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Title: The Protective Effect of Resveratrol Against Risperidone-induced Liver Damage Through an Action on FAS Gene Expression
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Abstract
The purpose of the study is to examine the protective effect of resveratrol (RSV) on the fatty acid synthase (FAS) gene expression against the side-effects of risperidone (RIS) in an experimental model in rat liver. In this study, thirty-five female Spraque-Dawley rats were divided into five groups (n=7): control, RIS (2 mg/kg), RIS+RSV-1 (20 mg/kg), RIS+RSV-2 (40 mg/kg), and RIS+RSV-3 (80 mg/kg) for 14 days. On treatment day 15, liver tissue was taken for analysis. The RSV treatment significantly reduced weight gain as opposed to the RIS administration. Moreover, the FAS gene expression level increased significantly with RSV-1 treatment ($p=0.011$). In addition, RSV enhanced the total antioxidant status (TAS), high-density lipoprotein cholesterol (HDL) levels and decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TCH), gamma glutamyl transpeptidase (GGT), low density lipoprotein cholesterol (LDL), oxidative stress index (OSI), triglycerides (TG), and total oxidant status (TOS) levels significantly ($p<0.05$). In conclusion, this study revealed that treatment with RSV might protect liver tissue against the side-effects of RIS over FAS gene expression. RSV could be an effective course of therapy for enhancing therapeutic efficacy.

Keywords: Risperidone; Resveratrol; FAS; Liver; Apoptosis

Changelog
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ABSTRACT

The purpose of the study is to examine the protective effect of resveratrol (RSV) on the fatty acid synthase (FAS) gene expression against the side-effects of risperidone (RIS) in an experimental model in rat liver. In this study, thirty-five female Spraque-Dawley rats were divided into five groups (n=7): control, RIS (2 mg/kg), RIS+RSV-1 (20 mg/kg), RIS+RSV-2 (40 mg/kg), and RIS+RSV-3 (80 mg/kg) for 14 days. On treatment day 15, liver tissue was taken for analysis. The RSV treatment significantly reduced weight gain as opposed to the RIS administration. Moreover, the FAS gene expression level increased significantly with RSV-1 treatment ($p=0.011$). In addition, RSV enhanced the total antioxidant status (TAS), high-density lipoprotein cholesterol (HDL) levels and decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TCH), gamma glutamyl transpeptidase (GGT), low density lipoprotein cholesterol (LDL), oxidative stress index (OSI), triglycerides (TG), and total oxidant status (TOS) levels significantly ($p<0.05$). In conclusion, this study revealed that treatment with RSV might protect liver tissue against the side-effects of RIS over FAS gene expression. RSV could be an effective course of therapy for enhancing therapeutic efficacy.

Keywords: risperidone, resveratrol, FAS, liver, apoptosis

Introduction

Atypical antipsychotics (AAPs) have been used in the treatment of schizophrenia. RIS is an AAPs prescribed for the treatment of bipolar disorder, schizophrenia, depression, and autism (Keck et al., 2000). On the other hand, AAPs are associated with metabolic syndrome (including weight gain, dyslipidemia, hyperglycemia, type II diabetes mellitus, insulin resistance) and cardiovascular disease (Bou-Khalil, 2012). However, the use of RIS has been restricted due to systemic side effects. Furthermore, RIS is the second most prescribed antipsychotic drug and causes significant changes in the metabolic parameters and weight gain in patients (Rummel-Kluge et al., 2010). The latest studies have shown that these drugs can change glucose and lipid metabolism unrelated of any effect on neurotransmitter receptors on expression on the periphery. CH and fatty acid biosynthesis transcriptionally activate by antipsychotic drugs in cultured human glioma cells, including FAS, HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), HMGCS1 (3-hydroxy3-methylglutaryl-coenzyme A synthase-1), and SREBP (Sterol regulatory element binding proteins) (Ferno et al., 2005).

FAS is a multifunctional protein enzyme encoded by the FASN gene that chiefly catalyzes fatty acids and regulates lipid metabolism (Wakil, 1989). The highest expression of FAS has been reported in hepatic tissues. Therefore, fatty acid production pathway in the liver tissue facilitates surplus energy storage and circulating TG rich lipoproteins (Jensen-Urstad and Semenkovich, 2012). The liver performs a considerable role in energy intake and the regulation of lipid metabolism. It has been suggested that antipsychotic drug-related lipogenic effects have metabolic side effects in the liver (Lauressergues et al., 2010). On the other hand, FAS is organized nutritionally and hormonally (Sul and Wang, 1998) to contribute to weight gain and the development of obesity (Mobbs and Makimura, 2002). More recent studies have demonstrated that RIS significantly increases expression of the FAS gene in rat hepatocyte cultures (Lauressergues et al., 2011).

Nowadays, medicinal plants are a major source of drug. The extensive use of herbal compounds has encouraged scientists to investigate therapeutic properties on health. RSV is a natural phytoalexin that exists in many different plants, especially in grapes (Pal et al., 2003). Phytoalexins are secondary constituents against UV rays and damage and infections in plants (Ozelci et al., 2007). RSV has antioxidant activity that prevents DNA damage and lipid peroxidation in the cell membrane. RSV has been indicated to have broad spectrum benefits on human health on, for example the hepatic, nervous, coronary, and cardiovascular systems (Martin et al., 2004). In addition, RSV is a natural compound and has been shown to exert protective effects on the liver preventing lipid accumulation. Because of the high effect and low toxicity of RSV upon human health, it is a hopeful alternative to traditional therapeutic drugs.

To our knowledge, there is no report regarding the protective and therapeutic effects of RSV against the effect of RIS over FAS gene expression. Thus, the objective of our work was to research the possible useful effect of oral supplementation with RSV against the effect of RIS over FAS gene expression. To reach our target, we investigated genetic, biochemical, and histological analyses on rats.

Materials and methods

Chemicals

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

Animals

Thirty-five female Sprague Dawley rats (12-16-week-old) initially weighing 220-260 g were used in our study. These rats were acquired from the Experimental Research Center of Firat University. The rats kept under standard conditions: 12:12 h light, dark-cycles. Food and tap water were provided ad libitum. The care and follow-up of the rats was done in this center. All procedures and protocols were conducted in accordance with the Ethical Committee of the Firat University Faculty of Medicine (Protocol # 2016/41).

Experimental design

All rats were randomly reserved into five groups (seven per group) as follows: control group (saline solution), RIS group (2 mg/kg RIS), RIS+RSV-1 group (2 mg/kg RIS and 20 mg/kg RSV), RIS+RSV-2 group (2 mg/kg RIS and 40 mg/kg RSV), and RIS+RSV-3 group (2 mg/kg RIS and 80 mg/kg RSV). The doses of RIS (2 mg/kg once a day for two weeks) and the doses of RSV (20, 40, and 80 mg/kg body weight/day for two weeks) were administered by gastric tube each day between 8:00 and 9:00. The doses of RIS (Zhang et al., 2007) and RSV (Zhao et al., 2014) were selected on the basis of previous study results.

Weights were recorded at the beginning and the end of the study. The rats' venous blood samples were collected. The animals were euthanized by exsanguination with diethyl ether anesthesia on the last day of the second week. The entire liver was excised and kept at -86 °C till analysis.

Biochemical Analysis

Blood samples were collected to determine liver enzyme activity, and serum samples were separated by centrifuge at 2800 g for 15 min; then, the samples were divided in Eppendorf tubes, and stored at -86 °C till biochemical analysis.

One of the samples was used for measuring serum levels of TCH (mg/dL), HDL (mg/dL), and TG (mg/dL) using routine enzymatic methods with an Olympus 2700 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). LDL (mg/dL) levels were calculated using Friedewald's formula. Standard liver function tests known as markers of liver injury, ALT (U/L), AST (U/L),and GGT (U/L) were measured using an autoanalyzer.

Another of the samples were used for measuring TAS, TOS, and OSI levels spectrophotometrically using the Erel method. Serum TAS and TOS levels were measured with kits (REL Assay Diagnostics, Gaziantep, Turkey). OSI value was calculated using the formula OSI=TOS/TAS (Erel, 2004; Erel, 2005; Harma et al., 2005).

Real-time PCR analysis

Rat livers were taken and divided. One of the samples of livers were stored in formaldehyde for TUNEL staining, and another of the samples of livers were stored at -86 °C until further analysis. Thirty mg of frozen liver tissues were homogenized in 500 µl Tissue Lizis Buffer for 1 min using homogenizer (Bioprep-24, Allsheng). Total RNA was obtained from liver samples using an ExiPrepTM Tissue Total RNA isolation kit (Bioneer, K-3325). The RNA concentration was determined from absorbance at 230-260 nm and 260/280 nm using a NanoDrop spectrophotometer (Denovix DS-11). The results were then reversely transcribed into cDNA using the AccuPower® RT PreMix (Bioneer, K-2041) according to the manufacturer's instructions.

Real-Time PCR was performed using AccuPower GreenStar qPCR PreMix according to the manufacturer's instructions (Bioneer, Cat No: K-6210). The level of mRNA expression of FAS

genes as detected using the ExiCyclerTM96 Real-Time Quantitative PCR system (Bioneer). The PCR reactions were performed as follows: 95 °C for 5 min, followed by 45 cycles at 95 °C for 15 sec, and then 60 °C for 25 second. The sequences primers used were: Forward, 5'-AGGTGCTAGAGGCCCTGCTA-3'; Reverse, 5'-GTGCACAGACACCTTCCCAT-3' (Bioneer, S-1001) (Ji et al., 2011; Fukunishi et al., 2014). The levels of each gene expression were calculated by the 2-ΔΔCt method.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

TUNEL staining was designed for the detection of apoptotic cells in liver tissue samples. The sections taken from the paraffin blocks at a thickness of 5 μm were taken into the polylysine lamella. Apoptotic cells were identified using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) according to the manufacturer's protocol. Preparations were analyzed and photographed by a research microscope (Leica DM500). In the evaluation of the TUNEL staining, Harris hematoxylin-stained nuclei were normalized, and cells demonstrating brown staining were evaluated as apoptotic. In ten randomly selected areas, the sections were analyzed at 400× magnification (Tas et al., 2015), and at least 500 normal and apoptotic cells were counted. The apoptotic index (AI) was calculated by the ratio of apoptotic cells to total (normal + apoptotic) cells. The degree of TUNEL staining was scored semiquantitatively as 0 (none), 1 (light), 2 (medium), and 3 (intense) (Can et al., 2015).

Statistical analysis

Statistical analyses were performed using Statistical Package 16.0 (SPSS, Chicago, IL, USA). The experimental data were expressed as mean ± standard error of mean (SEM). The Shapiro–Wilk test was used to determine the normality of variables in the groups. For the comparison of the mean weight of all groups, a paired T-test was performed. The groups were compared with the paired-samples T-test at the beginning and the end of the treatment. Two-way ANOVA was used to test the effect of RIS (control vs. RIS) and treatment (untreated vs. treated with RSV) as well as their interaction. The histopathological analysis was expressed as the means ± standard deviation (SD). The Mann-Whitney U test and the student's t test were used for statistical analysis. The significance was acceptable to a level of $p \leq 0.05$.

Results

Effects of RIS and RSV on weight gain/loss

Body weight measurements showed that, during the 14 days, weights increased from 238.28 g to 252.85 g for the control group, weights increased from 234.57 g to 248.00 g for the RIS group, weights increased from 225.28 g to 233.71 g for the RIS+RSV-1 group, weights decreased from 232.40 g to 226.80 g for the RIS+RSV-2 group, and weights increased from 244.80 g to 246.80 g for the RIS+RSV-3 group (Table 1: paired-samples T-test for the body weight at day 14, $p=0.000$, $p=0.005$, $p=0.005$, $p=0.071$, and $p=0.537$, respectively). Overall, in the control, RIS, and RIS+RSV-1 treatment groups ($p<0.05$) weight gain was statistically significant. On the other hand, the fact that the RIS+RSV-2 group was observed to have a weight loss and the RIS+RSV-3 group a weight gain had no significant effect on these measurements ($p>0.05$) (Table 1, Figure 1).

Effects of RIS and RSV on biochemical and oxidative stress parameters

We measured levels of biochemical parameters in the serum, and the results are shown in Table 2. ALT, AST, GGT, LDL, TG, and CH levels significantly increased in the RIS group compared to the control, RIS+RSV-1, RIS+RSV-2, and RIS+RSV-3 groups while the HDL level decreased ($p<0.01$). ALT, GGT, TG, and CH levels were significantly lower in the RIS+RSV-2 group compared to the RIS+RSV-1 group ($p<0.01$). ALT, AST, GGT, and LDL levels were significantly lower in the RIS+RSV-3 group compared to the RIS+RSV-1 group while the HDL level increased ($p<0.02$). The LDL level was significantly lower in the RIS+RSV-3 group compared to the RIS+RSV-2 group ($p<0.03$). LDL, TG, and CH levels were significantly lower in the RIS+RSV-2 group compared to

the control group ($p<0.02$). ALT, AST, LDL, TG, and CH levels were significantly lower in the RIS+RSV-3 group compared to the control group while the HDL level increased ($p<0.04$) (Table 2, Figures 2 and 3).
Treatment with RSV against RIS administration while increased the TAS level, decreased TOS and OSI levels ($p<0.05$). The TAS level was significantly increased in control group when compared to the RIS group ($p=0.024$). The TAS level was significantly increased in RIS+RSV-1 group when compared to the RIS, RIS+RSV-2, and RIS+RSV-3 groups ($p<0.04$). Also, the TAS level was significantly higher RIS+RSV-2 group when compared to the RIS+RSV-3 group ($p=0.019$). Conversely, the TOS level was significantly increased in RIS group when compared to the control, RIS+RSV-1, RIS+RSV-2, and RIS+RSV-3 groups ($p<0.001$). The TOS level was significantly increased in RIS+RSV-1 group when compared to the RIS+RSV-3 group ($p=0.006$). The OSI level was significantly higher in the RIS group when compared to the control, RIS+RSV-1, and RIS+RSV-2 groups ($p<0.05$) (Table 2, Figure 4).

Effect of RIS and RSV on expression of the FAS gene
Table 3 shows the effects of RSV treatment against the RIS administration on the mRNA expression of FAS gene level in all study groups and control. FAS gene expression significantly increased in RIS group compared to the control group. The RIS+RSV-1 group had a significantly lower expression of FAS gene level compared to the RIS group ($p\leq0.01$) (Table 3, Figure 5).

Effect of RIS and RSV on apoptosis in rat liver
The results of the apoptotic index are demonstrated in Table 4, Figure 6. Using TUNEL for the detection of apoptotic cells in the liver sections, the control (Figure 6A) group showed only a few TUNEL-positive cells. The count of TUNEL-positive cells significantly increased in the RIS (Figure 6B) group compared with that in control group ($p<0.05$). The RIS+RSV-1 (Figure 6C), RIS+RSV-2 (Figure 6D), and RIS+RSV-3 (Figure 6E) groups were similar and showed rare TUNEL-positive cells. Treatment with RSV (RIS+RSV-1, RIS+RSV-2, and RIS+RSV-3 groups) (Figure 6C, 6D, and 6E) reduced the count of TUNEL-positive cells compared to the RIS group ($p<0.05$).

Discussion
AAPs are used to treat serious mental disorders. Though they have many beneficial effects, they also have many serious side effects (Eder et al., 2001). RIS is one of the AAPs that has led to weight gain and obesity side-effects, and other metabolic disorders in patients (Yoon et al., 2016). Therefore, it is extremely important to prevent side effects and other metabolic disorders induced by RIS. Many authors have suggested a co-treatment between RIS and compounds that regulate its metabolic adverse effects. Through antioxidant and radical scavenger properties of natural compounds, may prevent and treat diseases. Dietary intake of natural compounds, including RSV, can inhibit the metabolic side effects of RIS and thereby may reduce the risk factors in the liver (Walton et al., 1999). Hence, the purpose of the current study was to investigate the protective and therapeutic effects of RSV against the effect of RIS over FAS gene expression and RIS-induced liver damage.
The liver is responsible for many vital life functions and is involved in uptake, secretion, synthesis, catabolism and storage. Fatty acids increase in the liver by hepatocellular uptake from the plasma and by de novo biosynthesis. Hepatic FAS is the synthesizing of fatty acids for the partitioning and storage of excess energy (Jensen-Urstad and Semenkovich, 2012). According to clinical experiences, an accumulation of extreme intracellular triglycerides often comes before the improvement of obesity (Riediger and Clara, 2011). This study shows that RIS significantly increases the expression of the FAS gene, and there are highly meaningful correlations between the expression of this gene and the final body weight of animals. This effect of RIS was formerly presented in different experimental models of the liver (Lauressergues et al., 2011, Cope et al., 2005). In addition, high triglycerides observed in rats subjected to RIS are a result of elevated

hepatic FAS expression. Similarly, previous studies reported that rodent models with high triglyceride levels are related to increased hepatic FAS expression (Morgan et al., 2008). In this study, we conclude that the increase in observed body weight can be partially elevated levels of circulating and stored triglycerides. Taken together, RIS exposure can cause long-term hypertriglyceridemia due to the FAS-dependent pathway to the synthesis of de novo triglycerides. Thus, RIS-induced weight gain could be the result of the effect of RIS associated alterations on the central nervous system, including on body temperature, on food intake, on locomotor activity.
RSV, a natural compound in superfoods like wine, and has a beneficial effect on glucose and lipid metabolism. In fact, many clinical trials have recently demonstrated that using animal models of diet-induced obesity has displayed the beneficial effects of RSV on reducing obesity and oxidative stress (G´omez-Zorita et al., 2012; Farag et al., 2017). In addition, RSV performs a considerable role in lipid metabolism. In the current study, RSV co-treatment decreased antipsychotic-induced weight gain significantly with only a 20 mg/kg dose. Also, RSV attenuated hepatic triacylglycerol and fatty acid synthesis in rats. This data suggest that the RSV had protective effects against the adverse effects of RIS and decreased the risk of obesity. These results imply that the mechanism of effect of RSV occurs by increasing energy consumption, inhibition of energy intake, and reducing energy storage. This weight-decreasing effect of RSV is estimated to be attributable, in part, to its effects on adipocytes and expression of the FAS gene (Baur et al., 2006, Naderali, 2009). Therefore RSV is a reliable compound for co-administration with RIS for decrease of antipsychotic-induced weight gain and obesity without effecting its therapeutic action.
In the present study, RIS exposure produced a significant increase in the activity of liver enzymes. ALT, AST, and GGT indicate a damaged functional and structural hepatic integrity. Oral supplementation of RSV reduces liver injury and improve the elevated serum ALT, AST and GGT activities. While RSV co-treatment curable these changes in all doses, it had the most obvious effect in high doses. Our study results are confirmed by data from the literature (Miguel et al., 2016). In addition, we demonstrated that RSV prevented the increase in TG, TCH, and LDL as well as a decrease in HDL caused by RIS consumption. All doses of RSV caused dose-dependent decreases in serum lipids compared to RIS administrated rats. However, RSV co-treatment curable these changes with more obvious effect and but with a major decrease in the 80 mg/kg dose. The effect of RSV on serum lipids has been reported in earlier experiments (Panico et al., 2017). This finding is probably a consequence of feeding behavior and the increase in body weight. Although underlying physiological pathways are not fully understood, the present findings indicate that RIS increases and RSV decreases serum lipids.
In this study, RSV significantly affected the RIS load on the liver, enhanced the reduced TAS, inhibited the elevated TOS and OSI levels, healed impaired hepatic function, and reformed the histopathological changes in the liver. RIS-mediated ROS formation by diminished antioxidant levels and oxidative stress and antioxidant depletion can lead to apoptotic cell death (Armstrong and Jones, 2002). In this study, we found that RSV had a significant protective role in apoptotic cell death, which might be due to the ROS scavenging property. Taking the previous findings and suggestions together, it can be concluded that RSV could prevent RIS-induced liver injury and histological perturbations through the enhancement of antioxidant defense systems, suppression of oxidative stress, and attenuation of apoptosis. Oxidative stress has a vital role in the chain of initiation and progression of liver diseases. In this study, in RIS administration rats, a reduction in TAS level was observed resulting in a rise in TOS and OSI levels as in previous studies (Li et al., 2015). On the other hand, we observed that RSV protected against RIS-induced liver damage by suppressing oxidative stress and apoptosis. In addition, our results demonstrated that TAS levels increased and TOS, OSI levels conspicuously reduced with RSV treatment as reported in prior studies (Faghihzadeh et al., 2015). Additionally, the level of antioxidant TAS significantly elevated with 20 mg/kg doses by RSV co-operation. Several studies have demonstrated that the hepatoprotective effect of RSV against liver damage is mediated by its antioxidant and anti-inflammatory properties (Bishayee et al., 2010). A few recent studies have shown that RSV administered to mice in their diet significantly reduced lipids and depressed the expression of genes

related to hepatic lipid metabolism (Ahn et al., 2008).
Histopathological findings support above oxidative results. The TUNEL assay used for determine apoptotic cells in the liver sections. Histopathological assessment of the liver showed serious damage follow by detrimental effects on the normal structure of the liver in RIS administrated rats including vacuolar degeneration of hepatocytes and fatty changes. RIS-induced toxic effects were prevented through the powerful antioxidant capacity and other biological effects of RSV. Among the three doses, 80 mg of RSV/kg body weight was found to provide optimum protective effect on the liver against RIS induced abnormal changes. Histological observations added more evidence supporting the protective effect of RSV. The present study demonstrated that RIS damaged the histological structure and function and inhibited the endogenous antioxidant defense system in rat liver tissue as reported in previous studies (Radzik et al., 2005). In addition, our results showed, at the first time, that RSV oral supplementation, at safe dose levels, has a noteworthy protective effect against RIS-induced liver damage in rats. This protection makes RSV a promising agent in a variety of conditions in which cellular damage occurs as a result of oxidative stress. RIS-induced liver injury causes increased ROS formation and subsequent toxic events. Accordingly, in our study, with RSV treatment of the cells against RIS exposure, the apoptotic cell injury and death were greatly reduced. The underlying mechanism of the protective quality of RSV may be associated with the suppression of apoptosis via death receptor-mediated pathways. Therefore, previous studies show that antioxidant activity of RSV can be possible because of the effect on mitochondria-independent apoptotic pathways. Hence, RSV may be the best choice against RIS induced side effects.
In conclusion, RSV may be a promising agent to mitigate the adverse effects of RIS, oxidative stress, and apoptotic status and to reduce weight gain and the expression of the FAS gene and so prevent liver damage in patients. Thus, daily consumption of RSV should be considered as a promising way to prevent liver damage. Our results could be used to plan strategies to protect against the adverse effects of RIS in the liver and in other organs. Hence, further in vivo and clinical studies are required to confirm the protective effects of RSV in patients receiving RIS.
Disclosure statement
No potential conflicts of interest were reported.

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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. £,$,† 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.

Figure 2. Effects of risperidone, resveratrol, and their coadministration on the liver level of serum ALT, AST and GGT in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. 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. Abbreviations: RIS: risperidone; RSV: resveratrol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; 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 risperidone, resveratrol, and their coadministration on the liver level of serum HDL, LDL, TG and CH in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. 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. Abbreviations: RIS: risperidone; RSV: resveratrol; HDL: high-density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TG: triglycerides; TC: cholesterol. 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 4. Effects of risperidone, resveratrol, and their coadministration on the level of TAS, TOS and OSI in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. 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. Abbreviations: RIS: risperidone; RSV: resveratrol; TAS: total antioxidant

status; TOS: total oxidant status; OSI: Oxidative stress index; 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; AU: Arbutrary Units.

Figure 5. Effects of RIS and RSV on the expression of FAS gene in rat liver. Data are means ± SEM (n = 7). Different letters over the bars represent significant differences, $p<0.05$.

Figure Legends

Figure 6. Representative photomicrographs of TUNEL staining in all five groups (scale bars=100 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 liver section (two sections/animal), and the sections were analyzed at 400× 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 (no), 1 (light), 2 (medium), and 3 (intense).

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The Protective Effect of Resveratrol Against Risperidone-induced Liver Damage Through an Action on FAS Gene Expression

ABSTRACT

The purpose of the study is to examine the protective effect of resveratrol (RSV) on the fatty acid synthase (FAS) gene expression against the side-effects of risperidone (RIS) in an experimental model in rat liver. In this study, thirty-five female Spraque-Dawley rats were divided into five groups (n=7): control, RIS (2 mg/kg), RIS+RSV-1 (20 mg/kg), RIS+RSV-2 (40 mg/kg), and RIS+RSV-3 (80 mg/kg) for 14 days. On treatment day 15, liver tissue was taken for analysis. The RSV treatment significantly reduced weight gain as opposed to the RIS administration. Moreover, the FAS gene expression level increased significantly with RSV-1 treatment ($p=0.011$). In addition, RSV enhanced the total antioxidant status (TAS), high-density lipoprotein cholesterol (HDL) levels and decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TCH), gamma glutamyl transpeptidase (GGT), low density lipoprotein cholesterol (LDL), oxidative stress index (OSI), triglycerides (TG), and total oxidant status (TOS) levels significantly ($p<0.05$). In conclusion, this study revealed that treatment with RSV might protect liver tissue against the side-effects of RIS over FAS gene expression. RSV could be an effective course of therapy for enhancing therapeutic efficacy.

Keywords: risperidone, resveratrol, FAS, liver, apoptosis

Introduction

Atypical antipsychotics (AAPs) have been used in the treatment of schizophrenia. RIS is an AAPs prescribed for the treatment of bipolar disorder, schizophrenia, depression, and autism (Keck et al., 2000). On the other hand, AAPs are associated with metabolic syndrome (including weight gain, dyslipidemia, hyperglycemia, type II diabetes mellitus, insulin resistance) and cardiovascular disease (Bou-Khalil, 2012). However, the use of RIS has been restricted due to systemic side effects. Furthermore, RIS is the second most prescribed antipsychotic drug and causes significant changes in the metabolic parameters and weight gain in patients (Rummel-Kluge et al., 2010).

The latest studies have shown that these drugs can change glucose and lipid metabolism unrelated of any effect on neurotransmitter receptors on expression on the

periphery. CH and fatty acid biosynthesis transcriptionally activate by antipsychotic drugs in cultured human glioma cells, including FAS, HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), HMGCS1 (3-hydroxy3-methylglutaryl-coenzyme A synthase-1), and SREBP (Sterol regulatory element binding proteins) (Ferno et al., 2005).

FAS is a multifunctional protein enzyme encoded by the FASN gene that chiefly catalyzes fatty acids and regulates lipid metabolism (Wakil, 1989). The highest expression of FAS has been reported in hepatic tissues. Therefore, fatty acid production pathway in the liver tissue facilitates surplus energy storage and circulating TG rich lipoproteins (Jensen-Urstad and Semenkovich, 2012). The liver performs a considerable role in energy intake and the regulation of lipid metabolism. It has been suggested that antipsychotic drug-related lipogenic effects have metabolic side effects in the liver (Lauressergues et al., 2010). On the other hand, FAS is organized nutritionally and hormonally (Sul and Wang, 1998) to contribute to weight gain and the development of obesity (Mobbs and Makimura, 2002). More recent studies have demonstrated that RIS significantly increases expression of the FAS gene in rat hepatocyte cultures (Lauressergues et al., 2011).

Nowadays, medicinal plants are a major source of drug. The extensive use of herbal compounds has encouraged scientists to investigate therapeutic properties on health. RSV is a natural phytoalexin that exists in many different plants, especially in grapes (Pal et al., 2003). Phytoalexins are secondary constituents against UV rays and damage and infections in plants (Ozelci et al., 2007). RSV has antioxidant activity that prevents DNA damage and lipid peroxidation in the cell membrane. RSV has been indicated to have broad spectrum benefits on human health on, for example the hepatic, nervous, coronary, and cardiovascular systems (Martin et al., 2004). In addition, RSV is a natural compound and has been shown to exert protective effects on the liver preventing lipid accumulation. Because of the high effect and low toxicity of RSV upon human health, it is a hopeful alternative to traditional therapeutic drugs.

To our knowledge, there is no report regarding the protective and therapeutic effects of RSV against the effect of RIS over FAS gene expression. Thus, the objective of our work was to research the possible useful effect of oral supplementation with RSV against the effect of RIS over FAS gene expression. To reach our target, we investigated genetic, biochemical, and histological analyses on rats.

Materials and methods

Chemicals

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

Animals

Thirty-five female Sprague Dawley rats (12-16-week-old) initially weighing 220-260 g were used in our study. These rats were acquired from the Experimental Research Center of Firat University. The rats kept under standard conditions: 12:12 h light, dark-cycles. Food and tap water were provided ad libitum. The care and follow-up of the rats was done in this center. All procedures and protocols were conducted in accordance with the Ethical Committee of the Firat University Faculty of Medicine (Protocol # 2016/41).

Experimental design

All rats were randomly reserved into five groups (seven per group) as follows: control group (saline solution), RIS group (2 mg/kg RIS), RIS+RSV-1 group (2 mg/kg RIS and 20 mg/kg RSV), RIS+RSV-2 group (2 mg/kg RIS and 40 mg/kg RSV), and RIS+RSV-3 group (2 mg/kg RIS and 80 mg/kg RSV). The doses of RIS (2 mg/kg once a day for two weeks) and the doses of RSV (20, 40, and 80 mg/kg body weight/day for two weeks) were administered by gastric tube each day between 8:00 and 9:00. The doses of RIS (Zhang et al., 2007) and RSV (Zhao et al., 2014) were selected on the basis of previous study results.

Weights were recorded at the beginning and the end of the study. The rats' venous blood samples were collected. The animals were euthanized by exsanguination with diethyl ether anesthesia on the last day of the second week. The entire liver was excised and kept at -86 °C till analysis.

Biochemical Analysis

Blood samples were collected to determine liver enzyme activity, and serum samples were separated by centrifuge at 2800 *g* for 15 min; then, the samples were divided in Eppendorf tubes, and stored at -86 °C till biochemical analysis.

One of the samples was used for measuring serum levels of TCH (mg/dL), HDL (mg/dL), and TG (mg/dL) using routine enzymatic methods with an Olympus 2700 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). LDL (mg/dL) levels were calculated using Friedewald's formula. Standard liver function tests known as markers of liver injury, ALT (U/L), AST (U/L),and GGT (U/L) were measured using an autoanalyzer.

Another of the samples were used for measuring TAS, TOS, and OSI levels spectrophotometrically using the Erel method. Serum TAS and TOS levels were measured with kits (REL Assay Diagnostics, Gaziantep, Turkey). OSI value was calculated using the formula OSI=TOS/TAS (Erel, 2004; Erel, 2005; Harma et al., 2005).

Real-time PCR analysis

Rat livers were taken and divided. One of the samples of livers were stored in formaldehyde for TUNEL staining, and another of the samples of livers were stored at -86 °C until further analysis. Thirty mg of frozen liver tissues were homogenized in 500 µl Tissue Lizis Buffer for 1 min using homogenizer (Bioprep-24, Allsheng). Total RNA was obtained from liver samples using an ExiPrepTM Tissue Total RNA isolation kit (Bioneer, K-3325). The RNA concentration was determined from absorbance at 230-260 nm and 260/280 nm using a NanoDrop spectrophotometer (Denovix DS-11). The results were then reversely transcribed into cDNA using the AccuPower® RT PreMix (Bioneer, K-2041) according to the manufacturer's instructions.

Real-Time PCR was performed using AccuPower GreenStar qPCR PreMix according to the manufacturer's instructions (Bioneer, Cat No: K-6210). The level of mRNA expression of FAS genes as detected using the ExiCyclerTM96 Real-Time Quantitative PCR system (Bioneer). The PCR reactions were performed as follows: 95 °C for 5 min, followed by 45 cycles at 95 °C for 15 sec, and then 60 °C for 25 second. The sequences primers used were: Forward, 5'- AGGTGCTAGAGGCCCTGCTA-3'; Reverse, 5'-GTGCACAGACACCTTCCCAT-3' (Bioneer, S-1001) (Ji et al., 2011; Fukunishi et al., 2014). The levels of each gene expression were calculated by the $2^{-\Delta\Delta Ct}$ method.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

TUNEL staining was designed for the detection of apoptotic cells in liver tissue samples. The sections taken from the paraffin blocks at a thickness of 5 µm were taken into the polylysine lamella. Apoptotic cells were identified using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) according to the manufacturer's protocol.

Preparations were analyzed and photographed by a research microscope (Leica DM500). In the evaluation of the TUNEL staining, Harris hematoxylin-stained nuclei were normalized, and cells demonstrating brown staining were evaluated as apoptotic. In ten randomly selected areas, the sections were analyzed at 400× magnification (Tas et al., 2015), and at least 500 normal and apoptotic cells were counted. The apoptotic index (AI) was

calculated by the ratio of apoptotic cells to total (normal + apoptotic) cells. The degree of TUNEL staining was scored semiquantitatively as 0 (none), 1 (light), 2 (medium), and 3 (intense) (Can et al., 2015).

Statistical analysis

Statistical analyses were performed using Statistical Package 16.0 (SPSS, Chicago, IL, USA). The experimental data were expressed as mean ± standard error of mean (SEM). The Shapiro–Wilk test was used to determine the normality of variables in the groups. For the comparison of the mean weight of all groups, a paired T-test was performed. The groups were compared with the paired-samples T-test at the beginning and the end of the treatment. Two-way ANOVA was used to test the effect of RIS (control vs. RIS) and treatment (untreated vs. treated with RSV) as well as their interaction. The histopathological analysis was expressed as the means ± standard deviation (SD). The Mann-Whitney *U* test and the student's t test were used for statistical analysis. The significance was acceptable to a level of $p \leq 0.05$.

Results

Effects of RIS and RSV on weight gain/loss

Body weight measurements showed that, during the 14 days, weights increased from 238.28 g to 252.85 g for the control group, weights increased from 234.57 g to 248.00 g for the RIS group, weights increased from 225.28 g to 233.71 g for the RIS+RSV-1 group, weights decreased from 232.40 g to 226.80 g for the RIS+RSV-2 group, and weights increased from 244.80 g to 246.80 g for the RIS+RSV-3 group (Table 1: paired-samples T-test for the body weight at day 14, p=0.000, p=0.005, p=0.005, p=0.071, and p=0.537, respectively). Overall, in the control, RIS, and RIS+RSV-1 treatment groups ($p<0.05$) weight gain was statistically significant. On the other hand, the fact that the RIS+RSV-2 group was observed to have a weight loss and the RIS+RSV-3 group a weight gain had no significant effect on these measurements ($p>0.05$) (Table 1, Figure 1).

Effects of RIS and RSV on biochemical and oxidative stress parameters

We measured levels of biochemical parameters in the serum, and the results are shown in Table 2. ALT, AST, GGT, LDL, TG, and CH levels significantly increased in the RIS group compared to the control, RIS+RSV-1, RIS+RSV-2, and RIS+RSV-3 groups while the HDL level decreased ($p<0.01$). ALT, GGT, TG, and CH levels were significantly lower in the

RIS+RSV-2 group compared to the RIS+RSV-1 group ($p<0.01$). ALT, AST, GGT, and LDL levels were significantly lower in the RIS+RSV-3 group compared to the RIS+RSV-1 group while the HDL level increased ($p<0.02$). The LDL level was significantly lower in the RIS+RSV-3 group compared to the RIS+RSV-2 group ($p<0.03$). LDL, TG, and CH levels were significantly lower in the RIS+RSV-2 group compared to the control group ($p<0.02$). ALT, AST, LDL, TG, and CH levels were significantly lower in the RIS+RSV-3 group compared to the control group while the HDL level increased ($p<0.04$) (Table 2, Figures 2 and 3).

Treatment with RSV against RIS administration while increased the TAS level, decreased TOS and OSI levels ($p<0.05$). The TAS level was significantly increased in control group when compared to the RIS group ($p=0.024$). The TAS level was significantly increased in RIS+RSV-1 group when compared to the RIS, RIS+RSV-2, and RIS+RSV-3 groups ($p<0.04$). Also, the TAS level was significantly higher RIS+RSV-2 group when compared to the RIS+RSV-3 group ($p=0.019$). Conversely, the TOS level was significantly increased in RIS group when compared to the control, RIS+RSV-1, RIS+RSV-2, and RIS+RSV-3 groups ($p<0.001$). The TOS level was significantly increased in RIS+RSV-1 group when compared to the RIS+RSV-3 group ($p=0.006$). The OSI level was significantly higher in the RIS group when compared to the control, RIS+RSV-1, and RIS+RSV-2 groups ($p<0.05$) (Table 2, Figure 4).

Effect of RIS and RSV on expression of the FAS gene

Table 3 shows the effects of RSV treatment against the RIS administration on the mRNA expression of FAS gene level in all study groups and control. FAS gene expression significantly increased in RIS group compared to the control group. The RIS+RSV-1 group had a significantly lower expression of FAS gene level compared to the RIS group ($p\leq0.01$) (Table 3, Figure 5).

Effect of RIS and RSV on apoptosis in rat liver

The results of the apoptotic index are demonstrated in Table 4, Figure 6. Using TUNEL for the detection of apoptotic cells in the liver sections, the control (Figure 6A) group showed only a few TUNEL-positive cells. The count of TUNEL-positive cells significantly increased in the RIS (Figure 6B) group compared with that in control group ($p<0.05$). The RIS+RSV-1 (Figure 6C), RIS+RSV-2 (Figure 6D), and RIS+RSV-3 (Figure 6E) groups were similar and showed rare TUNEL-positive cells. Treatment with RSV (RIS+RSV-1, RIS+RSV-2, and

RIS+RSV-3 groups) (Figure 6C, 6D, and 6E) reduced the count of TUNEL-positive cells compared to the RIS group ($p<0.05$).

Discussion

AAPs are used to treat serious mental disorders. Though they have many beneficial effects, they also have many serious side effects (Eder et al., 2001). RIS is one of the AAPs that has led to weight gain and obesity side-effects, and other metabolic disorders in patients (Yoon et al., 2016). Therefore, it is extremely important to prevent side effects and other metabolic disorders induced by RIS. Many authors have suggested a co-treatment between RIS and compounds that regulate its metabolic adverse effects. Through antioxidant and radical scavenger properties of natural compounds, may prevent and treat diseases. Dietary intake of natural compounds, including RSV, can inhibit the metabolic side effects of RIS and thereby may reduce the risk factors in the liver (Walton et al., 1999). Hence, the purpose of the current study was to investigate the protective and therapeutic effects of RSV against the effect of RIS over FAS gene expression and RIS-induced liver damage.

The liver is responsible for many vital life functions and is involved in uptake, secretion, synthesis, catabolism and storage. Fatty acids increase in the liver by hepatocellular uptake from the plasma and by de novo biosynthesis. Hepatic FAS is the synthesizing of fatty acids for the partitioning and storage of excess energy (Jensen-Urstad and Semenkovich, 2012). According to clinical experiences, an accumulation of extreme intracellular triglycerides often comes before the improvement of obesity (Riediger and Clara, 2011). This study shows that RIS significantly increases the expression of the FAS gene, and there are highly meaningful correlations between the expression of this gene and the final body weight of animals. This effect of RIS was formerly presented in different experimental models of the liver (Lauressergues et al., 2011, Cope et al., 2005). In addition, high triglycerides observed in rats subjected to RIS are a result of elevated hepatic FAS expression. Similarly, previous studies reported that rodent models with high triglyceride levels are related to increased hepatic FAS expression (Morgan et al., 2008). In this study, we conclude that the increase in observed body weight can be partially elevated levels of circulating and stored triglycerides. Taken together, RIS exposure can cause long-term hypertriglyceridemia due to the FAS-dependent pathway to the synthesis of de novo triglycerides. Thus, RIS-induced weight gain

could be the result of the effect of RIS associated alterations on the central nervous system, including on body temperature, on food intake, on locomotor activity.

RSV, a natural compound in superfoods like wine, and has a beneficial effect on glucose and lipid metabolism. In fact, many clinical trials have recently demonstrated that using animal models of diet-induced obesity has displayed the beneficial effects of RSV on reducing obesity and oxidative stress (G´omez-Zorita et al., 2012; Farag et al., 2017). In addition, RSV performs a considerable role in lipid metabolism. In the current study, RSV co-treatment decreased antipsychotic-induced weight gain significantly with only a 20 mg/kg dose. Also, RSV attenuated hepatic triacylglycerol and fatty acid synthesis in rats. This data suggest that the RSV had protective effects against the adverse effects of RIS and decreased the risk of obesity. These results imply that the mechanism of effect of RSV occurs by increasing energy consumption, inhibition of energy intake, and reducing energy storage. This weight-decreasing effect of RSV is estimated to be attributable, in part, to its effects on adipocytes and expression of the FAS gene (Baur et al., 2006, Naderali, 2009). Therefore RSV is a reliable compound for co-administration with RIS for decrease of antipsychotic-induced weight gain and obesity without effecting its therapeutic action.

In the present study, RIS exposure produced a significant increase in the activity of liver enzymes. ALT, AST, and GGT indicate a damaged functional and structural hepatic integrity. Oral supplementation of RSV reduces liver injury and improve the elevated serum ALT, AST and GGT activities. While RSV co-treatment curable these changes in all doses, it had the most obvious effect in high doses. Our study results are confirmed by data from the literature (Miguel et al., 2016). In addition, we demonstrated that RSV prevented the increase in TG, TCH, and LDL as well as a decrease in HDL caused by RIS consumption. All doses of RSV caused dose-dependent decreases in serum lipids compared to RIS administrated rats. However, RSV co-treatment curable these changes with more obvious effect and but with a major decrease in the 80 mg/kg dose. The effect of RSV on serum lipids has been reported in earlier experiments (Panico et al., 2017). This finding is probably a consequence of feeding behavior and the increase in body weight. Although underlying physiological pathways are not fully understood, the present findings indicate that RIS increases and RSV decreases serum lipids.

In this study, RSV significantly affected the RIS load on the liver, enhanced the reduced TAS, inhibited the elevated TOS and OSI levels, healed impaired hepatic function, and reformed the histopathological changes in the liver. RIS-mediated ROS formation by diminished antioxidant levels and oxidative stress and antioxidant depletion can lead to

apoptotic cell death (Armstrong and Jones, 2002). In this study, we found that RSV had a significant protective role in apoptotic cell death, which might be due to the ROS scavenging property. Taking the previous findings and suggestions together, it can be concluded that RSV could prevent RIS-induced liver injury and histological perturbations through the enhancement of antioxidant defense systems, suppression of oxidative stress, and attenuation of apoptosis. Oxidative stress has a vital role in the chain of initiation and progression of liver diseases. In this study, in RIS administration rats, a reduction in TAS level was observed resulting in a rise in TOS and OSI levels as in previous studies (Li et al., 2015). On the other hand, we observed that RSV protected against RIS-induced liver damage by suppressing oxidative stress and apoptosis. In addition, our results demonstrated that TAS levels increased and TOS, OSI levels conspicuously reduced with RSV treatment as reported in prior studies (Faghihzadeh et al., 2015). Additionally, the level of antioxidant TAS significantly elevated with 20 mg/kg doses by RSV co-operation. Several studies have demonstrated that the hepatoprotective effect of RSV against liver damage is mediated by its antioxidant and anti-inflammatory properties (Bishayee et al., 2010). A few recent studies have shown that RSV administered to mice in their diet significantly reduced lipids and depressed the expression of genes related to hepatic lipid metabolism (Ahn et al., 2008).

Histopathological findings support above oxidative results. The TUNEL assay used for determine apoptotic cells in the liver sections. Histopathological assessment of the liver showed serious damage follow by detrimental effects on the normal structure of the liver in RIS administrated rats including vacuolar degeneration of hepatocytes and fatty changes. RIS-induced toxic effects were prevented through the powerful antioxidant capacity and other biological effects of RSV. Among the three doses, 80 mg of RSV/kg body weight was found to provide optimum protective effect on the liver against RIS induced abnormal changes. Histological observations added more evidence supporting the protective effect of RSV. The present study demonstrated that RIS damaged the histological structure and function and inhibited the endogenous antioxidant defense system in rat liver tissue as reported in previous studies (Radzik et al., 2005). In addition, our results showed, at the first time, that RSV oral supplementation, at safe dose levels, has a noteworthy protective effect against RIS-induced liver damage in rats. This protection makes RSV a promising agent in a variety of conditions in which cellular damage occurs as a result of oxidative stress. RIS-induced liver injury causes increased ROS formation and subsequent toxic events. Accordingly, in our study, with RSV treatment of the cells against RIS exposure, the apoptotic cell injury and death were greatly reduced. The underlying mechanism of the protective quality of RSV may be

associated with the suppression of apoptosis via death receptor-mediated pathways. Therefore, previous studies show that antioxidant activity of RSV can be possible because of the effect on mitochondria-independent apoptotic pathways. Hence, RSV may be the best choice against RIS induced side effects.

In conclusion, RSV may be a promising agent to mitigate the adverse effects of RIS, oxidative stress, and apoptotic status and to reduce weight gain and the expression of the FAS gene and so prevent liver damage in patients. Thus, daily consumption of RSV should be considered as a promising way to prevent liver damage. Our results could be used to plan strategies to protect against the adverse effects of RIS in the liver and in other organs. Hence, further in vivo and clinical studies are required to confirm the protective effects of RSV in patients receiving RIS.

Disclosure statement

No potential conflicts of interest were reported.

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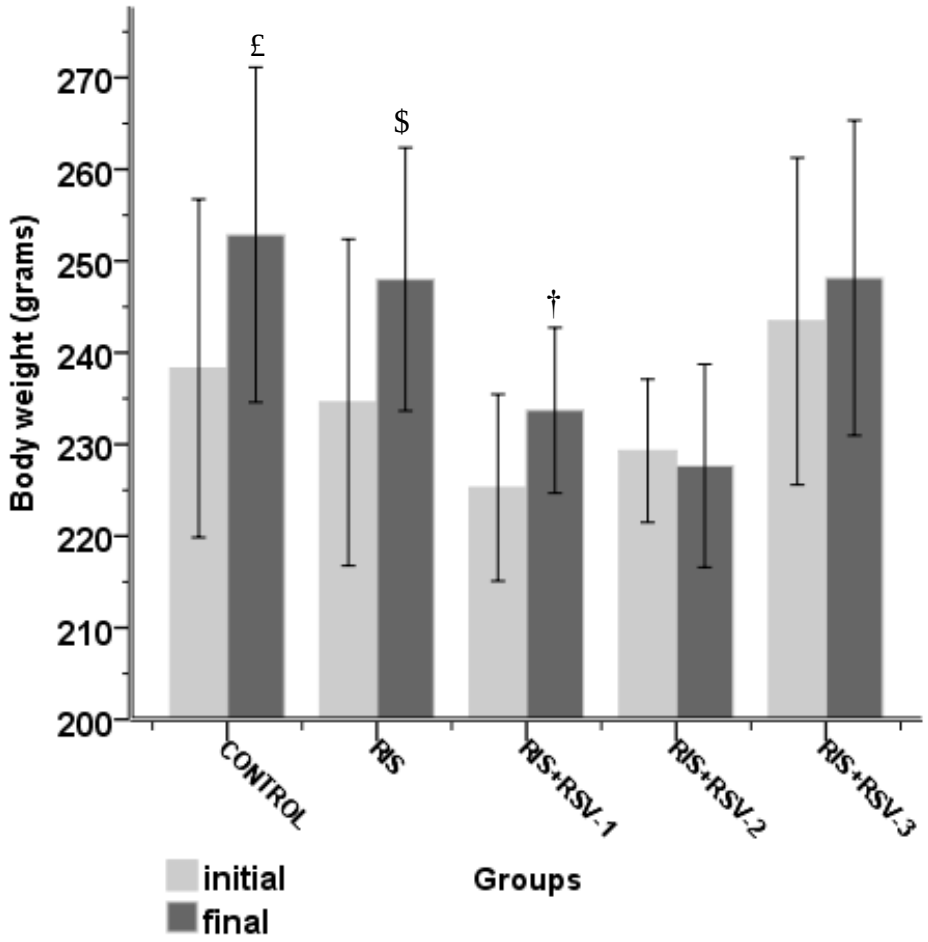

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446 **Figure 1.** Changes in the body weight of experimental rats. Values are expressed as mean ±
447 SEM of seven animals. The groups were compared with the paired-samples T-test at the
448 beginning and end of the treatment. [£,$,†] In each column, different superscript letters mean
449 significant differences at $p<0.05$. Abbreviations: RIS: risperidone; RSV: resveratrol;
450 RIS+RSV-1: 2 mg/kg RIS+20 mg/kg RSV; RIS+RSV-2: 2 mg/kg RIS+40 mg/kg RSV;
451 RIS+RSV-3: 2 mg/kg RIS+80 mg/kg RSV.

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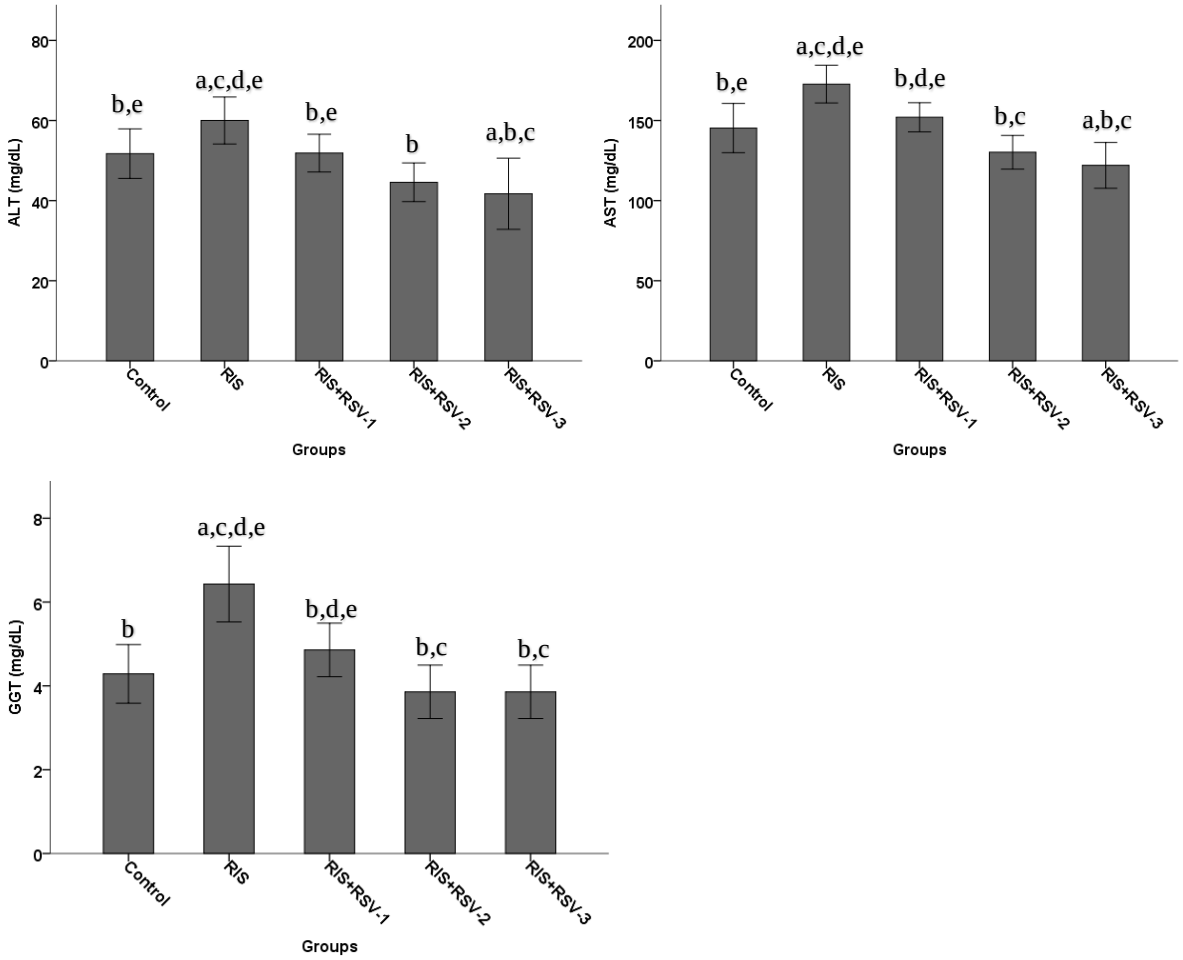


Figure 2. Effects of risperidone, resveratrol, and their coadministration on the liver level of serum ALT, AST and GGT in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. [a] $p<0.05$ versus control; [b] $p<0.05$ versus RIS-treated rats; [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. Abbreviations: RIS: risperidone; RSV: resveratrol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; 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.

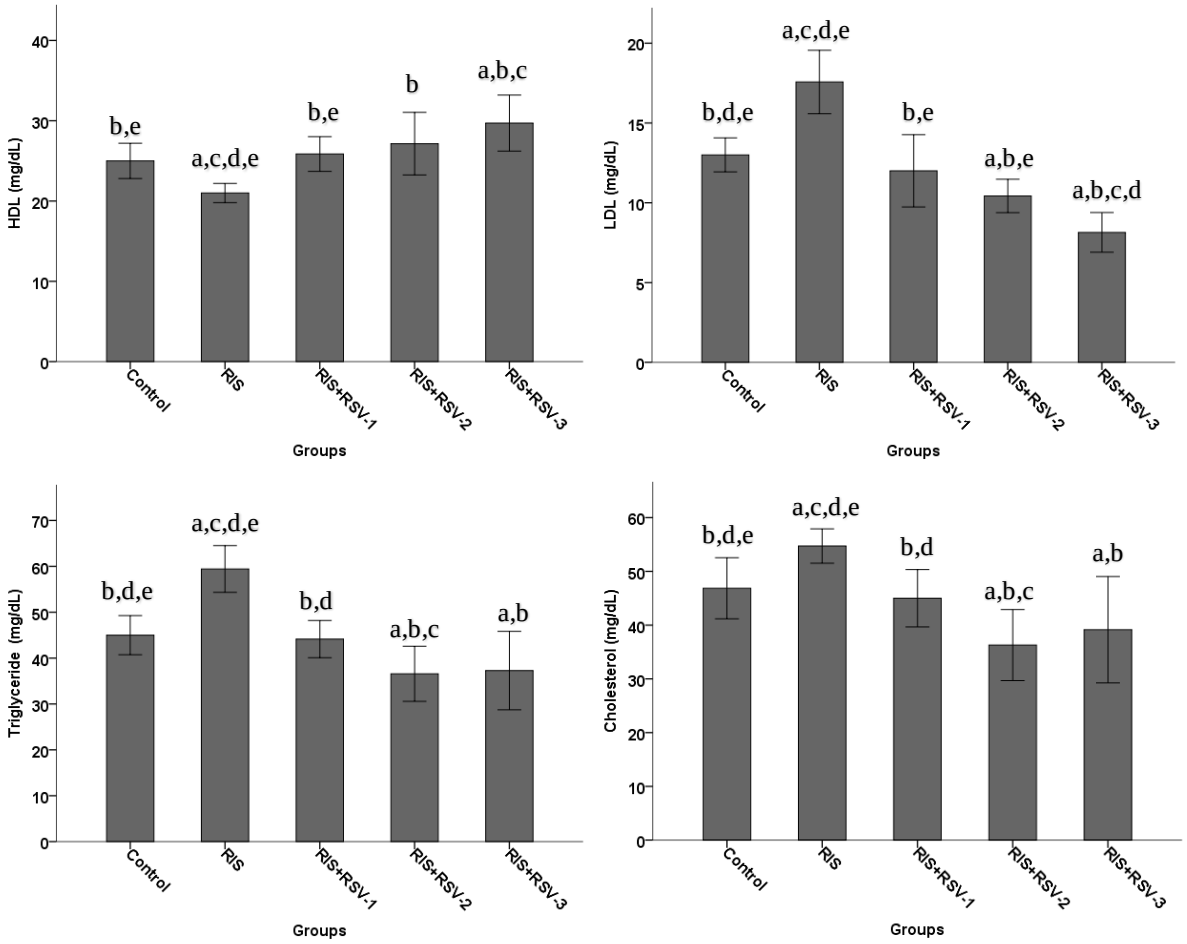


Figure 3. Effects of risperidone, resveratrol, and their coadministration on the liver level of serum HDL, LDL, TG and CH in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. [a] $p<0.05$ versus control; [b] $p<0.05$ versus RIS-treated rats; [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. Abbreviations: RIS: risperidone; RSV: resveratrol; HDL: high-density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TG: triglycerides; TC: cholesterol. 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.

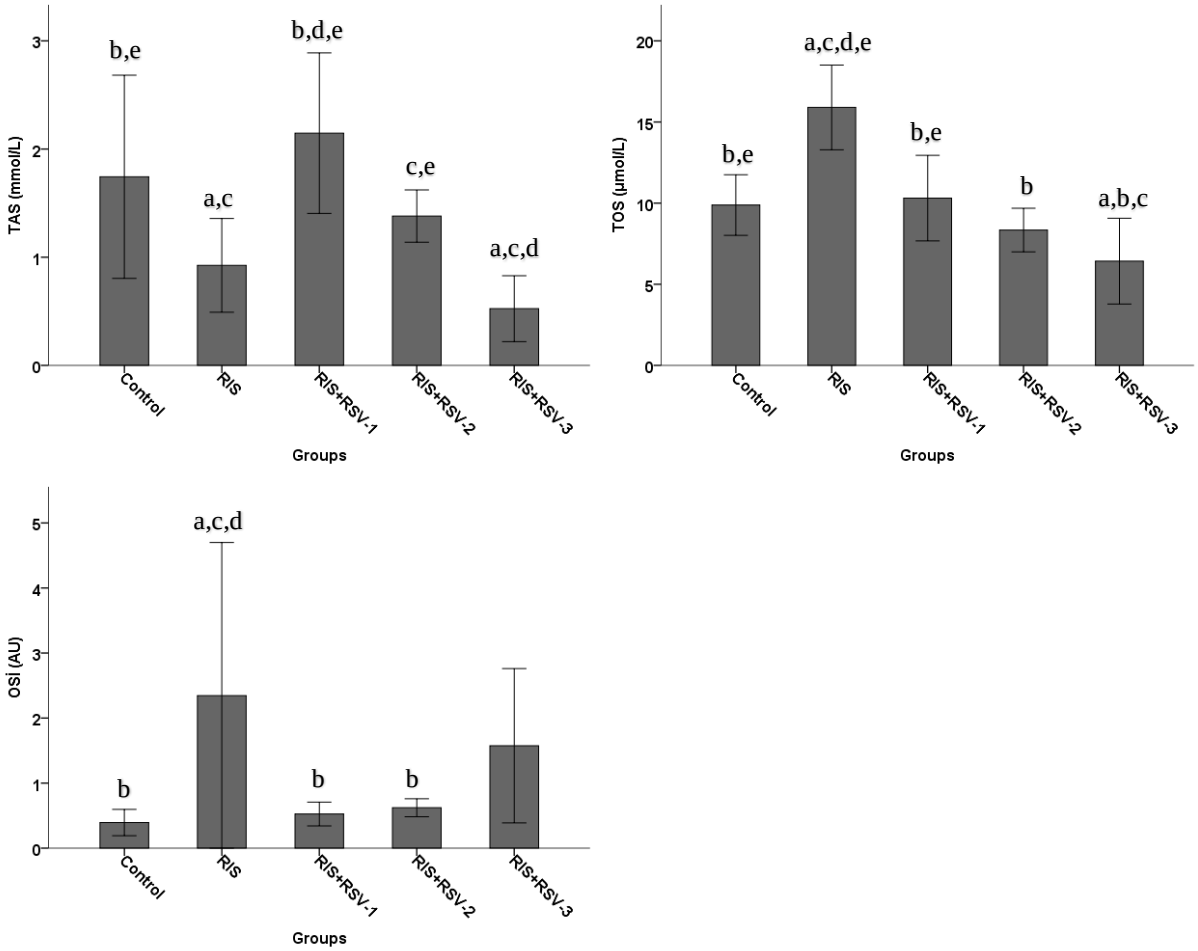
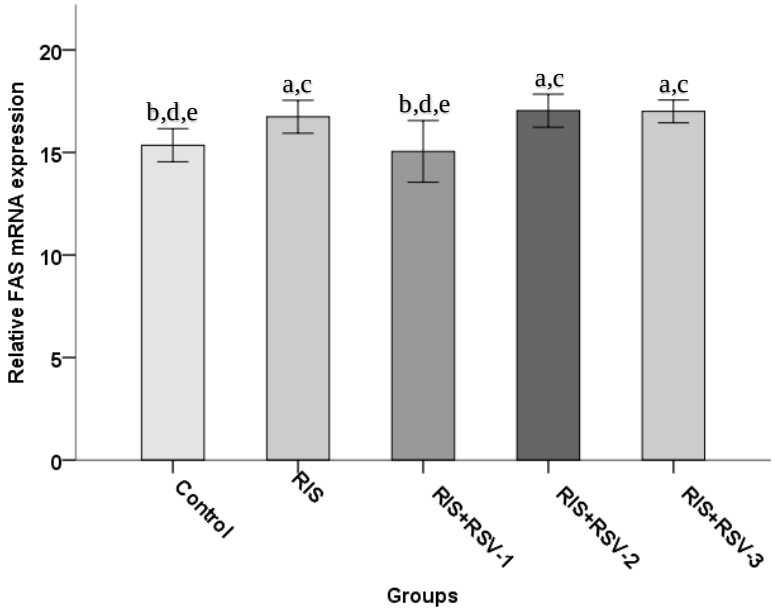


Figure 4. Effects of risperidone, resveratrol, and their coadministration on the level of TAS, TOS and OSI in rats after two weeks. Values are expressed as mean ± SEM of seven animals. Data were subjected to two-way ANOVA. [a] $p<0.05$ versus control; [b] $p<0.05$ versus RIS-treated rats; [c] $p<0.05$ 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. Abbreviations: RIS: risperidone; RSV: resveratrol; TAS: total antioxidant status; TOS: total oxidant status; OSI: Oxidative stress index; 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; AU: Arbutrary Units.



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505 **Figure 5.** Effects of RIS and RSV on the expression of FAS gene in rat liver. Data are means
506 ± SEM (n = 7). Different letters over the bars represent significant differences, $p<0.05$.

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Figure Legends

Figure 6. Representative photomicrographs of TUNEL staining in all five groups (scale bars=100 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 liver section (two sections/animal), and the sections were analyzed at 400× 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 (no), 1 (light), 2 (medium), and 3 (intense).

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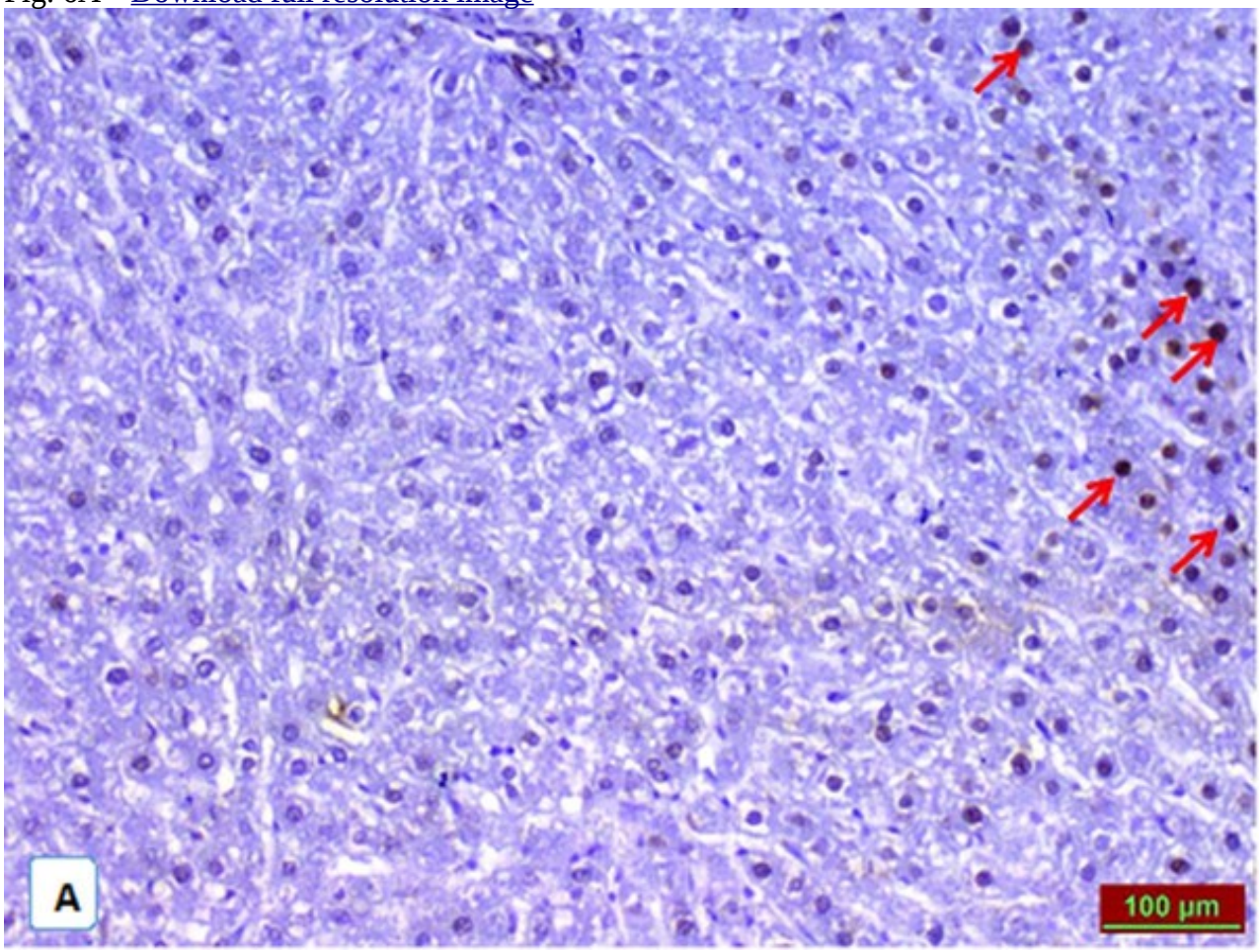
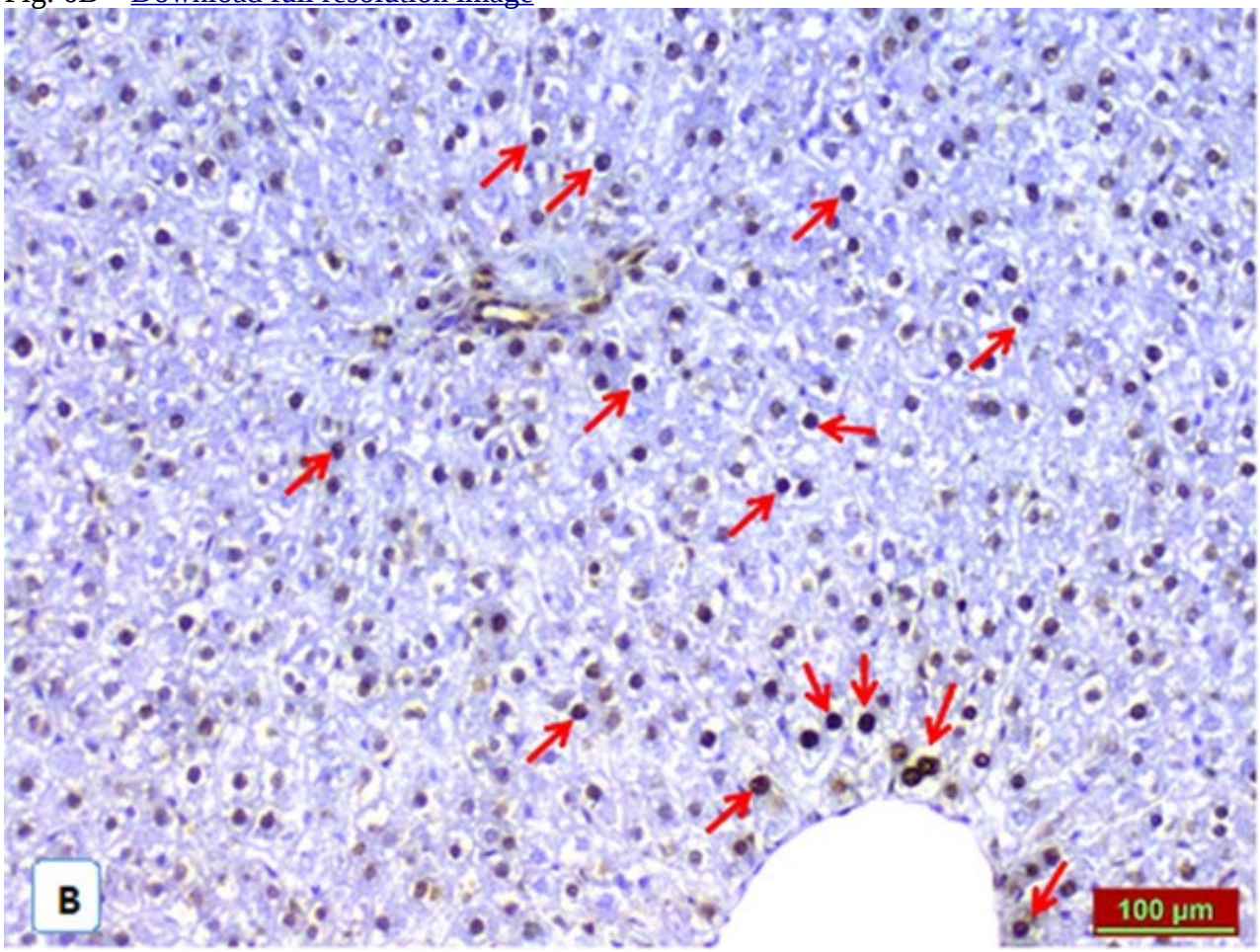


Fig. 6B Download full resolution image



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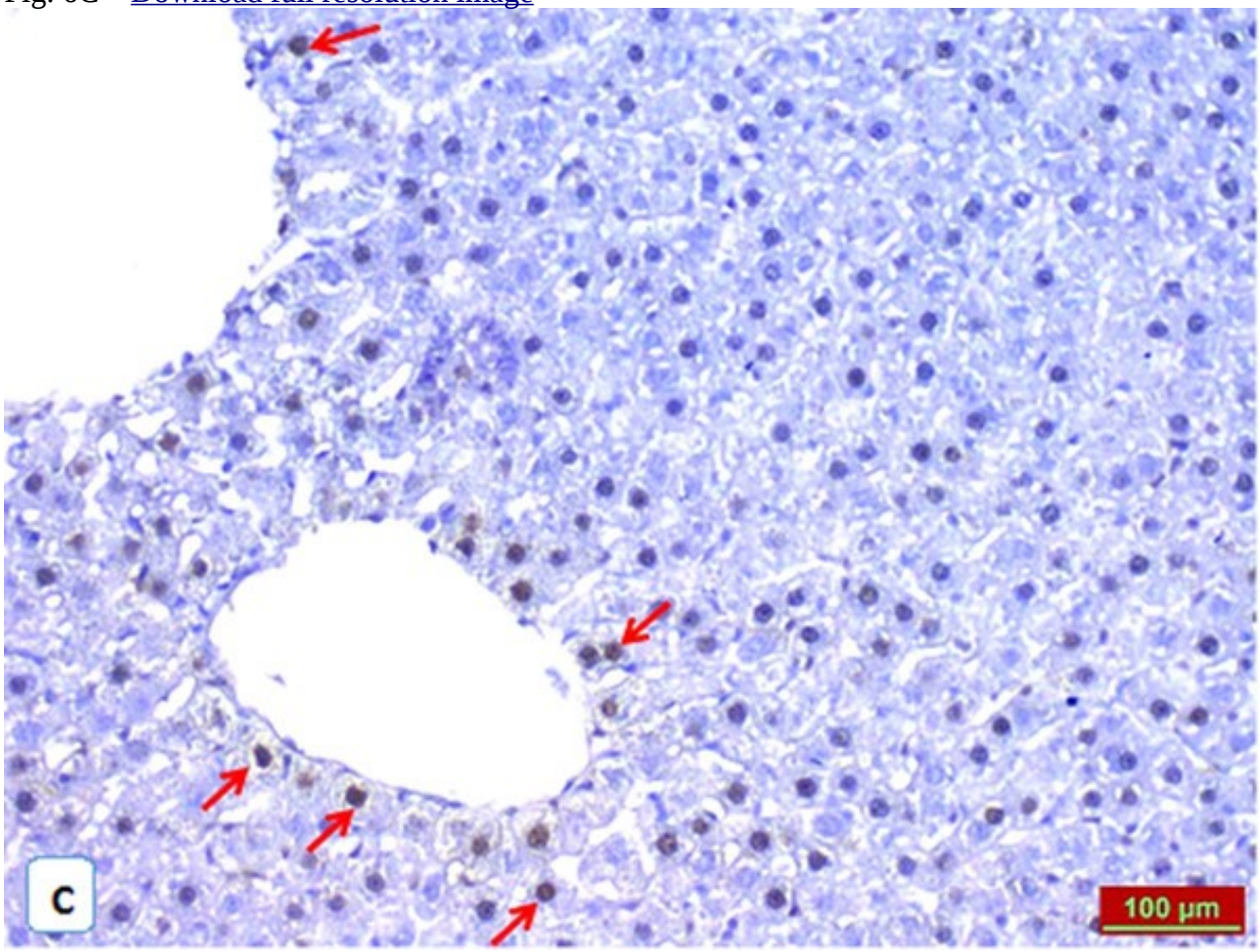

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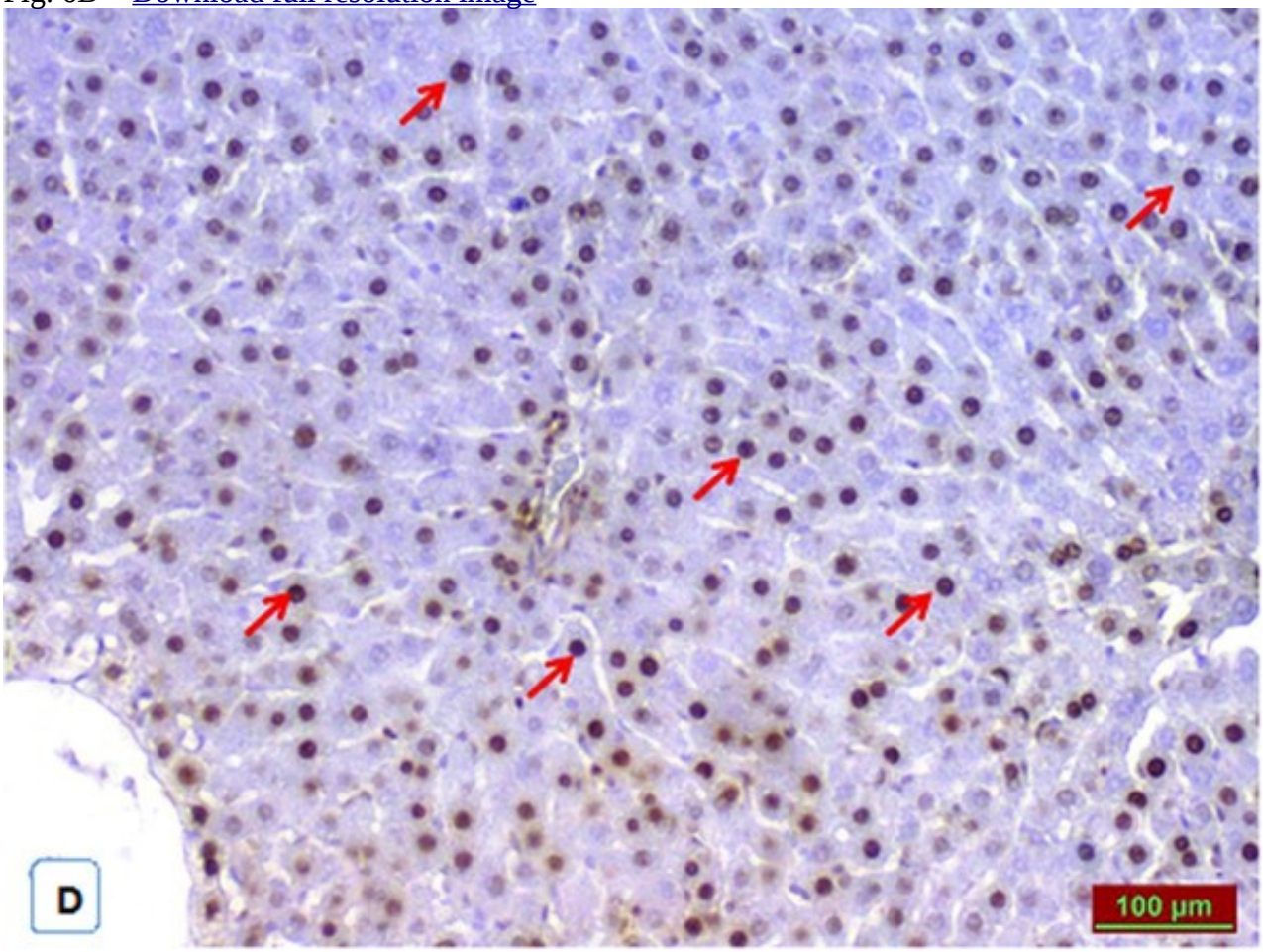

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