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RESEARCH ARTICLE

IN VITRO EFFECTS OF ANTIOXIDANT AND PROAPOPTOTIC ACTIVITIES OF THYMOQUINONE IRON COMPLEX

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ABSTRACT

Introduction: This study aimed to investigate the proapoptotic and antioxidant effects of the Thymoquinone (TQ) iron complex on the SW480 cell line. This study investigates the proapoptotic and antioxi-dant effects of the TQ iron complex on the SW480 cell line.
Material and Methods: The SW480 cells were routinely cultured in a medium for 48 h. and incubated at 37°C in a 5% CO2 in the air. After the incubation period, the cells were washed with buffer, and 100 ml of the denaturing lysis buffer per 0.5 was added to $2x10^7$ cells for 15 min, and supernatants were tak-en. ELISA test was used to examine the expression and activity of GADD153, Wee1, cleaved Caspase-3, Bax, GRP78, and Bcl-2 proteins in SW480 cells. In this study, to measure activities of total antioxidant capacity (TAS), catalase (CAT), total oxidant capacity (TOS), and superox-ide dismutase (SOD) activities were investigated by the ELISA method in cell lines SW480 treated with the TQ iron complex.
Results: ELISA test results indicated that the activities of apoptotic proteins Bax, Wee1 Caspase-3, GADD153, GRP78, and Bcl-2 in human SW480 cell lines were significantly increased in the 48-hour treatment.
Conclusion: Our results of this study demonstrated that in untreated cultures, high TAS, SOD and CAT ac-tivities were found in SW480 cell lines than in control cell lines.

Key Words: TQ, iron chelation, bax, caspase-3, bcl-2, iron, drug

INTRODUCTION

Iron is an essential element for almost all living organisms, including humans, plants, bacteria, and humans. However, as it is a transition metal, it can participate in the Fenton reaction and the generation of free radicals and reactive oxygen species (ROS) (1,2). Iron is an essential element in the human body involved in numerous metabolic processes such as ATP production, oxygen transport, deoxyribonucleic acid (DNA) synthesis, mitochondrial respiration, electron transport and oxygen consumption (3,4,5).
The amount of iron in the body is tightly regulated physiologically; however, the body does not have a mechanism to expel excess iron. Iron overload occurs when excess iron builds up in the body. In such situations, for example, in repeatedly transfused patients suffering from β-thalassemia or sickle cell anemia, iron chelation therapy is required(6,7,8). At the same time, Free iron can easily accept and donate electrons, and catalyze the Fenton reaction to generate hydroxyl radicals. Hydroxyl radicals are highly reactive species attacking most of the organic molecules. Hydroxyl radicals can damage important classes of biological molecules, including lipids, polypeptides, proteins, and nucleic acids, especially thiamine and guanosine (9-14).

Iron chelation therapy with desferrioxamine (DFO) has been available since the late 1960s. Nevertheless, it does not fit the ideal drug properties discussed above. Recently, Deferasiroks (DFX), oral iron chelator, has been successful in clinical trials patients and implemented as an alternative to DFO, but there are limited data on its long-term efficacy and safety in children. During the last few years, the oral chelator deferiprone (DFP) has been approved in many coun-tries. It has side effects that limit its usefulness, which the most adverse ones are musculoskele-tal, gastric, and hematologic effects (15-19).

Iron chelators have recently been demonstrated to be beneficial for cancer treatment. Colorectal cancer (CRC) is one of the most common cancers globally. It maintains a high mortality rate despite the latest methodological treatment approaches adopted in various academic establish-ments. Currently, there is a trend of searching for anticancer chemicals in natural sources (20-23).

TQ is the active compound extracted from Nigella sativa. Several pharmacological actions of TQ have been investigated, including anti-tumor anti-histaminic, anti-inflammatory, and anti-oxidant effects. Numerous preclinical studies have been conducted to determine the anticancer effects of TQ. (24-29).

This study aimed to investigate the proapoptotic effect of the TQ iron complex on SW480. In this study, CAT, SOD, TOS, and TAS activities were investigated by the ELISA method in SW480 cell lines. However, we investigated the iron chelation of TQ that may be used in the treatment of iron overload.

MATERIAL AND METHODS

Cell Culture

SW480 cells were maintained under 37°C in 5% atmospheric CO2 and cultured in a medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% fetal bovine serum (FBS) in 75 cm2 tissue culture flasks. At the end of the treatment period, cells were treated with 3mM pantothenic acid [30].

Cell Homogenization

The cells (104/cm2) were exposed to a 99.2% medium and a 0.8% TQ for 48 h. The cell pellet was reconstituted in 3ml Radio-Immunoprecipitation Assay (RIPA) buffer, 30µl protease in-hibitör, 30µl phenylmethylsulfonyl fluoride (PMSF), and 30µl sodium vanadate, which were added to the cells and kept on ice for 10 minutes.

ELISA Test

The ELISA test was used to examine expression, and activity is used to examine the expression and activity of Wee1, Bax, Caspase-3, GADD153, GRP78, and Bcl-2 proteins.

Biochemical Analysis

Superoxide dismutase activity was determined by using its ability to inhibit the reduction of NBT by superoxide ions generated by the xanthine/xanthine oxidase system (33). CAT activity was determined by the method described by Aebi (34). TOS and TAS were spectrophotometrical-ly measured using a commercially available kit.

RESULTS

This study aimed to investigate the proapoptotic effect of the TQ iron complex on human SW480 cell lines. The TQ treatment significantly raised the levels of Caspase-3 and Bax and in SW480 cells when compared to the control group ($p<0.05$) (Fig. 1, 2). However, the TQ iron complex treatment reduced the Bcl-2 levels in SW480 cells. ($p<0.05$) (Fig 3). In SW480 cells treated with TQ, GRP78 AIF and Wee1 levels were also investigated. Treat-ment with the TQ iron complex resulted in statistically significant increased levels of Wee1, GRP78 and AIF in SW480 cells when compared to the control group ($p<0.05$) (Fig. 4, 5, 6).

SOD Activity

The SOD activity of the TQ iron complex in SW480 with and without treatment of the TQ iron complex was investigated. In untreated normal SW480 and treated normal SW480 cell lines, SOD activities were

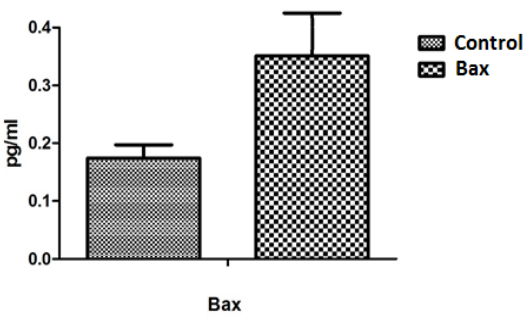

Figure 1. Effect of the TQ iron complex treatment on the Bax expression

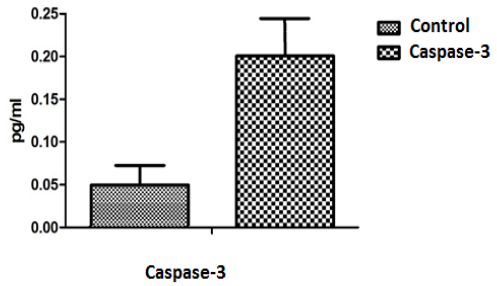

Figure 2. Effect of the TQ iron complex treatment on the Caspase-3 expression

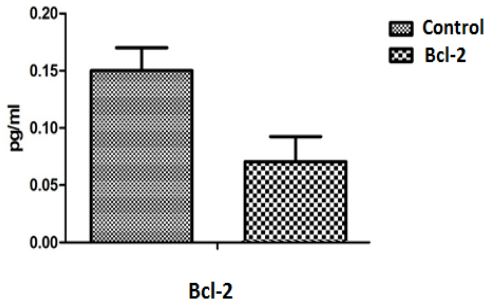

Figure 3. Effect of the TQ iron complex treatment on the Bcl-2 expression

82.12 ± 4.2 unit/mg protein and 145.45 ± 19.9 unit/mg protein, respective-ly. Additionally, the SOD activity was significantly reduced ($p < 0.05$).

Catalase Activity

The CAT activities in the TQ iron complex in SW480 cell lines with and without treatment of the TQ combination were examined. In the untreated SW480 cell line, the CAT activities were 0.75 ± 0.026 unit/mg protein and treated SW480 cell line 1.05 ± 0.18 unit/mg protein, respec-tively. The CAT activities significantly reduced ($p< 0.05$) TQ in SW480 cell lines when com-pared to the untreated culture.

TOS and TAS Activity

The TAS level was significantly increased in the treated TQ iron complex group than in the con-trol group ($P< 0.05$). The total oxidant capacity was found higher in the treated TQ than in un-treated cell lines ($P< 0.05$). At the end of the study, the TQ iron complex significantly reduced levels of TOS (15.45±3.9 compared to 18.58±6.02 (mmol H2O2/L)), and TAS (0.85± 0,46 compared to 1.46±0,44 (mmol Trolox equiv./L).

DISCUSSION

The present study is the first one demonstrating an increase in the expression of GRP78, Wee 1, Bax, Caspase-3 and AIF protein in SW480 cells after the TQ treatment. In this study, TQ sig-nificantly increased the concentration of Wee 1 and GADD153 and Bax, Caspase-3, AIF, Wee 1, and GRP78, while it significantly reduced the amount of Bcl-2 in SW480. Furthermore, this study revealed that the TQ iron complex was effective in inducing apoptosis. The Bcl-2 family and Caspase are the main mediators in the apoptotic pathway. This study indi-cated that the TQ iron complex promoted the Bax expression, decreased the Bcl-2 protein expression, and increases the Caspase-3 activation in cells. It also showed that reduced Bcl-2 ex-pression could enhance the anticancer effects of the TQ iron complex on cells. The Bcl-2 family can function against Bcl-2-related proteins like Bax. In this study, treatment of SW480 cells with the TQ iron complex significantly increased Bax levels, while it significantly lowered Bcl-2 levels. The TQ iron complex can also activate apoptosis by regulating Bcl-2 and Bax genes.

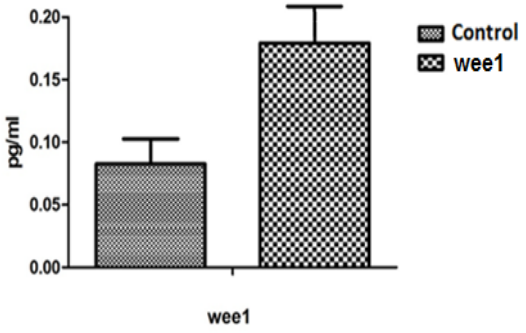

Figure 4. Effect of the TQ iron complex treatment on the wee1 expression

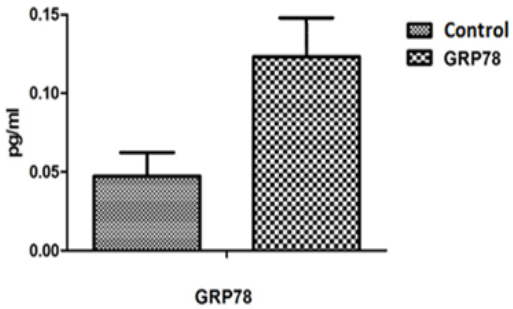

Figure 5. Effect of the TQ iron complex treatment on the GRP78 expression

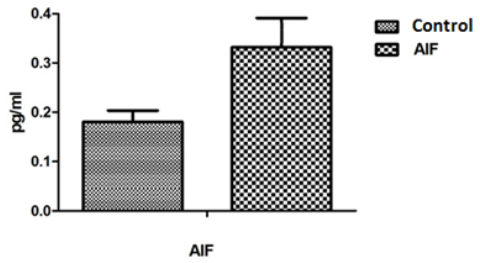

Figure 6. Effect of the TQ iron complex treatment on the AIF expression

AIF is exhibited in the induction of apoptosis. For example, in lung, breast, and colon cancer cell lines, AIF was induced by peroxides (30,31). This study showed an increase in the expres-sion of the AIF protein in SW480 cells after applying the TQ iron complex. Hassan et al. showed that TQ significantly induced apoptosis.

In this study, the TQ iron complex significantly increased the concentration of the GRP78 pro-tein in SW480 cells. The elevated GRP78 protein level on the tumor cell surface is associated with increased apoptosis.

In this study, measured the activities of SOD and CAT. The also explored the possible antioxi-dant effects of the TQ iron complex in the SW480 cell line by measuring antioxidant levels in this cell line when treated with TQ. We also studied the SOD activity of the TQ iron complex in SW480 with and without treatment of the TQ iron complex. In untreated normal SW480 and treated normal SW480 cell lines, SOD activities were 82.12 ± 4.2 unit/mg protein and 145.45 ± 19.9 unit/mg protein, respectively. In the untreated SW480 cell line, CAT activities were 0.75 + 0.026 unit/mg protein and treated SW480 cell line 1.05 + 0.18 unit/mg protein, respectively. CAT activities significantly reduced ($p< 0.05$) TQ in SW480 cell lines when compared to the untreated culture.

The TAS and TOS results were expressed in μmol H2O2 Eq/L and μmol Trolox Eq/L. The TAS was increased, and the serum TOS was decreased compared to the control levels when TQ was administered concomitantly with iron. Meanwhile, TQ is known to exhibit a strong antioxi-dant activity.

In this study, I demonstrated that the TQ iron complex significantly increased the proapoptotic protein expression in SW480 cell lines, while it significantly reduced the Bcl-2 expression. We also showed that the complex exhibited a catalase activity. In conclusion, We study confirms previous reports on the therapeutic potential of TQ.

CONCLUSION

In this study, I demonstrated that the TQ iron complex significantly increased the proapoptotic protein expression in SW480 cell lines, while it significantly reduced the Bcl-2 expression. We also showed that the complex exhibited a catalase activity. In conclusion, We study confirms previous reports on the therapeutic potential of TQ.

Author contributions: All authors discussed the results and contributed to the final manuscript. G.Ö. and A. A designed the study. G.Ö and A.A. analyzed the data. A.A.carried out the experiment. G.Ö and A. A. wrote the manuscript.

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