

EXPERIMENTAL STUDY

The protective effect of thymoquinone over olanzapine-induced side effects in liver, and metabolic side effects

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ABSTRACT

OBJECTIVES: The aim of the study was to investigate the possible protective qualities of thymoquinone (TQ) against the side-effects of olanzapine (OLZ) in an experimental model in rat liver with histologic and biochemical assessments.

METHODS: Experimental procedures were performed on 35 female Sprague Dawley rats. Rats were randomly divided into five groups as: group 1: control; group 2: OLZ; group 3: OLZ+TQ-1; group 4: OLZ+TQ-2; and group 5: OLZ+TQ-3.

RESULTS: The results showed that a 2-week administration of OLZ (4 mg/kg, once a day for the first week, 8 mg/kg once a day for the second week, p.o.) and treatment with TQ (25, 50, 100 mg/kg, once daily, p.o.) significantly reduced weight gain induced by OLZ. In addition, TQ increased the total antioxidant status (TAS), high-density lipoprotein cholesterol (HDL), insulin levels and decreased serum oxidative stress index (OSI), total oxidant status (TOS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), low density lipoprotein cholesterol (LDL), glucose, triglycerides (TG) and total cholesterol (CH) levels significantly ($p < 0.05$).

CONCLUSION: This study revealed that treatment with TQ might protect liver tissue against the side-effects of OLZ. TQ could be an effective course of therapy to enhance therapeutic efficacy (Tab. 4, Fig. 4, Ref. 47).

Text in PDF www.ejis.sk.**KEY WORDS:** thymoquinone, olanzapine, adverse effects, liver, weight gain/loss, apoptosis.

Abbreviations: ALT – alanine aminotransferase, AST – aspartate aminotransferase, CH – total cholesterol, DAB – diamino benzidine, GGT – gamma glutamyl transpeptidase, HDL-cholesterol – high-density lipoprotein cholesterol, LDL-cholesterol – low-density lipoprotein cholesterol, OLZ – olanzapine, OSI – oxidative stress index, PBS – phosphate buffered saline, ROS – reactive oxygen species, TAS – serum total antioxidant status, TG – triglycerides, TOS – total oxidant status, TQ – thymoquinone, TUNEL – terminal deoxynucleotidyl transferase dUTP nick-end labeling.

Introduction

OLZ is one of the atypical antipsychotics, drugs widely used in the treatment of psychotic disorders that have also been used as monotherapy or adjunctively with antidepressants to treat depressive disorders with or without psychotic symptoms (1). Clinical assay suggests that OLZ reduces both positive and negative symptoms, and that it is associated with a low incidence of extra pyramidal side effects. In addition, it is related to significant weight

gain and metabolic alterations such as dyslipidemias, diabetes, and other problems involving the metabolic syndrome (2, 3). There are few cases declaring OLZ associated hepatotoxicity in patients, but the mechanism that leads to OLZ-related liver toxicity is not well known (4). The primary site for OLZ metabolism is the liver, where it is metabolized into inactive metabolites primarily by the cytochrome P450 system. However, although treatments with atypical antipsychotics are effective in stress-associated psychiatric diseases like schizophrenia and mood and anxiety disorders, the influence of OLZ on the liver requires further research (5).

Medicinal plants nowadays are an important source of drug synthesis. The wide utilization of herbal drugs has encouraged scientists to research their impressive effects on health, and a large number of medicinal plants and their active extracted ingredients are extensively studied for their potentials to protect cells from injuries. *Nigella sativa* (also called black cumin, black seed) is among the promising medicinal plants. *N. sativa* Linn as a member of the botanical family of Ranunculaceae is an annual herbaceous plant (6). TQ (2-isopropyl-5-methyl-1,4-benzoquinone), the main active component of the essential oil of *Nigella sativa* seeds, has various pharmacological effects. It has been reported to display various pharmacological activities such as antioxidant (7), antidiabetic (8), cardioprotective (9), hepatoprotective (10), neuroprotective (11), nephroprotective (12), anti-inflammatory (13), anti-mutagenic (14), and anticancer (15) effects. TQ is of low toxicity (LD50 2.4 g/kg) and is well tolerated when given subchronically till 90 mg/

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kg/day for 90 days (16). TQ supplementation considerably protected several organs including liver, against oxidative damage induced by a variety of free radical generating agents (17). The high potency and low systemic toxicity of TQ make it a promising alternative to conventional therapeutic drugs (18). The fact that OLZ exposure induces an excessive increase in metabolic alterations suggests that TQ could be used as an alternative therapy.

To our knowledge, there is no report regarding the protective and therapeutic effects of TQ against OLZ-induced liver toxicity. The influence of TQ on OLZ-induced liver injury has not been studied until now. Therefore, the present study was designed to investigate, for the first time, the possible beneficial impact of oral supplementation with TQ against OLZ-induced liver damage in rats. To achieve our goal, we performed several biochemical and histological analyses in female rats.

Materials and methods

Chemicals

OLZ was obtained from Ali Arif Ilac Sanayi (ARIS), Istanbul, Turkey. TQ (purity > 98 %) was purchased from Sigma. All other chemicals used were of the best analytical grade.

Animals

In this study, 35 female Sprague Dawley rats were used. Healthy adult (4 months old) rats, weighing 250-300 g obtained from the Firat University Laboratory Animal Production and Research Center. Entire animal care and follow-up were performed at this Center. The experiments were performed in accordance with the protocol approved by Firat University Faculty of Medicine, Laboratory Animals Ethics Committee (Protocol # 2015/23). The rats were kept at 21 ± 1 °C, for 12 h in a light-dark cycle, and were fed with standard rat chow and drunk tap water ad libitum. OLZ and TQ were administered to rats for 2 weeks.

Experimental design

In our study, 35 rats were randomly assigned to one of five groups, with an equal number of rats contained within each group. Simple randomization technique was used in this experimental study. Thirty-five female Sprague Dawley rats were divided into five groups as follows: group 1: control; group 2: OLZ; group 3: OLZ+TQ-1; group 4: OLZ+TQ-2; group 5: OLZ+TQ-3. The control group was given physiological saline solution by gavage once a day. Except for control, OLZ was given to all groups in amount of 4 mg/kg once a day for the first week, and 8 mg/kg once a day for the second week. OLZ+TQ-1 group was given TQ in amount of 25 mg/kg body weight/day. OLZ+TQ-2 group was given TQ in amount of 50 mg/kg body weight/day. OLZ+TQ-3 group was given TQ in amount of 100 mg/kg body weight/day. The assigned dosage of powdered OLZ was administered according to a previous report which demonstrated a significant weight gain in female Sprague Dawley rats (19). TQ was administered by gastric tube daily between 8:00 and 9:00 a.m. The dose and duration of TQ was selected according to results from previous study (20). All compounds were suspended in physiological saline solution and

administered by gavage once a day. Body weight was recorded at the beginning and at the end of the study. The treatment course lasted 2 weeks for all groups. At the end of week 2 of the treatment period, the animals were euthanized by exsanguination through cardiac puncture under diethyl ether anesthesia. The entire liver was excised and stored at -80 °C for subsequent biochemical analysis. Blood samples were immediately collected from the retro-orbital venous plexus in a tube free from any anticoagulant agent for separation of serum samples for biochemical analysis.

Liver enzyme assays

Blood samples collected from each rat were used to determine liver enzyme activity. Serum concentrations of CH (mg/dL), HDL-cholesterol (mg/dL), and TG (mg/dl) were measured using routine enzymatic methods with Olympus 2700 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). LDL-cholesterol (mg/dL) levels were calculated using Friedewald's equation. Standard liver function tests, namely ALT (U/L), AST (U/L), GGT (U/L) were measured on the same day with an autoanalyzer. We examined the glucose level (Architect ci16200) and insulin level (Beckman Coulter) according to manufacturer's instructions.

TAS and TOS determination

The automated calorimetric measurement methods developed by Erel were used to determine TAS (mmol/L) and TOS (μ mol/L) (a serum oxidant parameter). Measurements of TAS and TOS in serum samples were defined by TAS and TOS kit (REL Assay Diagnostics) (21,22).

OSI determination

OSI was defined as TOS-to-TAS ratio and calculated as follows: $OSI \text{ (arbitrary unit)} = ((TOS, \mu\text{mol H}_2\text{O}_2 \text{ eq/L}) / (TAS, \mu\text{mol Trolox eq/L}))$ (23).

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

Apoptotic cells were defined using the ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Chemicon, Cat no: S7101, USA) according to the manufacturer's instructions. Sections (5 μ m) taken from the paraffin blocks were entrenched onto polylysine-coated slides, deparaffinized using xylene, dehydrated with a series of alcohol rinses, and then washed with phosphate-buffered saline (PBS). The tissues were then incubated with a proteinase K solution (0.05 %) and with 3 % hydrogen peroxide for 5 min to forestall endogenous peroxidase activity. After washing with PBS, the tissues were incubated with equilibration buffer for 6 min and in working solution (70 % μ L reaction Buffer + 30 % TdT enzyme) at 37 °C under moist conditions for 60 min. Tissues were then incubated in stop/wash buffer for 10 min and incubated in anti-digoxigenin-peroxidase for 30 min. Apoptotic cells were examined using the diaminobenzidine substrate. Cross-sections contrast-stained with methyl green were sealed using a proper covering solution. Mammary tissue was used as a positive control. PBS was used instead of TdT enzyme in the negative control tissue. Preparations were examined and assessed using a research micro-

Tab. 1. Body weight (g) of animals during treatment.

Design of treatment	Control	OLZ	OLZ+TQ-1	OLZ+TQ-2	OLZ+TQ-3
Start of Study	258.57±8.39	240.33±8.67	255.20±5.23	260.83±5.95	250.40±5.50
End of Study	269.14±8.17	249.50±9.02	245.80±3.55	249.16±9.80	234.20±7.95
Statistical comparison (Start of study vs end of study) (P)					
Control	0.011				
OLZ	0.001				
OLZ+TQ-1	0.015				
OLZ+TQ-2	0.312				
OLZ+TQ-3	0.127				

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. $p \leq 0.05$. OLZ – olanzapine; TQ – thymoquinone; OLZ+TQ-1 – OLZ+25 mg/kg TQ; OLZ+TQ-2 – OLZ+50 mg/kg TQ; OLZ+TQ-3 – OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group

Tab. 2. Comparison of serum oxidative stress parameters among the groups.

Parameters	Control	OLZ	OLZ+TQ-1	OLZ+TQ-2	OLZ+TQ-3	p
TOS ($\mu\text{mol/L}$)	9.75±1.66 ^{b,c}	17.32±1.99 ^{a,c,d,e}	4.91±0.93 ^{a,b,e}	7.56±0.42 ^{b,e}	12.23±0.66 ^{b,c,d}	0.000
TAS (mmol/L)	1.69±0.26 ^{b,c,e}	0.62±0.11 ^{a,c,d}	3.55±0.11 ^{a,b,d,e}	1.45±0.18 ^{b,c,e}	0.76±0.13 ^{a,c,d}	0.000
OSI (AU)	6.07±0.82 ^b	44.01±17.45 ^{a,c,d}	1.36±0.23 ^b	6.07±1.17 ^b	17.83±2.94	0.009

Each group represents the mean ± SEM for seven rats. ^a $p < 0.01$ vs control group; ^b $p < 0.01$ vs OLZ group; ^c $p < 0.01$ vs OLZ+TQ-1 group; ^d $p < 0.03$ vs OLZ+TQ-2 group; ^e $p < 0.03$ vs OLZ+TQ-3 group.

OLZ – olanzapine; TQ – thymoquinone; TAS – total antioxidant status; TOS – total oxidant status; OSI – oxidative stress index; OLZ+TQ-1 – OLZ+25 mg/kg TQ; OLZ+TQ-2 – OLZ+50 mg/kg TQ; OLZ+TQ-3 – OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group. AU – arbitrary units

scope (Olympus BH2 light microscope) and then photographed. To assess the TUNEL staining, after staining with methyl green, cells with green nuclei were considered normal whereas those with brown nuclei were considered apoptotic. Apoptotic (TUNEL-positive) cells were counted and statistically assessed. This analysis was made in at least eight areas of each liver section (two sections/animal), and the sections were analyzed at 400× magnification (24).

The evaluation of TUNEL staining was made 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) (25).

Statistical analysis

Statistical analyses were performed using a statistical software package (SPSS version 20.0, SPSS, Chicago, IL). Normality for variables in the groups was determined by the Shapiro-Wilk test. All values are given as means ± standard error of mean (SEM). Body weight gain data were analyzed by paired-samples T-test. The groups were compared with the paired-samples T-test at the beginning of study and end of the study. Biochemical parameters showed a normal distribution. One-way analysis of variance (ANOVA) and post-hoc LSD tests were used to compare the groups. For histopathological analysis, results were expressed as means ± standard deviation. The statistical significant difference was determined by ANOVA followed by Tukey's multiple comparison test. Probability values (p) less than 0.05 were considered to be statistically significant.

Results

Body weight measurements showed that during the 2 weeks animals grew from 258.57 g at day 1 to 268.14 g for the control

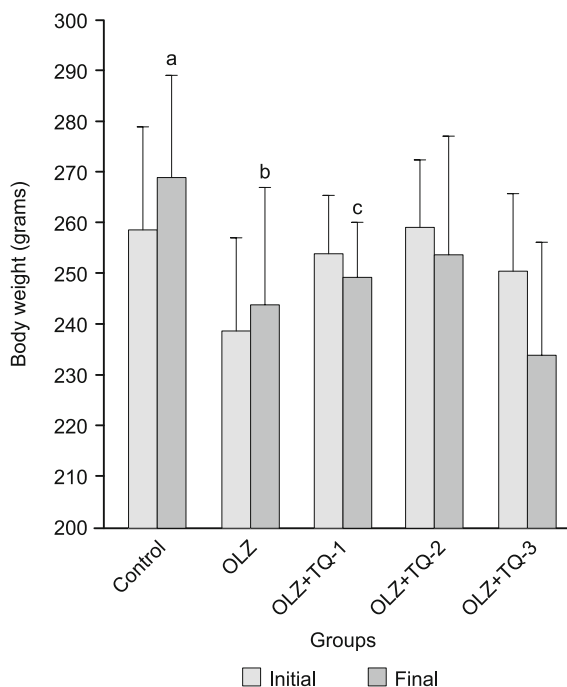


Fig. 1. Changes in the body weight of experimental rats. Values are expressed as mean ± SEM of seven animals. The groups were compared with the paired-samples T-test at the beginning and end of the treatment. a,b,c In each column, different superscript letters mean significant differences at $p < 0.05$. OLZ: olanzapine; TQ: thymoquinone; OLZ+TQ-1: OLZ+25 mg/kg TQ; OLZ+TQ-2: OLZ+50 mg/kg TQ; OLZ+TQ-3: OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group.

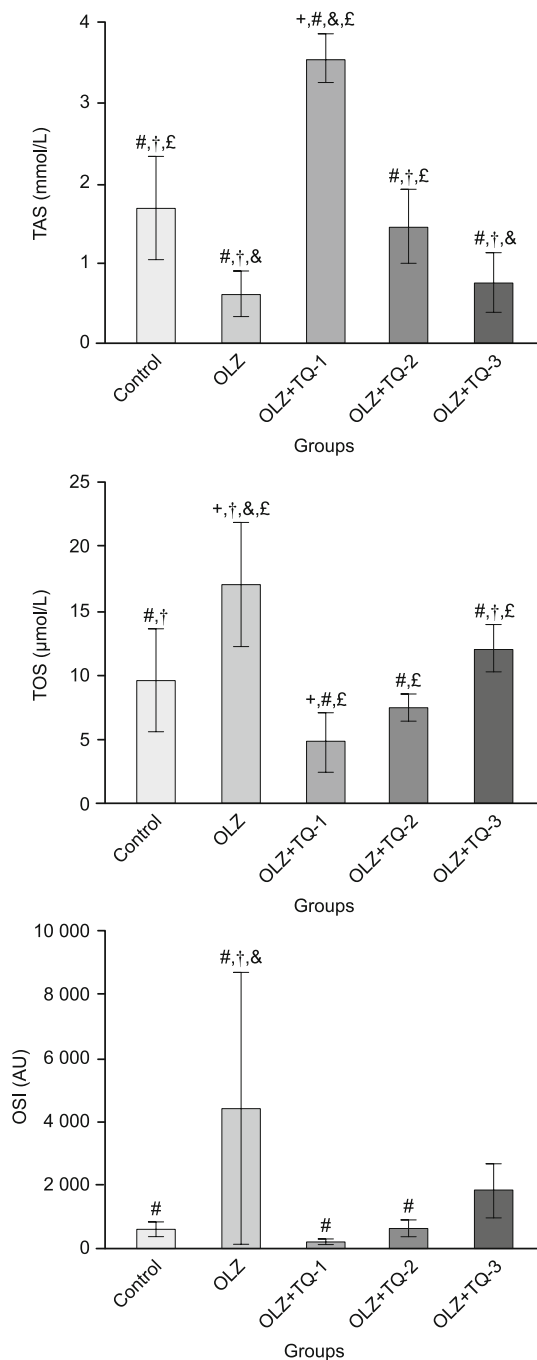


Fig. 2. Effects of olanzapine, thymoquinone, and their coadministration on the liver level of TAS (total antioxidant status), TOS (total oxidant status), and OSI (oxidative stress index) in rats after two weeks. Values are expressed as mean \pm SEM of seven animals. ANOVA followed by the LSD post hoc test were used. * $p < 0.05$ versus control; # $p < 0.05$ versus OLZ-treated rats; † $p < 0.05$ versus OLZ+TQ-1 treated rats; & $p < 0.05$ versus OLZ+TQ-2 treated rats; £ $p < 0.05$ versus OLZ+TQ-3 treated rats. OLZ: olanzapine; TQ: thymoquinone; OLZ+TQ-1: OLZ+25 mg/kg TQ; OLZ+TQ-2: OLZ+50 mg/kg TQ; OLZ+TQ-3: OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group. AU: arbitrary units.

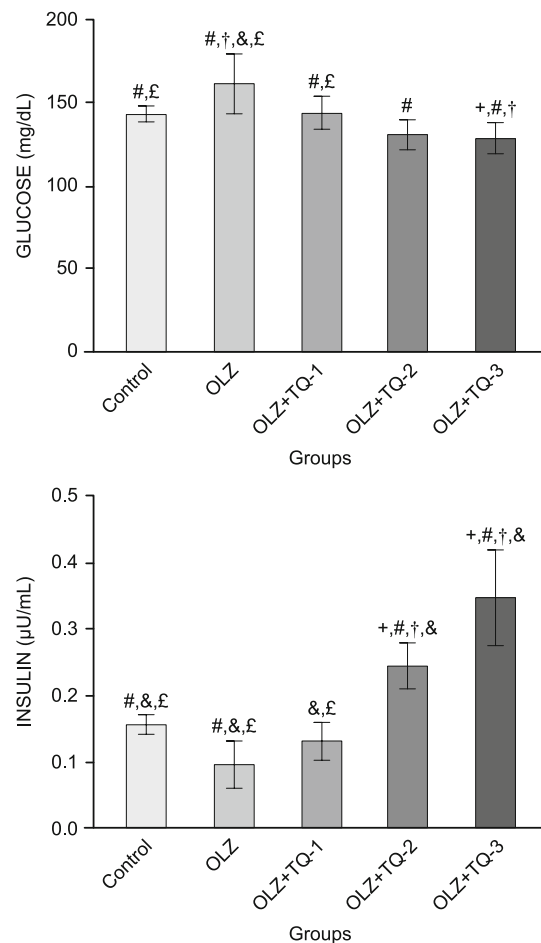


Fig. 3. Effects of olanzapine, thymoquinone, and their coadministration on the liver level of serum biochemical parameters in rats after two weeks. Values are expressed as mean \pm SEM of seven animals. ANOVA followed by the LSD post hoc test were used. * $p < 0.05$ versus control; # $p < 0.05$ versus OLZ-treated rats; † $p < 0.05$ versus OLZ+TQ-1 treated rats; & $p < 0.05$ versus OLZ+TQ-2 treated rats; £ $p < 0.05$ versus OLZ+TQ-3 treated rats. OLZ: olanzapine; TQ: thymoquinone; OLZ+TQ-1: OLZ+25 mg/kg TQ; OLZ+TQ-2: OLZ+50 mg/kg TQ; OLZ+TQ-3: OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group.

group, 240.33 g at day 1 to 249.50 g for the OLZ group, 255.20 g at day 1 to 245.80 g for the OLZ+TQ-1 group, 260.83 g at day 1 to 249.16 g for the OLZ+TQ-2 group, and 250.40 g at day 1 to 234.20 g for the OLZ+TQ-3 group at day 14 ($p = 0.011$, $p = 0.001$, $p = 0.015$, $p = 0.312$, $p = 0.127$, respectively). There was a significantly increased total body weight gain in the control, OLZ groups and a significantly decreased body weight gain in the OLZ+TQ-1 treatment group ($p = 0.011$, $p = 0.001$, $p = 0.015$, respectively). However, the OLZ+TQ-2 and OLZ+TQ-3 groups were observed to have a decreased weight gain and had no significant effect on these measurements ($p = 0.312$, $p = 0.127$, respectively) (Tab. 1, Fig. 1, paired-samples T-test for the body weight at day 14).

Tab. 3. Levels of serum biochemical parameters for all groups.

Parameters	Control	OLZ	OLZ+TQ-1	OLZ+TQ-2	OLZ+TQ-3	p
ALT (U/L)	64.00±1.77 ^{b,d,e}	74.28±0.99 ^{a,c,d,e}	67.33±0.76 ^{b,d,e}	60.00±0.65 ^{a,b,c}	56.40±1.50 ^{a,b,c}	0.000
AST (U/L)	178.85±2.85 ^b	221.85±3.46 ^{a,c,d,e}	182.50±7.64 ^{b,e}	170.28±3.27 ^b	166.00±1.64 ^{b,c}	0.000
GGT (U/L)	4.00±0.57 ^b	8.00±0.57 ^{a,c,d,e}	5.16±0.87 ^b	4.71±0.83 ^b	3.60±1.12 ^b	0.003
HDL (mg/dL)	21.28±0.96 ^b	13.57±1.04 ^{a,c,d,e}	19.33±0.76 ^b	21.28±1.86 ^b	19.40±1.20 ^b	0.001
LDL (mg/dL)	10.85±1.10 ^{b,e}	16.14±1.31 ^{a,c,d}	10.00±0.68 ^{b,e}	10.57±2.40 ^{b,e}	15.60±1.28 ^{a,c,d}	0.017
TG (mg/dL)	57.00±2.29 ^{b,e}	76.71±2.34 ^{a,c,d,e}	55.50±3.54 ^b	51.28±2.25 ^b	48.00±4.74 ^{a,b}	0.000
CH (mg/dL)	46.71±1.61 ^{b,d,e}	58.00±1.75 ^{a,c,d,e}	40.83±1.97 ^{b,d,e}	29.85±2.87 ^{a,b,c}	28.20±2.55 ^{a,b,c}	0.000
Glucose (mg/dL)	141.57±1.90 ^{b,e}	160.42±7.05 ^{b,c,d,e}	142.16±3.99 ^{b,e}	129.42±3.84 ^b	127.60±3.52 ^{a,b,c}	0.000
Insulin (µU/mL)	0.15±0.02 ^{b,d,e}	0.09±0.01 ^{b,d,e}	0.13±0.01 ^{d,e}	0.24±0.01 ^{a,b,c,d}	0.34±0.01 ^{a,b,c,d}	0.000

Each group represents the mean ± SEM for seven rats. ^ap < 0.05 vs control group; ^bp < 0.02 vs OLZ group; ^cp < 0.03 vs OLZ+TQ-1 group; ^dp < 0.04 vs OLZ+TQ-2 group; ^ep < 0.05 vs OLZ+TQ-3 group. OLZ – olanzapine; TQ – thymoquinone; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma glutamyl transpeptidase; HDL – high-density lipoprotein cholesterol; LDL – low density lipoprotein cholesterol; TG – triglycerides; CH – total cholesterol; OLZ+TQ-1 – OLZ+25 mg/kg TQ; OLZ+TQ-2 – OLZ+50 mg/kg TQ; OLZ+TQ-3 – OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group

Tab. 4. Effects of olanzapine and thymoquinone on apoptotic index (%) in rat liver.

Groups	Apoptotic Index (%) (AI; mean±SD)
Control	2.60±0.89 ^{b,c,d,e}
OLZ	17.60±1.14 ^{a,c,d,e}
OLZ+TQ-1	7.80±1.30 ^{a,b}
OLZ+TQ-2	7.40±1.14 ^{a,b}
OLZ+TQ-3	8.50±5.05 ^{a,b}

The apoptotic index of all the groups. Values are mean ± SD for seven rats in each group. a – Significant from control; b – Significant from OLZ; c – Significant from OLZ+TQ-1; d – Significant from OLZ+TQ-2; e – Significant from OLZ+TQ-3 (p≤0.05). OLZ – olanzapine; TQ – thymoquinone; OLZ+TQ-1 – OLZ+25 mg/kg TQ; OLZ+TQ-2 – OLZ+50 mg/kg TQ; OLZ+TQ-3 – OLZ+100 mg/kg TQ. Amounts of 4 mg/kg once a day for the first week, 8 mg/kg once a day for the second week of OLZ were given to all groups, except for control group

Ameliorative effects of TQ treatment against OLZ administration significantly increased the serum TAS level and decreased TOS, OSI levels (p < 0.05). The control group had significantly higher TAS level compared to both OLZ and OLZ+TQ-3 groups (p = 0.000 and p = 0.002, respectively). The OLZ+TQ-1 group

had significantly higher TAS level compared to control, OLZ, OLZ+TQ-2 and OLZ+TQ-3 groups (p < 0.001). The OLZ+TQ-2 group had significantly higher TAS level than both OLZ and OLZ+TQ-3 groups (p = 0.002 and p = 0.018, respectively) (Tab. 2, Fig. 2). TOS level was significantly higher in OLZ group compared to control, OLZ+TQ-1, OLZ+TQ-2 and OLZ+TQ-3 groups (p < 0.002). The OLZ+TQ-1 group had a significantly lower TOS level than both control and OLZ+TQ-3 group (p = 0.019 and p = 0.002, respectively). The OLZ+TQ-3 group had a significantly higher TOS level compared to OLZ+TQ-2 group (p < 0.030) (Tab. 2, Fig. 2). OSI level was significantly higher in OLZ group compared to control, OLZ+TQ-1 and OLZ+TQ-2 groups (p = 0.003, p = 0.002 and p = 0.003, respectively) (Tab. 2, Fig. 2).

ALT, AST, GGT, LDL, glucose, TG, and CH levels were significantly increased in the OLZ group compared to the control, OLZ+TQ-1, OLZ+TQ-2 and OLZ+TQ-3 groups, while the HDL and insulin levels were decreased (p < 0.01). ALT and CH levels were significantly lower in the OLZ+TQ-2 group compared to the

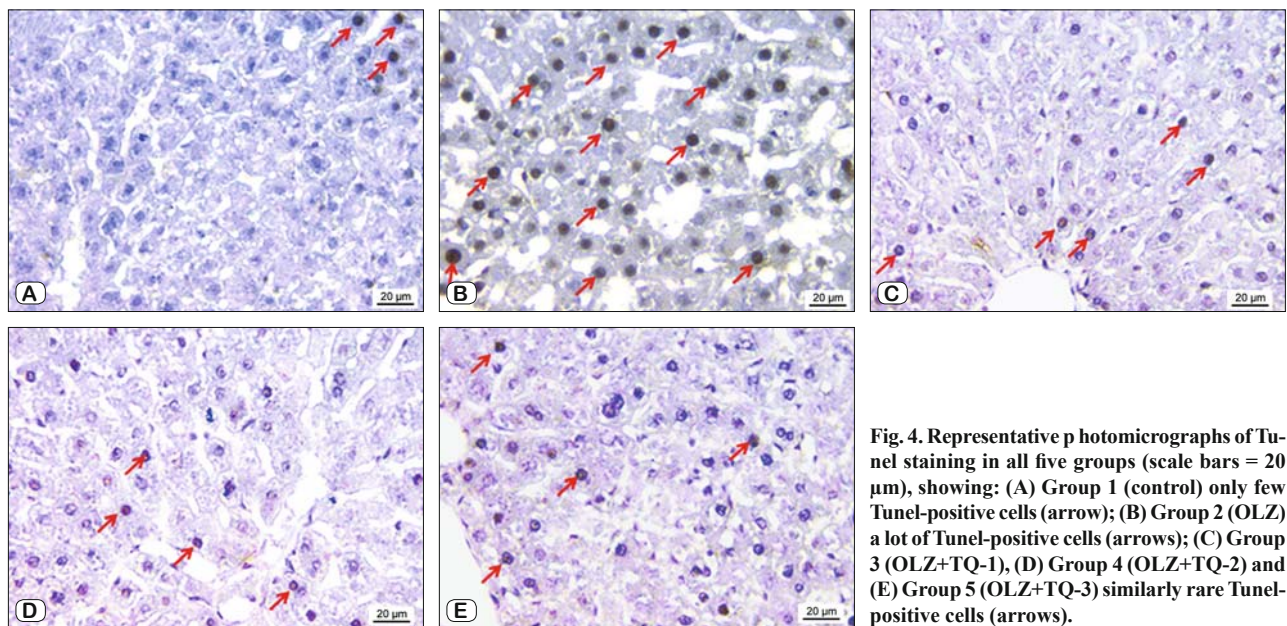


Fig. 4. Representative photomicrographs of TUNEL staining in all five groups (scale bars = 20 µm), showing: (A) Group 1 (control) only few TUNEL-positive cells (arrow); (B) Group 2 (OLZ) a lot of TUNEL-positive cells (arrows); (C) Group 3 (OLZ+TQ-1), (D) Group 4 (OLZ+TQ-2) and (E) Group 5 (OLZ+TQ-3) similarly rare TUNEL-positive cells (arrows).

OLZ+TQ-1 group ($p < 0.01$). ALT, AST and CH levels were significantly lower in the OLZ+TQ-3 group compared to the OLZ+TQ-1 group, while the LDL level was increased ($p < 0.02$). LDL level was significantly lower in the OLZ+TQ-2 group compared to the OLZ+TQ-3 group ($p < 0.03$). ALT and CH levels were significantly lower in the OLZ+TQ-2 group compared to the control group ($p < 0.02$). ALT, TG and CH levels were significantly lower in the OLZ+TQ-3 group compared to the control group, while the LDL level was increased ($p < 0.05$) (Tab. 3, Fig. 3).

Evaluation of apoptosis in liver tissue

The results of the apoptotic index are shown in Table 4 and Figure 4. Using TUNEL assay to detect apoptotic hepatic cells in the liver sections, the control (Fig. 4A) group showed only a few TUNEL-positive cells. The number of TUNEL-positive cells markedly increased in OLZ (Fig. 4B) group compared with the control group ($p < 0.05$). OLZ+TQ-1 (Fig. 4C), OLZ+TQ-2 (Fig. 4D) and OLZ+TQ-3 (Fig. 4E) groups were similar and showed rare TUNEL-positive cells. Treatment with TQ (OLZ+TQ-1, OLZ+TQ-2 and OLZ+TQ-3 groups) (Fig. 4C, 4D and 4E) reduced the number of TUNEL-positive cells as compared with the OLZ group ($p < 0.05$).

Discussion

Many efforts have been ongoing for the identification and development of adjunctive medications to prevent the side effects induced by antipsychotics, including OLZ. Despite the advantages of treatment with OLZ, the reported side effects including, weight gain, impaired glucose metabolism and dyslipidemia, elevated liver enzymes and hepatotoxicity should be taken into account (26, 27). Therefore, it is extremely important to prevent adverse effects and other metabolic disorders induced by OLZ. In addition, its effects on the liver, the primary organ for drug activation and detoxification, still remains unclear. Dietary intake of natural compounds, including TQ, can inhibit metabolic adverse effects of OLZ and thereby may reduce the risk factors in the liver. Hence, the aim of the current study was to investigate the effect of TQ treatment against administration of OLZ in liver of rats (28, 29).

The increased energy storage induced by OLZ was extensively considered a result of both increased energy intake (such as hyperphagia) (30, 31) and reduced energy expenditure (such as decreased thermogenesis) (32–34). In a study performed by Ader et al showed that dogs treated with OLZ for 4–6 weeks and assessed parameters related to weight gain, adiposity and insulin resistance (35, 36). In the present study, initial and final body weight of experimental animals were measured. All doses of TQ caused dose-dependent decreases in body weight of the animals as compared to OLZ administrated rats. However, TQ co-treatment ameliorated these changes with a more obvious effect and significantly decreased only in group with 25 mg/kg dose. So far multiple *in vivo* studies have reported excellent effectiveness of TQ treatment on liver diseases (39). Hence TQ, a dietary supplement, significantly prevents OLZ-induced weight gain, which might suggest a potential effectiveness on human subjects. Therefore, TQ is a safe compound for co-administration with OLZ for mitigating antipsychotic-induced

weight gain/obesity without influencing their therapeutic action. In addition, these results are certain to influence the choice of protective compound when several options exist and to institute preemptive strategies for weight management.

We investigated whether OLZ causes alterations in glucose metabolism and insulin secretion. Our findings confirmed that administration of OLZ caused a major impairment of glucose metabolism and a diminution of insulin action in liver. However, there are several other explanations possible. The explanation that fits best with the current data is that OLZ might be acting directly or indirectly to increase glucose level. Accordingly, a study conducted by Chintoh et al (2009) demonstrated that increased glucose was observed in OLZ-treated rats (37). On the other hand, in our study, TQ prevented the increase in fasting plasma glucose, impaired glucose tolerance and alteration in insulin sensitivity. Accordingly, the antihyperglycemic effect of TQ has been reported earlier in streptozotocin–nicotinamide-induced diabetic rats (38). The exact mechanisms of action of TQ against OLZ, impaired glucose tolerance and insulin resistance are not known. A further molecular study is required to find out the exact mechanisms of action of TQ against OLZ-induced impaired glucose tolerance and insulin resistance.

Our results indicated that OLZ exposure produced a significant increase in the activity of liver enzymes. ALT, AST and GGT indicating a damaged structural and functional hepatic integrity. Oral supplementation of TQ lowers the liver injury scores, restores the elevated serum ALT, AST and GGT activities. TQ co-treatment ameliorated these changes in all doses, especially the more obvious effect in high dose. These results are supported by literature data (40). In addition, we demonstrated that TQ prevented the increase in TG, total CH and LDL as well as decrease in HDL caused by OLZ consumption. All doses of TQ caused dose-dependent decreases in serum lipids as compared to OLZ administrated rats. However, TQ co-treatment ameliorated these changes with more obvious effect and significantly decreased only in group with 100 mg/kg dose. The effect of TQ on serum lipids has been reported earlier in experimental conditions (20). This finding is probably a consequence of feeding behavior and increase in body weight. Although underlying physiological pathways are not fully understood, the present findings indicate that OLZ increases and TQ decreases the serum lipids.

Oxidative stress is one of the key mechanisms responsible for liver damage and disease progression. TAS measurement has been used to evaluate the overall performance of the antioxidant system. TOS measurement provides a sensitive index of lipid peroxidation and oxidative stress (41). The TOS/TAS ratio is termed as “OSI”, which is an indicator of the oxidative stress degree (42). In the present study, we have observed that TQ could protect the liver from OLZ-induced liver injury. We measured TAS, TOS, and OSI levels at the same time to evaluate the oxidative stress status more accurately, and found that serum TAS levels increased and TOS/OSI levels prominently decreased with TQ treatment as in the previous studies (44). Likewise, OLZ administration resulted in a decrease in the liver TAS level and have also reported a decline in TAS after OLZ exposure (43). The beneficial effects of

TQ, obtained in the current work, are very likely due to its strong antioxidant properties. The mechanisms of TQ antioxidant action are not yet clear. Nevertheless, TQ is known to reduce oxidative stress not only through the direct antioxidant effect but also indirectly. Previous studies reported that TQ acted as a strong reactive oxygen species (ROS) scavenger (45, 46).

Histopathological evaluation of liver showed severe damage ensued by loss of liver normal architecture which include vacuolar degeneration of hepatocytes and fatty changes in OLZ-administrated rats. These toxic effects were effectively prevented by antioxidant TQ administration. Among the 3 doses, 100 mg of TQ/kg body weight was found to provide optimum protective effect on liver against OLZ-induced abnormal changes. Histological observations added more evidence to the protective effect of TQ. The present study indicates that OLZ damaged the histological structure, impaired the function, inhibited the endogenous antioxidant defense system and stimulated TOS in the rat liver tissue. Besides, our results demonstrate, for the first time, that TQ oral supplementation, at safe doses, has a remarkable protective effect against OLZ-induced liver damage in rats. This protection makes TQ a promising agent in a variety of conditions where cellular damage is a consequence of oxidative stress. Therefore, OLZ-induced liver injury causes increased ROS formation and subsequent toxic events. Accordingly, in our study, TQ treatment of the cells against OLZ exposure had a great effect on apoptotic cell injury and death. The underlying protective mechanism of TQ may be associated with the suppression of apoptosis via death receptor-mediated pathways. Hence, it can be assumed that the antioxidant activity of TQ may be due to the effect on mitochondria-independent apoptotic pathway which has to be supported with further experimentations. Therefore, TQ may be the best choice against OLZ-induced side effects.

On the one hand, co-treatment of OLZ-exposed rats with TQ in the present study significantly affected the liver OLZ burden. On the other hand, it improved the histopathological changes, ameliorated the impaired hepatic function, enhanced the reduced TAS, and inhibited the elevated TOS, and OSI levels in the liver. OLZ mediated ROS formation by diminishing antioxidant levels. Oxidative stress and antioxidant depletion could lead to apoptotic cell death (47). In this study, we found that TQ had a significant protective role in apoptotic cell death, which might be due to the ROS scavenging property. Taken the previous findings and suggestions together, it can be concluded that TQ could prevent OLZ-induced liver injury and histological perturbations through the enhancement antioxidant defense system, suppression of oxidative stress, and attenuation of apoptosis.

In conclusion, TQ may be a promising agent to improve OLZ adverse effects, oxidative stress, and apoptotic status, and so reduce weight gain and prevent liver damage in patients. Thus, daily consumption of TQ should be considered as a promising way to prevent liver damage. Our results could be used for planning strategies to protect against adverse effects of OLZ in the liver and also in other organs. Hence, further *in vivo* and clinical studies are needed to confirm the protective effects of TQ in patients receiving OLZ.

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