

Resveratrol Attenuates Benzo(a)pyrene-Induced Dysfunctions, Oxidative Stress and Apoptosis in Pancreatic Beta-Cells

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Abstract

Background: Diabetes mellitus is one of the major health problems for people all over the world today. According to international diabetes federation reports, diabetes affects 382 million people worldwide. Environmental pollutants have deleterious effects on glucose metabolism and cause insulin resistance. We aimed to investigate the effects of the environmental pollutants benzo(a)pyrene, and the therapeutic potential of resveratrol. **Methods:** 20 µM of benzo(a)pyrene was administered after 48 h of resveratrol (80 µM) application for 24 h in INS-1 (832/13) insulinoma cells. The cells were treated with 20 µM benzo(a)pyrene for 24 hours after 48 hours initial preconditions with 10 µM resveratrol. Oxidative stress status, insulin secretion and apoptosis were analyzed by molecular techniques. **Results:** Though resveratrol increased the antioxidant status which was decreased by benzo(a)pyrene, interestingly, it increased the oxidative status. Resveratrol increased benzo(a)pyrene-depleted reduced glutathione levels to the control level. The mRNA expression levels of beta-cell functions associated with genes insulin-1, insulin-2 and sirtuin-1 were upregulated by resveratrol. Resveratrol treatment elevated the insulin concentration of culture medium, and the mRNA expression of forkhead box protein-1 gene. Resveratrol upregulated benzo(a)pyrene-downregulated p53 gene expression. On the other hand, benzo(a)pyrene-downregulated mRNA expression of B-cell lymphoma-2 was induced by resveratrol treatment. **Conclusion:** The data showed that resveratrol could reverse the oxidative alterations, functional impairments and the carcinogenetic effects of benzo(a)pyrene in pancreas beta-cells.

Keywords

Benzo(a)pyrene, INS-1 (832/13) Insulinoma Cell, Insulin, Apoptosis,

1. Introduction

Diabetes mellitus (DM) is characterized by exclusively or partially deficiency of insulin released from the pancreas. Type 2 DM (T2DM) is closely related with insulin resistance and is life threatening as significant public health problem in adults, children and adolescents [1] [2] [3]. Diabetes ranks seventh in the causes of death. In the early stages of T2DM, the beta cells produce excessive amounts of insulin to ensure the continuation of normal blood glucose levels. However, beta cells can not cope with mitochondrial dysfunction, resulting in endoplasmic reticulum stress and apoptosis [4]. Consequently, a reduction occurs in the beta cell mass [5].

The oxidative stress may develop due to the production of reactive oxygen species (ROS) in high levels and/or lack of antioxidant defense system [6]. The activities of antioxidant enzymes are increased in diabetes. It is thought that increases of hydrogen peroxide and superoxide by the events including non-enzymatic glycosylation and auto-oxidation of glucose are the basis of this phenomenon [7]. Significant decreases in plasma reduced glutathione (GSH) levels occur in diabetes [8]. The GSH is the most important bio-molecule in defense against chemicals. It also acts as a free radical scavenger and can repair cell damage caused by radicals [9].

The essential factors for the progression of the T2DM are obesity and insulin resistance. However, this results in inadequate insulin secretion from beta cells to compensate for insulin deficiency. In either case, it is regarded as the main pathway of the beta cell death, apoptosis. There are many potentially effective stimulants in the apoptosis of the beta cell. These are Fas ligand and Fas receptors as death receptors, perforins, cytokines [10] [11], ROS [12], reactive nitrogen species [13], alkylating agents [14], ceramide [15]; lack of growth factors [16].

It has been hypothesized by Longnecker and Daniels [17] that chemical contaminants may play an important role in the etiology of T2DM. This hypothesis was supported by epidemiological studies; this is an indication that the increase in insulin resistance or incidence of T2DM is a consequence of exposure to high levels of arsenic, organochlorine contaminants and air pollution [18] [19] [20] [21].

Pollution has harmful effects on human health directly. Approximately 40% of human deaths in the world depend on environmental pollution. Even people who have never smoked in their lifetime are suffering from respiratory diseases such as lung cancer due to air pollution. Polluted waters cause many diseases. More than 1.2 million people in the world cannot find clean water for their lives. Contaminated soil carries chemicals and numerous toxins. Benzo(a)pyrene [B(a)P] is a compound with a pentacyclic polycyclic aromatic hydrocarbon

structure. The most important source of atmospheric B(a)P is wood burning. It is also found in coal tar, in automobile exhaust fumes (especially in diesel engines) and in all fumes from organic materials and grilled food burning [22]. The metabolites of B(a)P are carcinogenic and cause DNA degradation. For example, Benzo(a)pyran-8-diol-9,10-epoxide forms further DNA products [23] [24].

Resveratrol (RES) is a natural phytoalex produced by many fruits and plants, including grapes. It prevents DNA damage and lipid peroxidation in cell membranes. Various research studies [25] [26] [27] have indicated that it is necessary to carry out further studies on how RES can be used in cancer treatment. Resveratrol activates sirtuin (Sirt)-1 [28] which plays an important role in many different physiological events such as cell cycle regulation, metabolism and inflammation [29]. Previous studies have demonstrated that Sirt-1 has a positive regulatory role in insulin secretion and the persistence of beta cell function [30] [31] [32].

However, to our knowledge, there is no previous report on the direct effect of environmental pollutant B(a)P on insulin levels produced by pancreatic beta cells. Therefore, the present study was designed to evaluate the possible effects of RES on oxidative stress, dysfunction and apoptosis in the pancreatic beta cells exposed to B(a)P *in vitro*.

2. Materials and Methods

2.1. Materials

All chemicals except resveratrol (Cayman Chemical, Tallin, Estonia) were purchased from Sigma-Aldrich (Germany). INS1-E pancreatic cell line was obtained from Lisa Poppe (Duke University Medical Center, USA).

2.2. Cell Culture and Treatments

Rat pancreatic INS-1 (832/13) cell line was cultured in RPMI 1640 supplemented with 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin, 1 mM sodium pyruvate, 5 mM glutamine, 50 µM 2-mercaptoethanol and 10 mM HEPES buffer. Cells were incubated at 37°C in a humidified atmosphere (95% air, 5% CO₂). INS-1 cells were seeded at a density of 2.1×10^6 cells/ml in a 75-cm² culture flask and grown for 2 - 3 days before use.

2.3. Experimental Groups

INS-1 β -cells were divided into four groups: control group, resveratrol administered group, benzo(a)pyrene treated group, and benzo(a)pyrene treated followed by resveratrol administered group. Resveratrol was administered in an amount of 10 µM for 48 h in RES and RES + B(a)P groups [33]. After first incubation, 20 µM B(a)P [34] was added into the flasks in B(a)P and RES + B(a)P groups, and this second incubation was carried out for 24 h. After last incubation, the cells were separated from medium and used for biochemical analysis. Each assay was performed in triplicate and repeated three times.

2.4. Protein Analysis

The medium was removed, and the cells were harvested by trypsin-EDTA treatment and washed twice with PBS. The harvested cells were lysed in lysis buffer. The lysates were centrifuged at 12.000 g (4°C, 10 min). The protein concentrations were determined in supernatants by Bradford method [35].

2.5. Glucose Stimulated Insulin Secretion Analysis

After incubating with resveratrol and B(a)P, the cells were washed in Krebs-Ringer bicarbonate (KRB) solution containing 2.8 mM glucose for 1 h, with subsequent incubation in KRB solution, containing 2.8 mM glucose for 30 min at 37°C. At the end of the experiment, secreted insulin levels were quantified in the medium samples by the Rat/Mouse Insulin ELISA kit (EZRFMI-13K, Merck Millipore, Merck Darmstadt, Germany).

2.6. Measurements for Oxidative Stress-Related Parameters

The total antioxidant status (TAS) of the cell lysate was determined by the test kit from Rel Assay Diagnostics (Gaziantep, Turkey). This colorimetric method based on the bleaching of color characteristics of a more stable ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation by antioxidants [36].

The total oxidant status (TOS) levels were determined by the test kit from Rel Assay Diagnostics (Gaziantep, Turkey) using a novel automated method, which was developed by Erel [37], in the cell lysates. In this method, oxidants presented in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity was measured spectrophotometrically at 530 nm wavelength. The results were calibrated with hydrogen peroxide and expressed as $\mu\text{mol H}_2\text{O}_2$ equiv./mg prot).

The Oxidative Stress Index (OSI), which is an indicator of the degree of oxidative stress, was calculated according to the formula: $\text{OSI} = \text{TOS} (\mu\text{mol/mg prot}) / (\text{TAS} (\text{mmolTroloxEquiv/L}) \times 100$ [38].

The Griess reaction was used for determining the NO levels in the cell lysates [39]. The results were expressed as $\mu\text{mol/mg protein}$.

The GSH level of samples was determined by spectrophotometer at 412 nm after the reaction between sulfhydryl groups present in supernatant and DTNB (5,5'-2-dithiobis nitrobenzoic acid) [40]. The results of the analysis were expressed as $\mu\text{mol/mg protein}$.

2.7. Total RNA Isolation and Real Time PCR Analysis

The cells were seeded in 25-cm² culture flasks and grown for 1 - 2 days before use. Cells were collected and washed with PBS after completion of incubations given above. Total RNA was isolated by RNAeasy kit according to manufacturer's instructions (Qiagen, Valencia, CA). cDNA was generated with a First Strand

cDNA Synthesis kit according to manufacturer's instructions (Thermo Scientific, Waltham, MA USA). Briefly, 1 µg of total RNA was used as template in RT reaction, which was carried out with RevertAid™ H(-) M-MuLV Reverse Transcriptase (MBI Fermentas) following instructions provided by the manufacturers. RNA was mixed with 10 µM primer (oligo dT, random hexanucleotides or specific primers), incubated 5 min at 65°C, and kept on ice for 2 min to allow hybridization. Then, RT reaction Mix (buffer 5X, dNTPs mix 10 mM each one, RNase inhibitor RNaseOUT (40 U/µl) (Invitrogen)) and Reverse Transcriptase were added following manufacturer instructions. After 60 min incubation at 42°C (M-MuLV) the RT enzyme was heat inactivated at 70°C for 5 min.

The real-time PCR reactions were performed using a Stratagene Mx3005P QPCR system (Agilent, CA, USA). The reaction mix contained 12.5 µl of Maxima SYBR Green qPCR Master Mix (2×), no ROX (Thermo Scientific, Massachusetts, USA), 400 nM of each primer, 2 µl of cDNA and nuclease-free water to reach a final volume of 25 µl. The thermal cycling conditions comprised an initial denaturation step of 5 min at 95°C and 35 - 40 amplification cycles which included denaturation at 95°C for 30 s and annealing at 54°C - 70°C for 60 s (Table 1). A melt curve analysis was performed after the last cycle, in order to investigate the specificity of the amplicon and the presence of reaction artefacts such as primer dimer, using a temperature gradient from 60°C to 100°C and a ramp speed of 0.5°C·s⁻¹ (for 10 s) and continuous fluorescence measurement. Expression levels of the target genes were normalized to the housekeeping gene GAPDH. Gene expression values were then calculated based on the $\Delta\Delta C_t$ method using the equation: $RQ = 2^{-\Delta\Delta C_t}$ [41]. The primer sequences used in PCR reactions and PCR conditions are described in Table 1. Each assay was performed in triplicate and repeated three times.

Table 1. Oligonucleotide primer sequences and PCR programs.

Transcripts	Primer Sequences	PCR Programs	Cycles
p53	F-5'CGGAGGTCGTGAGACGCTG'3 R-5'CACATGTACTTGTAGTGGATGGTGG'3	94°C-1m/59°C-1m/72°C-1m	40
Foxo1	F-5'GTGAACACCATGCCTCACAC'3 R-5'CACAGTCCAAGCGCTCAATA'3	95°C-15s/60°C-1m/72°C-1m	40
Ins-1	F-5'CCTGCTCGTCCTCTGGGAGCCCAAG'3 R-5'CTCCAGTGCCAAGGTCTGAAGATCC'3	94°C-1m/68°C-1m/72°C-1m	35
Ins-2	F-5'CCTGCTCATCCTCTGGGAGCCCCGC'3 R-5'CTCCAGTGCCAAGGTCTGAAGGTCA'3	94°C-1m/70°C-1m/72°C-1m	40
Sirt-1	F-5'AGGGAACCTCTGCCTCATCTAC'3 R-5'GGCATACTCGCCACCTAACCT'3	94°C-1m/60°C-1m/72°C-1m	35
Bcl-2	F-5'CAGCTGCACCTGACGCCCTT'3 R-5'CCCAGCCTCCGTTATTCTGGA'3	94°C-1dk/58°C-1dk/72°C-1dk	40
GAPDH	F-5'GTCGTGGAGTCTACTGGCGTC'3 R-5'GATGACCCTTTGGCACCACC'3	94°C-1m/54°C-1m/72°C-1m	35

p53, Tumor protein 53; Foxo-1, Forkhead box protein O1; Ins-1, Insulin-1; Ins-2, Insulin-2; Sirt-1, Sirtuin-1; Bcl-2, B-cell lymphoma-2; GAPDH, Glucose-6-phosphate dehydrogenase.

2.8. Statistical Analysis

Since normal distribution was not observed Kruskal Wallis test was used followed by the Conover-Iman test. $P < 0.05$ was considered as statistically significant between the groups. The results were expressed as mean \pm standard deviation. Data were analyzed with the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows.

3. Results

The oxidative stress-related parameters such as NO, TAS, TOS, GSH and OSI are outlined in **Table 2**. Interestingly, resveratrol pretreatment significantly ($p < 0.05$) increased NO production in RES + B(a)P group compared to B(a)P-treated cells. Resveratrol significantly ($p < 0.05$) increased the TAS in both RES treated groups. On the other hand, B(a)P ($p < 0.05$) decreased the TAS level significantly in B(a)P group compared to the CTRL group. These results demonstrated that resveratrol supported the cellular antioxidant defense against B(a)P-induced oxidative events. In OSI levels, there was no significant difference between the RES + B(a)P and B(a)P groups. The GSH levels were significantly ($p < 0.05$) increased both in the RES group and RES-B(a)P group in comparison with the control and B(a)P group, respectively.

Resveratrol pretreatment increased insulin production significantly ($p < 0.05$) in RES and RES + B(a)P groups compared to the CTRL and B(a)P groups, respectively (**Table 3**). Comparison of mRNA expression levels of genes in INS1-E beta cells is presented in **Figure 1**. Resveratrol upregulated the FoxO1, a gene related with cell proliferation, by approximately 1.43-fold in RES group in comparison with the control group. The similar upregulation (1.23-fold) of FoxO1 was seen in the B(a)P group too. On the other hand, resveratrol increased the FoxO1 mRNA expression by 1.23-fold in the RES + B(a)P group compared to the B(a)P group (**Figure 1**). mRNA expression level of Sirt-1, that has an effective role in the glucose-mediated insulin synthesis, was upregulated by resveratrol in all resveratrol treated groups. This increasing was occurred in the RES + B(a)P group compared to the B(a)P group (**Figure 1**). In our study, B(a)P treatment suppressed the mRNA expression of ins-1 gene by 1.70-fold in the B(a)P group compared to the control. The suppression of ins-2 gene expression was 2.17-fold in the same group. These results demonstrate that B(a)P significantly reduces the expression of insulin-encoding genes in this study. On the other hand, resveratrol increased the expression of these two genes in all groups treated with resveratrol (**Figure 1**).

The expression of p53 mRNA was 1.40-fold reduced by the B(a)P application in group B(a)P compared to the control group. On the other hand, an increase of 1.60-fold was detected in the B(a)P + RES group in comparison with the group which was administrated only with benzo(a)pyrene (**Figure 1**).

The antiapoptotic gene Bcl-2 was downregulated by 1.15-fold in the B(a)P group in comparison with the control group, while resveratrol increased the ex-

pression of this gene by 1.20-fold in B(a)P + RES group comparison with B(a)P group (**Figure 1**).

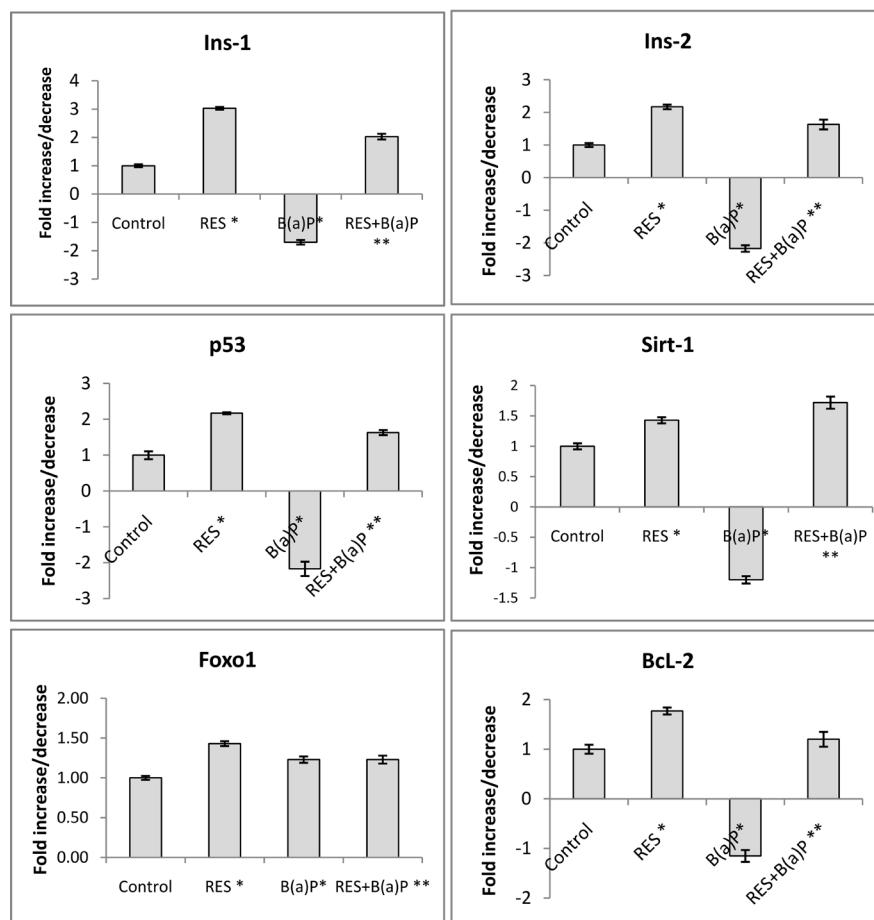


Figure 1. mRNA expression levels of various genes related with proliferation, apoptosis and activity of beta cell. Foxo-1, Forkhead box protein O1; Sirt-1, Sirtuin-1; Ins-1, Insulin-1; Ins-2, Insulin-2; p53, Tumor protein53; Bcl-2, B-cell lymphoma-2. *: Compared with control group, **: Compared with B(a)P group.

Table 2. Effect of resveratrol on the levels of NO, TAS, TOS, OSI and GSH in cell lysates.

Groups	Mean (SD)				
	NO ($\mu\text{mol/g prot}$)	TAS ($\mu\text{mol H}_2\text{O}_2$ Equiv/mg prot)	TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv/mg prot)	OSI	GSH ($\mu\text{mol/mg prot}$)
Control	10.78 (0.53) ^a	5.48 (0.83) ^a	1.26 (0.11) ^a	23.39 (3.27) ^a	5.80 (0.63) ^a
RES	15.50 (1.20) ^{bc}	8.36 (1.09) ^b	2.85 (0.40) ^b	34.40 (6.23) ^b	9.57 (0.99) ^b
B(a)P	12.30 (0.90) ^a	3.04 (1.35) ^a	1.09 (0.36) ^a	13.60 (3.30) ^c	3.77 (0.28) ^c
RES + B(a)P	17.42 (1.76) ^c	17.05 (1.53) ^c	2.88 (0.46) ^c	16.95 (2.01) ^c	5.64 (1.50) ^a

^{a,b,c}: The mean values in a different row are significantly different ($p < 0.05$). RES: Resveratrol, B(a)P: Benzo(a)pyrene, NO: Nitric oxide, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, GSH: Reduced Glutathione.

Table 3. Effect of resveratrol on secreted insulin levels.

Groups	n	Insulin (ng/ml) Mean (SD)	P-value *
Control	3	0.49 (0.05) ^a	
RES	3	0.75 (0.09) ^{bc}	<0.05
B(a)P	3	0.60 (0.01) ^b	<0.05
RES + B(a)P	3	0.99 (0.22) ^c	<0.05

*Kruskal Wallis test followed by Conover-Iman test; ^{a,b,c}The mean values in a different row are significantly different (p < 0.05). RES: Resveratrol, B(a)P: Benzo(a)pyrene.

4. Discussion

Benzo(a)pyrene is known to occur after several combustion reactions [42]. It has been revealed that the air pollution was closely related to diabetes [43].

The B(a)P metabolism causes overexpression of ROS, which are effective in cancer development, by acting as a second messenger for certain cytokines and growth factors in cells [44]. It was reported that ROS could initiate the peroxidation of lipids [45].

It is well known that GSH protects the cells against oxidative damage via its thiol groups [46]. It was reported that the B(a)P application significantly reduced the levels of GSH [47]. Similarly, the level of GSH was reduced by B(a)P in our study. This could be explained by the excessive use of GSH in the process of detoxifying of peroxide radicals that were generated by lipid peroxidation. Likewise, this result was supported by the reducing effects of B(a)P on the TAS in beta cells.

A research study has reported that resveratrol causes an increase in GSH levels in the protective role against oxidative injury [48]. In our study, increase in GSH levels was detected by resveratrol treatment in both RES and B(a)P + RES groups compared to the control and B(a)P groups, respectively. In addition, it has been reported that resveratrol acts as an antioxidant function such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [49]. This effect of resveratrol is based on its ability to protect the cell membranes against oxidative damage [50]. Resveratrol is known to have potent antioxidant properties and protective capacity against harmful effects induced by lipid peroxidation [51] [52]. Interestingly, there are some studies showing that resveratrol increases iNOS expression and NO production [53] [54]. Consistent with the literature, the level of NO was increased by resveratrol treatments in this study. This effect may be related to the activation of iNOS.

Resveratrol has been reported to reduce blood sugar and triglyceride levels, and also insulinemia in rats [55]. In consistent with previous studies, RES improved the insulin levels in this study. This effect of RES on insulin secretion could be related to the increased expression of Sirt-1 gene. Kong *et al.* [56] reported that RES improved glycemic control and insulin levels which was reduced in rats fed with high-fat diets for eight weeks period [56]. From these re-

sults, researchers have concluded that RES increased the expression of Sirt-1 gene. The results obtained in our study support the literature data.

In a study used Zucker diabetic rats, Sirt-1 gene activator improved the homeostasis of glucose and positively affected the sensitivity of insulin in fat, liver and muscle tissues. It was stated in the same study that the activation of Sirt-1 in the treatment of age-related disease and T2DM was a promising new treatment strategy [57].

It was previously demonstrated that RES increased Sirt-1 gene expression in human endothelial cell line EA.HY926 [58]. The same investigators suggested that the anti-inflammatory effect of RES could be achieved with Sirt-1 and AMP-activated kinase (AMPK). In our study, RES increased the mRNA expression level by 1.34-fold in the RES group compared to the control, and by 1.72-fold in the B(a)P + RES group compared to the B(a)P group. In this context, it is suggested that the underlying mechanism in action of RES on GSH regulation due to upregulation of Sirt-1 gene expression as described before. Sirt-1 accelerates the detoxification of ROS by upregulating cellular antioxidant enzymes, including SOD-1, catalase, GPx-1, and thioredoxin-1. GPx-1 regulates cellular oxidant status directly through elimination of hydroperoxides and *via* oxidation of GSH, the major low-molecular-weight thiol in cells [59] [60]. Thus, our findings suggest that Sirt-1 was upregulated by RES coordinately might increase GSH and GPx necessary for hydrogen peroxide reduction.

Alternatively, resveratrol may directly increase the level of glutathione with antioxidant effect. Resveratrol has been well known for its possible antioxidant role and protective effects against oxidant damage [61].

In the present study, we tested the ability of RES to attenuate the B(a)P mediated GSH depletion in INS-1E pancreatic cells. Treatment with RES + B(a)P significantly increased the level of GSH compared with treatment with B(a)P alone. It is possible that RES attenuates B(a)P-mediated depletion of GSH levels by increasing the biosynthesis of GSH and also by scavenging B(a)P-induced ROS. Polyphenolic compounds with hydroxyl groups at 4' and 5 positions, as seen in resveratrol, have the potential to scavenge free radicals [62] [63]. Our data corroborate previous observations of the ability of RES to scavenge free radicals such as OH and $O_2^{\bullet-}$ [33] and have been shown to possess the antioxidant property [64].

The tumor suppressor p53, a transcription factor that is activated by various forms of cellular stress, was one of the earliest detected cancer gene [65]. Sin *et al.* [65] reported the increased levels of p53 protein in rats treated with the combination of RES, and sirtinol which is a Sirt-1 inhibitor. In this context, the combination consisting of RES and sirtinol can be used for preventing the harmful effects of B(a)P. In our study, although RES was used alone, expression levels of p53 in RES group and B(a)P + RES group were increased by 2.07- and by 1.60-fold compared to the control and B(a)P group, respectively. Therefore, it can be said that apoptosis is stimulated via p53 mediated mechanism reported by

Soengas *et al.* [66], by RES in this study. The role of RES in the induction of apoptosis in various cells is controversial. The agent has been shown to induce apoptosis in cancer cells, such as skin cancer cells [67], and has been shown to play a protective role against apoptosis in human tenocytes [68], endothelial cells [69] and other cell types, particularly under oxidative stress [70]. And in this study, we confirmed the therapeutic role of RES against the reduced-apoptosis in INS-1 pancreatic cells exposure to B(a)P.

It has been reported that RES has anti-diabetic effects in different in vivo and in vitro studies [71] [72]. Xie *et al.* [72] showed that RES could induce the expression of several β -cell genes including insulin gene. Animal studies have shown similar beneficial effects of RES by increasing insulin secretion [73] or enhancing sensitivity to insulin in peripheral organs via activation of Sirt-1 [74]. In our study, RES increased the levels of Ins-1 and Ins-2 genes by 2.03- and 1.63-folds respectively, in RES + B(a)P group compared to the B(a)P group. In addition, insulin hormone secretion was significantly increasing in RES + B(a)P group compared with B(a)P group in this study. It has been suggested that these results are consistent with the results of previous studies mentioned above.

On the other hand, it was shown that RES increased the expression of key β -cell transcription factors such as Foxo1 and Ngn3 [72]. The increased expression of FoxO1 by resveratrol treatment in this study explains and supports the positive effect of RES on insulin levels.

The expression of the Bcl-2 gene has been reported to be significantly suppressed in the cerebral cortex of rats exposed to B(a)P. This effect of B(a)P was attributed its enhancing effect on apoptosis index [74]. In consistent with the literature, expression of Bcl-2 gene was suppressed by 1.15 fold in the B(a)P group in this study.

He *et al.* [75] applied RES as an activator for SIRT1 for preventing the effects of apoptosis induced by H₂O₂ in mouse osteoblastic cells MC3T3-E1 cell lines. With H₂O₂ administration, p53, bax and caspase-9 were stimulated while SIRT1 and Bcl-2 were inhibited. It was determined that RES stimulated the activity of both Bcl-2 and SIRT1 genes against this effect of H₂O₂ [75]. Similarly, RES increased the level of Bcl-2 expression in this study. This increase was also assumed to be effective in removing the reduction in Bcl-2 gene expression caused by B(a)P.

5. Conclusion

The harmful effects of B(a)P on beta cells of pancreas and therapeutic aspects of RES have been investigated for the first time in this study. For this reason, it is believed that the results obtained here may contribute to the literature. Based on all these results, it can be said that RES may be a very important therapeutic agent in the protection or treatment of adverse effects of carcinogenesis and oxidative changes that may occur due to B(a)P toxication in pancreatic beta cells. The demonstration of these effects of RES reveals how important RES is in

preventing the harmful effects of environmental pollutants. The importance of RES-rich foods has been once again revealed by scientific evidence. With further studies, it may be possible to illuminate the cellular or molecular mechanisms underlying these effects of RES.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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