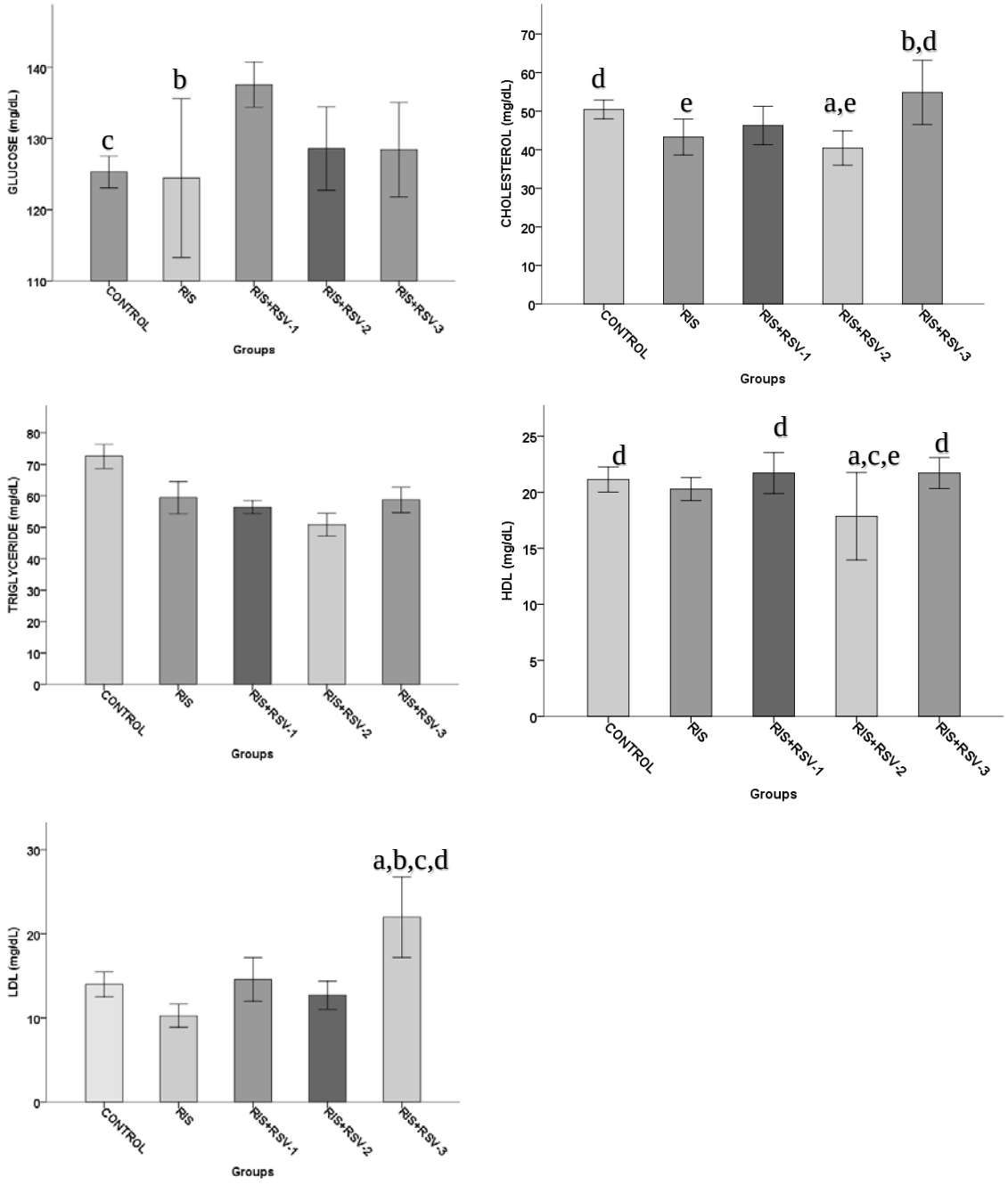

Figure 2. Effects of RIS, RSV, and their coadministration on the brain level of glucose, CH, TG, HDL, and LDL in rats after two weeks.

Values are expressed as mean ± SEM of seven animals. ANOVA followed by the LSD post hoc test were used. [a] $p < 0.05$ versus control; [b] $p < 0.05$ versus RIS-treated rats; [c] $p < 0.05$ versus RIS+RSV-1 treated rats; [d] $p < 0.05$ versus RIS+RSV-2 treated rats; [e] $p < 0.05$ versus RIS+RSV-3 treated rats. RIS: risperidone; RSV: resveratrol; RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV; RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

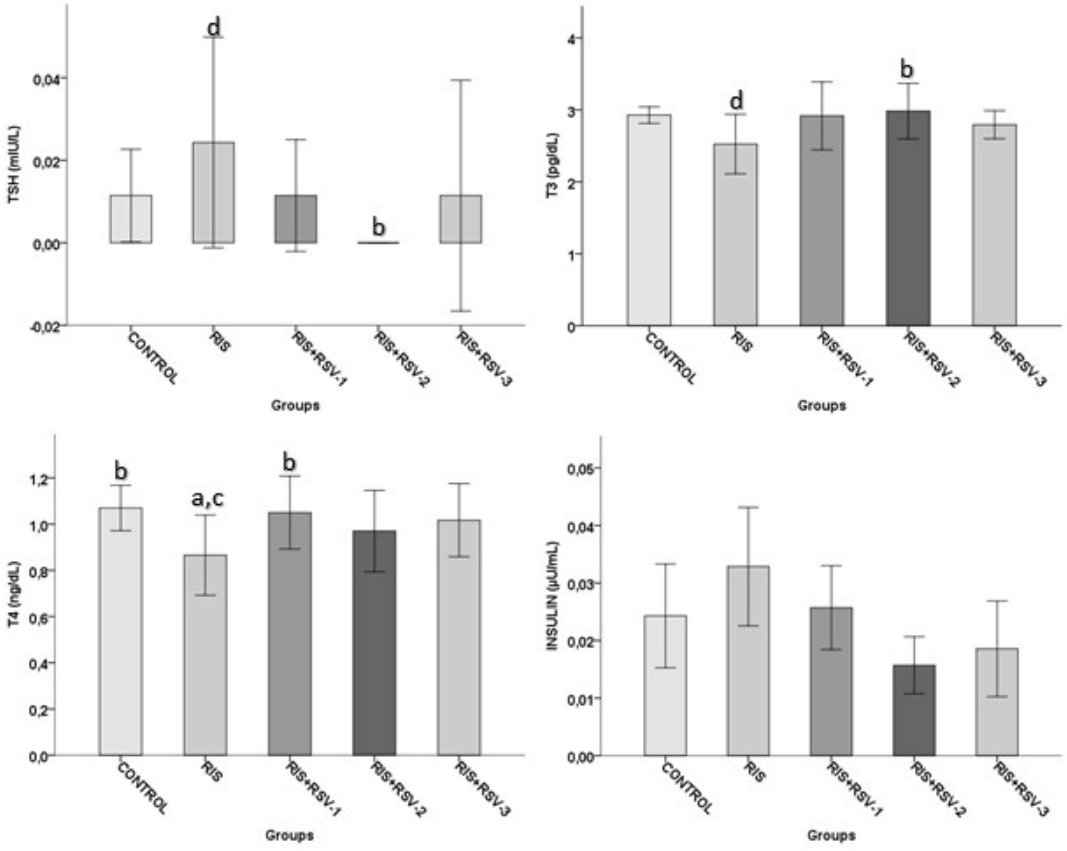

Figure 3. Effects of RIS, RSV, and their coadministration on the brain level of TSH, T3, T4, and insülin in rats after two weeks.

Values are expressed as mean ± SEM of seven animals. ANOVA followed by the LSD post hoc test were used. [a] $p < 0.05$ versus control; [b] $p < 0.05$ versus RIS-treated rats; [c] $p < 0.05$ versus RIS+RSV-1 treated rats; [d] $p < 0.05$ versus RIS+RSV-2 treated rats; [e] $p < 0.05$ versus RIS+RSV-3 treated rats. RIS: risperidone; RSV: resveratrol; RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV; RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

Table 5. Effects of RIS and RSV on AI (%) in rat brains

Groups	AI (%) (mean ± SD)
Control	$1.50 \pm 0.83^{b,c,d,e}$
RIS	$32.16 \pm 3.31^{a,c,d}$
RIS+RSV-1	$6.66 \pm 1.03^{a,b,d,e}$
RIS+RSV-2	$14.16 \pm 0.75^{a,b,c,e}$
RIS+RSV-3	$33.33 \pm 4.22^{a,c,d}$

The AI of all the groups. Values are mean ± SD for seven rats in each group. a: Significant from control; b: Significant from RIS; c: Significant from nRIS+RSV-1; d: Significant from RIS+RSV-2; e: Significant from RIS+RSV-3 ($p<0.05$)

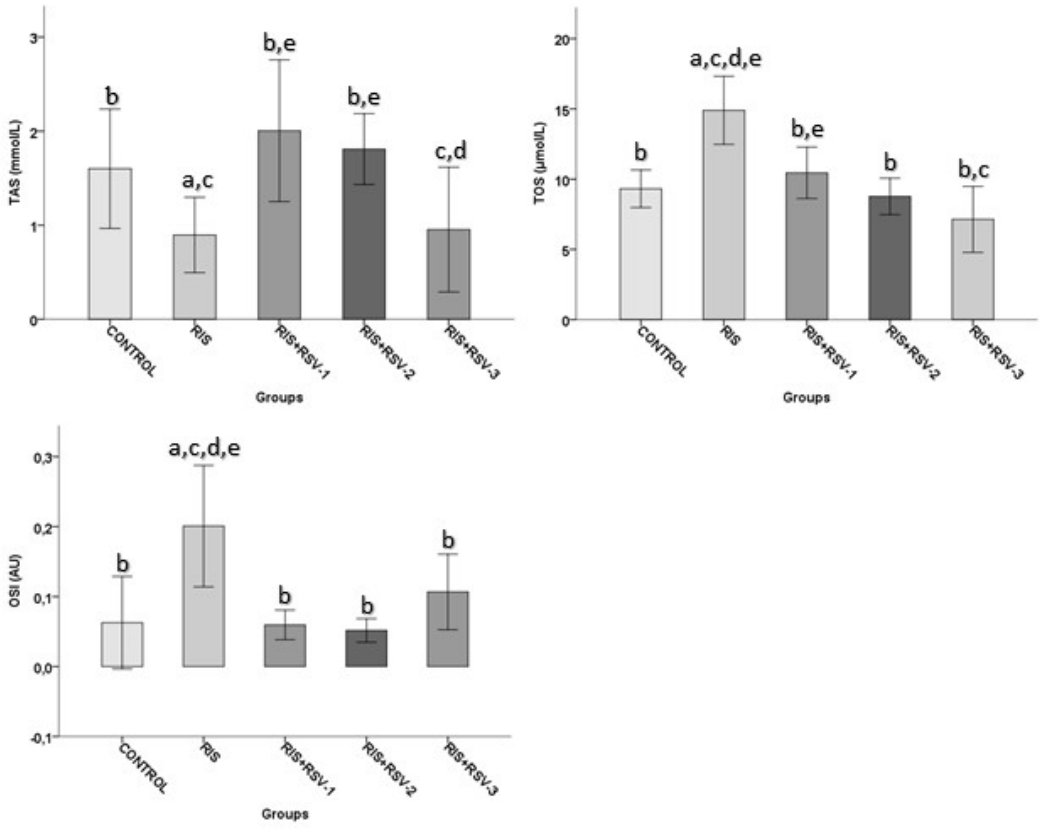

Figure 4. Effects of RIS, RSV, and their coadministration on the brain level of TAS, TOS, and OSI in rats after two weeks. Values are expressed as mean ± SEM of seven animals. ANOVA followed by the LSD post hoc test were used. [a] $p < 0.05$ versus control; [b] $p < 0.05$ versus RIS-treated rats; [c] $p < 0.05$ versus RIS+RSV-1 treated rats; [d] $p < 0.05$ versus RIS+RSV-2 treated rats; [e] $p < 0.05$ versus RIS+RSV-3 treated rats. RIS: risperidone; RSV: resveratrol; AU: arbitrary units; RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV; RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

DISCUSSION

The many countries in the industrialized world, SGAs have become the first line drug treatment for people with schizophrenia. The question as to whether, and if so how much the effects of the various SGAs differ, is a matter of debate [36]. RIS is one of the SGAs and has led to weight gain/obesity side-effects, and other metabolic disorders in patients [4,6,7,37,38]. However, the mechanism(s) responsible for antipsychotic drug-induced weight gain are not really understood [39]. Increased food intake, altered glucose homeostasis, and metabolism, have been proposed as the mechanisms responsible for antipsychotic drug-induced weight gain. The excessive accumulation of fat as the result of more energy intake and less energy expenditure is known as obesity. At present, a combination therapy of reducing calorie intake, increased energy expenditure, and pharmacotherapy is becoming more popular. A recent review on dietary polyphenols and obesity also confirmed that green tea catechins, RSV, and curcumin, all exert anti-obesity properties. It was reported that RSV had an antiobesogenic effect via regulating fatty acid β-oxidation in relation to preventing the degradation of intracellular cyclic adenosine monophosphate (cAMP) through the inhibition of cAMP phosphodiesterase enzymes [40]. In this study, we first used three different doses of RSV to determine the dose-dependent effects of the RSV against the RIS on the brain.

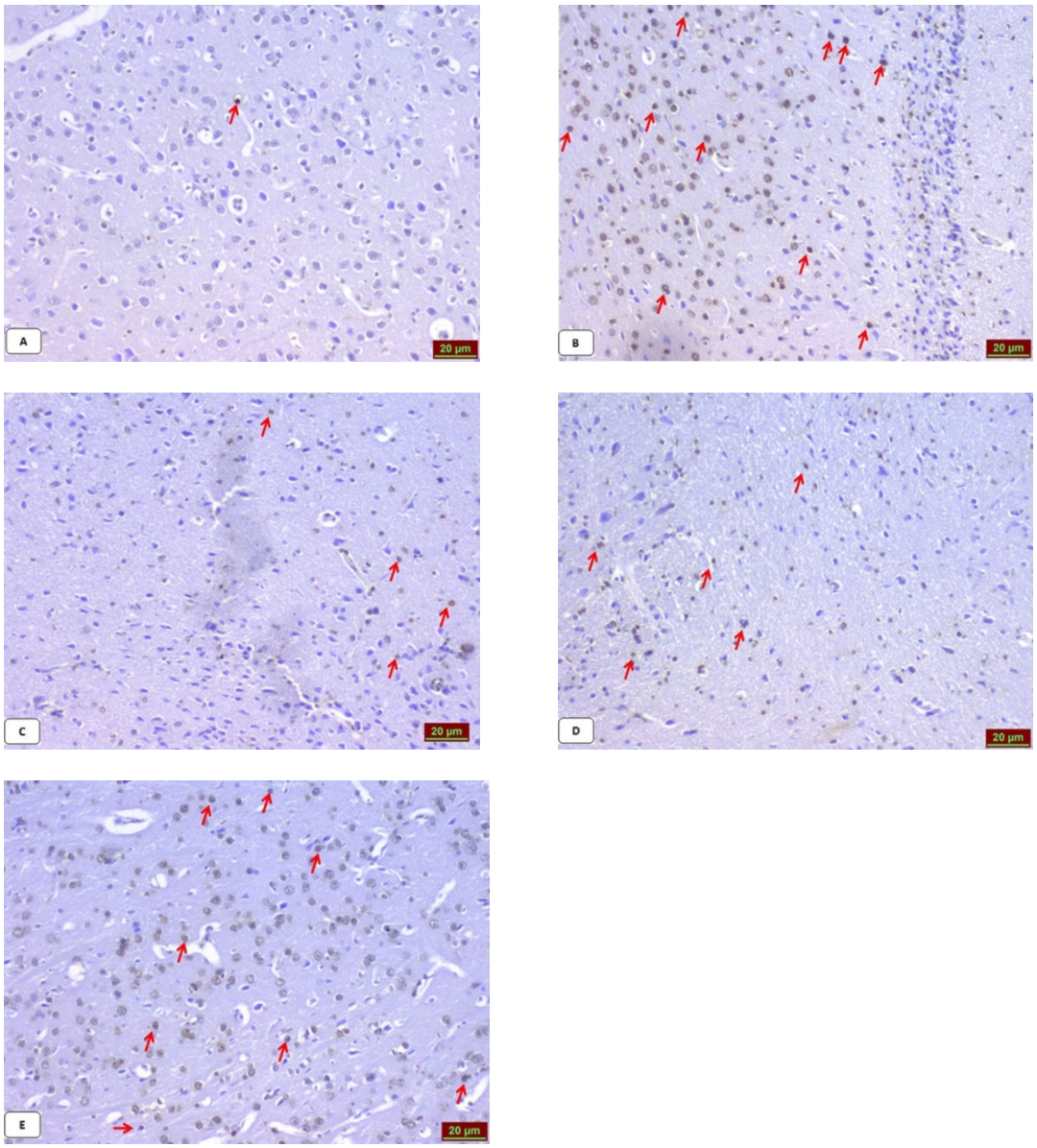

Figure 5. Representative photomicrographs of Tunel staining in all five groups (scale bars = 20 µm), showing: (A) Group 1 (control) only few Tunel-positive cells (arrow); (B) Group 2 (RIS) a lot of TUNEL-positive cells (arrows); (C) Group 3 (RIS+RSV-1), (D) Group 4 (RIS+RSV-2) and (E) Group 5 (RIS+RSV-3) similarly rare TUNEL positive cells (arrows). This analysis was exerted in at least eight areas of each brain section (two sections/animal), and the sections were analyzed at 400x magnification. The evaluation of TUNEL staining was exerted based on the extent of the staining of apoptotic cells. The extent of TUNEL staining was scored semiquantitatively as 0 (none), 1 (light), 2 (medium), and 3 (intense)

Body weight gain and metabolic alterations have been reported during treatment with atypical antipsychotic medications. Weight gain is associated with a reduction in insulin sensitivity and an increase

in glucose production. Therefore, we used a short-term oral supplementation of RIS to understand its potential direct effect on the glucose parameter. By using this methodology with an oral administration, it was shown that RIS did not increase the serum glucose level as in the previous studies [41]. A study conducted by Sikich et al. reported a non-significant increase in values of fasting blood sugar in RIS-treated patients after eight weeks of treatment [42]. There may be multiple physiologic mechanisms underlying the unchanging glucose and insulin levels seen in the RIS administration. According to the previous studies, RSV improves insulin sensitivity and lowers the hepatic glucose production in the rat models of obesity and diabetes, but the underlying mechanisms for these antidiabetic effects remain elusive [43]. Conversely, the present study demonstrated that RSV caused an increase (at a lower dose) in the level of glucose in the rats. There may be the low bioavailability of oral RSV on the tissues. It is not immediately clear how to reconcile these different outcomes, and further work may be needed to determine whether there is a differential mechanism involved.

The current study reported a non-significant decrease of CH, TG, HDL and LDL levels in the RIS-treated group after two weeks. Similar results have also been reported by Mc Evoy et al. (2007) and a cross-sectional study[44]. Mc Evoy et al. (2007)'s study showed a decrease in HDL levels. A cross-sectional study conducted by McCormack & Wiseman (2004) revealed no significant differences in the LDL levels in the RIS-treated individuals[45]. Another cross-sectional study conducted by Henderson et al. (2005) demonstrated no significant differences in the total CH levels attained after the RIS treatment[46]. The reason behind the different propensity of the RIS to cause the derangement of the lipid profile is not properly understood, but it may be due to the different affinities of the drugs attaching to the various receptors. The current study demonstrated a significant decrease of the CH, TG, HDL and LDL levels in the RSV-treatment. Our findings are in accordance with the findings of previous studies. It is reported that treatment with RSV significantly reduced the CH and LDL levels in the patients[18]. Treatment with RSV, 40 mg/kg, resulted in decreased CH and HDL levels compared to those of the control rats. This result is consistent with the findings reported by other works [47,48]. Consistent with former findings, our data strongly suggests that RSV may have promise as a new hypolipidemic compound. RSV can ameliorate these effects, and has a promising neuroprotective effect in RIS-induced cerebral complications.

In the current study, a significant decrease of T3, T4 and an increase of TSH levels in the RIS-treated group after two weeks were reported. A recent naturalistic study demonstrated that the treatment of acute psychotic episodes was associated with a reduction of T4 and T3 serum concentrations accompanied by an increase in TSH serum concentrations [49]. Similarly, other authors have also found that the antipsychotic treatment resulted in a reduction of serum T4 concentrations in acutely psychotic patients. It was hypothesized that antipsychotic medication may alter the thyroid hormone metabolism in the brain by enhancing the activity of the type II deiodinase enzyme in certain brain regions that consequentially leads to a diminished T4 serum concentration [50]. Treatment with the RSV, 40 mg/kg, resulted in decreased TSH and increased T3 levels compared to those of the RIS treated rats. Additionally, treatment with RSV, 20 mg/kg, resulted in an increased T4 level compared to the RIS group. This result is consistent with former findings. Ge et al. demonstrated that RSV treated rats exhibited a reduced plasma TSH level [51]. Böttner et al. (2006) reported that the serum levels of RSV of 1.0 and 8.1 μM resulted in a significant increase in the total T3 serum levels [52]. RSV may also alter T3 binding protein levels such as thransthyretin, and albumin. The increased levels of T3 after the oral treatment with RSV showed that this phytoestrogen may affect the deiodinase activity in yet unidentified organs. In conclusion, the addition of the RSV treatment to RIS is safe, and also associated with an accelerated treatment response as well as greater improvement of the symptom severity.

We measured the TAS, TOS, and OSI levels at the same time in order to more accurately evaluate the oxidative stress status. RSV is known to reduce oxidative stress not only through the direct antioxidant effect, but also indirectly. Previous studies reported that RSV acts as a strong Reactive Oxygen Species (ROS) scavenger. We suggest that RSV may also protect the brain tissues against the action of oxidative stress, which is mediated by the RIS-induced molecular changes. Also, previous in vitro studies showed that RIS had more potent mitochondrial toxicity among the other atypical antipsychotics 53. It is suggested that mitochondrial

ROS formation is the key mediator in RIS induced cytotoxicity. RIS-induced damage causes augmented ROS production 54. In the current study, we found that the serum TAS levels increased and the TOS/OSI levels, prominently decreased in the RSV given groups, as in the previous studies [55]. RIS administration resulted in a decrease in the TAS level and an increase in the TOS/OSI levels.

The RSV co-treatment ameliorated these changes with a more obvious effect in a 20 mg/kg dose. The beneficial effects of RSV obtained in the current work, are very likely due to its strong antioxidant properties, and may be associated with its constituent compounds. This implies that the RSV presented a neuroprotective activity probably due to its antioxidant capacity. The restoration of the tissue antioxidant function by the RSV may be attributed to its ability to upregulate the antioxidant gene expression.

Elevated ROS can act as a trigger for apoptosis. The cytotoxic effects of the RIS were in part mediated by oxidative stress since its action was partially blocked with glutathione; a reductant well established to protect against oxidative stress associated cytoxicity [56]. These changes were attenuated by the treatment with RSV, suggesting that RIS induced apoptosis through oxidative mitochondrial damage. More recently, Elmorsy et al. (2014) showed that some antipsychotics, including RIS, could induce apoptosis by increasing the caspases 3 and 8 levels in microvascular endothelial cells of the human brain, which are similar to the cells found in the blood-brain barrier [57]. The apoptosis effects triggered by the RIS appeared to be cell-type dependent [58]. ROS and amyloid-β peptide can also induce cell death via apoptosis in many cell types. Such an effect was also blocked by RSV [59]. Polyphenols reduce oxidative stress and promote neuronal survival signals in many models (in vitro and in vivo) of neuronal injury and neurodegenerative disease. Accordingly, in our study, the RSV treatment to the cell's exposure to RIS, the apoptotic cell injury, and death were significantly reduced. Therefore, RSV may be the best choice against RIS induced side effects.

We also studied the expressions of the D1R, D4R, 5-HT1a, 5-HT2c, NPY, H1R, and hypothalamic AMPKα genes to determine the effects of the RIS and RSV on the gene expression levels and the receptor(s) likely to be responsible for antipsychotic drug-induced weight gain. SGAs have binding affinities for various neurotransmitter receptors, such as D2R, serotonin 5-HT2a (5-HT2aR) and 5-HT2c receptors (5-HT2cR), and H1R. Lian et al. reported that RIS elevated appetite and body weight gain in juveniles via the regulation of the hypothalamic H1R, NPY and AgRP pathways, as well as by reducing activity [4]. In our study, we did not find different gene expression patterns in the D4R, H1R, 5-HT1a and hypothalamic AMPKα genes; however, while the expression levels of the D1R were increased by the RIS administration, it reduced with the 20 mg/kg and 40 mg/kg RSV treatment. The D1R gene encodes the D1 subtype of the dopamine receptor. The D1 subtype is the most abundant dopamine receptor in the central nervous system. D1 receptors regulate neuronal growth and development, mediate some behavioral responses, and modulate dopamine receptor D2-mediated events[60]. Both the D1 and the D2 receptors are strongly associated with food intake[61]. Atypical antipsychotics are antagonists for both serotonin (5-hydroxytryptamine, 5-HT) and dopamine receptors. It is known that RIS is a dopamine receptor antagonist, and in our study, we identified the RIS's negative effect on the D1R mRNA expression. In contrast, RSV decreased the D1R mRNA expression, and it helps in weight loss. Some reports showed that D1R has a low affinity in Dopamine and how it interacts with the D1R receptor and with the RIS and RSV, and how it helps to reduce weight gain in RIS+RSV groups is unclear [62, 63].

The process of the weight gain in obesity is more complex. Both leptin and NPY have led to a better understanding of the pathophysiology of obesity. Leptin, the obese gene product, is produced by adipocytes and secreted in plasma, which acts on hypothalamus in particular. It inhibits food intake and stimulates energy expenditure and reduces weight. In contrast, NPY, a hypothalamic neurotransmitter, is a potent stimulant of appetite and feeding, which also reduces energy expenditure. The expression and release of hypothalamic NPY are inhibited by leptin. The interaction between the leptin and NPY concentration has been considered an important factor in the regulation of body weight [4,64] reported that the hypothalamic NPY mRNA expressions were elevated by RIS treatment in young female rats, which were correlated with RIS-induced food intake and weight gain. NPY widely expressed in the central and peripheral nervous system and it participates in multiple physiological

and pathological processes through specific receptors [65], one of them is NPY1. In the present study, the NPY1 expression in RIS group was not different from control ($p>0.05$). On the other hand, it decreased with the all RSV groups, but only in 20 mg/kg RSV treatment, the decreased level was found statistically different from control and RIS groups ($p<0.05$). In short, it was seen that the reduced NPY1 expressions in all RSV treated rats led reduced weight. It was reported by Wang et al. (2015) that mechanisms in which RSV exerts anti-obesity effects are not completely understood, but several biological processes such as adipogenesis, lipogenesis, lipolysis, apoptosis, fatty acid oxidation, thermogenesis, and browning of white adipose tissue (WAT) have been implicated in the fat-lowering effects of RSV [66]. NPY1 receptor may be the candidate mechanism in this context.

In particular, it has been reported that 5-HT_{2C} is associated with weight gain and its absence leads to an excessive food intake and results in obesity [67]. RIS has a higher affinity towards the 5-HT_{2C} receptors. Prolonged treatment with olanzapine and possibly RIS decreased the labeling of the 5-HT_{2C} receptors in the hippocampus, and as a result weight gain eventually takes place [68]. It was reported that the mice deficient in 5-HT_{2C} develop hyperphagia leading to obesity, and diets rich in fat have been associated with the decreased *HTR2c* mRNA expression in rats [69]. In a study performed by Xu et al. (2010), it was shown that trans-RSV produced a significant increase in serotonin and noradrenaline levels at 40 or 80 mg/kg in the brain regions [29]. Also, they indicated that the antidepressant-like effect of trans-RSV might be related to serotonergic and noradrenergic activation. The molecular mechanisms of the RSV on the beneficial health effects are unknown because it is an ubiquitous activator of several intracellular pathways. In opposition to other studies, we found that the 5-HT_{2c} expression level decreased gradually in RSV groups and in the 80 mg/kg RSV treatment, there was a statistically different level of decrease from the control. According to our study, RSV was probably treated by acting as an agonist of 5-HT_{2c} or affects the pathways in which the 5-HT_{2c} is involved, and causes weight loss. Therefore, further studies are necessary to investigate the mechanisms underlying RIS-induced weight gain and RSV-induced weight loss.

In conclusion, antipsychotics such as RIS show a much greater risk of obesity and other metabolic disorders in adulthood, which pose as a major risk for cardiovascular diseases, cancer, toxicity and mortality in adults. RSV may have a promising agent for improving the negative effects of RIS, oxidative stress, apoptotic status, and so reduce weight gain. Thus, a daily consumption of RSV should be considered as a promising way to prevent weight gain and other adverse effects of RIS. Therefore, further in vivo and clinical studies showing the expression of different genes involved in carbohydrate and fat metabolisms should be performed for developing strategies to ameliorate RIS-induced weight gain and to reveal its underlying mechanisms. In this study, we had some limitation for all rats who might not have the same level of diet and exercise, which may have affected the results.

Acknowledgements

This work was funded by a grant from the University of Adıyaman, Turkey (SHMYOMAP/2015-0001).

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