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Research Article

Effect of Pterostilbene in induction of apoptosis through down regulation of caspase activation in ovarian cancer cells

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ABSTRACT

Introduction: Pterostilbene is a natural dietary compound present in the blue berries. It is a methylated analog of resveratrol with increased bioavailability and lipophilicity compared to that of resveratrol. So, recent research has been into much focus on this compound.**Objectives of the study:** Thus the present study was designed with an aim to elucidate the mechanism of action of pterostilbene against ovarian cancer cells.**Materials and methods:** This study was conducted at SIMS, Lahore during 2016 to 2017 with the approval of ethical committee of hospital. The 5cc blood was drawn for the analysis of ROS. Reactive oxygen species was determined using fluorescent probe DCF-DA.Pterostilbene-induced ROS generation was measured by DCF-DA.**Results:**The results showed that pterostilbene-induced ROS generation at 120%, 134% and 157% in 12, 24 and 48 h,respectively.**Conclusion:** The present study demonstrates for the first time that pterostilbene- induces apoptosis in ovarian cancer cell line, through ROS generation, mitochondrial depolarization, activation of caspase 9 and 3.

Key words: anticancer, ROS, oxidative stress, caspase

INTRODUCTION

Pterostilbene is a natural dietary compound present in the blue berries[1]. It is a methylated analog of resveratrol with increased bioavailability and lipophilicity compared to that of resveratrol. So, recent research has been into much focus on this compound[2]. Pterostilbene is an excellent antioxidant compound, which has been reported for various pharmacological properties like antifungal, anti-inflammatory, anti-diabetic and anti- cancer properties[3]. The anti - cancer role of pterostilbene has been reported in different cancers such as breast, gastric, prostate, hepatic etc., by both *in vitro* and *in vivo* studies[4]. The mechanism through which pterostilbene exerts anti- cancer potential has been reported to include both apoptosis and autophagy. Although it has been studied for its anti-cancer property in different cancers, its role in ovarian cancer has not been explored[5]. Pterostilbene (trans-3,5-dimethoxy-40-hydroxystilbene) is a naturally occurring phytoalexin identified in the genus Pterocarpus, leaves of Vitisvinifera, and some berries and grapes[6]. It has multiple pharmacologic activities, including antioxidant and cancer prevention activity and the capability to inhibit DNA synthesis[7]. Pterostilbene is also cytotoxic to various types of cancer cells, including breast cancer, melanoma, colon cancer, liver cancer, and

gastric cancer. Although anti-proliferative and pro- 0064apoptotic activities of pterostilbene have been demonstrated in vitro, the ability of pterostilbene to induce apoptosis in drug-resistant lymphoma cell lines[8].

Objectives of the study

Thus the present study was designed with an aim to elucidate the mechanism of action of pterostilbene against ovarian cancer cells. The study evaluated its cytotoxic potential and analyses its mechanism of cell death through oxidative stress parameters, ROS generation, mitochondrial membrane potential, caspase activation and cell cycle analysis.

MATERIALS AND METHODS

This study was conducted at SIMS, Lahore during 2016 to 2017 with the approval of ethical committee of hospital. The 5cc blood was drawn for the analysis of ROS. Reactive oxygen species was determined using fluorescent probe DCF-DA. Total antioxidant capacity **(**TAC) in the cells treated with pterostilbene was determined by the specific method. After an appropriate treatment period, the cells were sonicated and the cell lysate was used for the assay. The principle involves measurement of hydroxyl radical formation between the antioxidants in the sample against free radicals.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All biochemical experiments were performed thrice in triplicates to ensure reproducibility.

RESULTS

Pterostilbene-induced ROS generation was measured by DCF-DA. The results showed that pterostilbene-induced ROS generation at 120%, 134% and 157% in 12 , 24 and 48 h,respectively. Further results from Ca^{2+} levels also showed that pterostilbene caused significant levels of calcium release with maximum levels upto 180% ($p<0.001$) when compared to control cells (Figure 1).

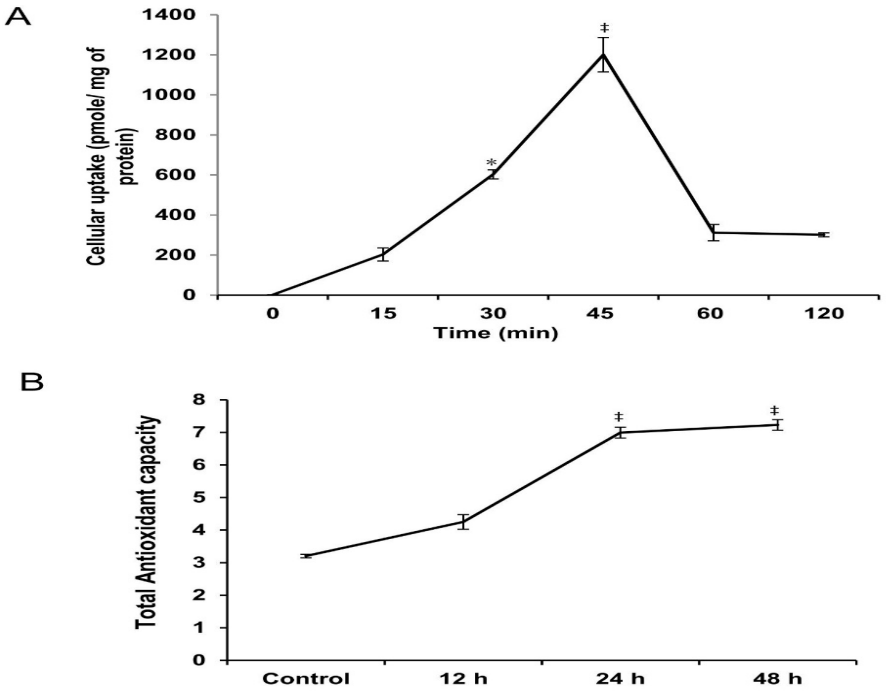

Figure 01: Pterostilbene enhances antioxidant statusA)Cellular uptake of Pterostilbene in SKOV-3 cells. Time-dependent study (15 mins, 30 mins, 45 mins, 1 hr and 2 hr) on pterostilbene uptake showed maximum levels at 45 mins when compared to control cells. **B) Pterostilbene- enhanced TAC.** Cells treated with pterostilbene at different

time points showed time dependent increase in antioxidant levels when compared to control cells(results shown as mean ± SEM). *p<0.05, †p<0.01, ‡p<0.001; NS – non significant when compared to control

DISCUSSION

Ovarian cancer is one of the common and lethal gynecologictumors worldwide. Chemo resistance plays major roadblocks for the treatment of ovarian cancer[9]. Reports suggest that compounds which sensitize these cells to cell death could act as a therapeutic strategy. The present study showed the cytotoxic effect of pterostilbene against ovarian cancer at a level of 55 uM[10]. Further, increase in LDH release during pterostilbene treatment reveals that it causes membrane damage and subsequent cell death[11].

It has been well known that reactive oxygen species mediates cell death mechanisms. The present study evidenced that pterostilbene caused a significant increase in ROS generation in a time dependent manner. However, these increases in ROS, did not affect the antioxidant status; as we observed a consistent increase in antioxidant status. Thus, this level of antioxidant activity could be explained as cellular stress mechanisms evoked to combat the cell death. It is observed in many cases, where the antioxidant enzymes are upregulated during cellular stress responses[12].

We also observed that ROS levels concomitantly increased oxidative stress markers like Nitrite levels and Lipid peroxide during pterostilbene treatment. Alterations in mitochondrial membrane potential and intracellular calcium levels play a key role in the induction of apoptosis[13]. In addition involvement of ROS and intracellular Ca $^{2+}$ levels play a major role in dissipation in mitochondrial membrane potential. The present study results show that pterostilbene resulted in a significant increase in ROS, Ca^{2+} levels and subsequent loss of membrane potential[14].

CONCLUSION

The present study demonstrates for the first time that pterostilbene- induces apoptosis in ovarian cancer cell line, through ROS generation, mitochondrial depolarization, activation of caspase 9 and 3. Dietary phenols and antioxidants play a major role in cancer prevention. However, compounds with increased biovailability have gained much importance. Owing to its higher bioavailability and apoptosis inducing capability, pterostilbene might act as promising dietary intervention in preventing ovarian cancer.

REFERENCES

1. Kapetanovic IM, Muzzio, M, Huang, Z, Thompson TN,McCormick DL. Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. Cancer ChemotherPharmacol 2011; 68:593-601.
2. Rimando AM,Kalt W,Magee JB,Dewey J,Ballington JR. Resveratrol, pterostilbene, and piceatannol inVacciniumberries.J Agric Food Chem 2004; 52:4713-9.
3. Rimando AM, Cuendet M, Desmarchelier C, Mehta RG, Pezzuto JM, Duke SO. Cancer Chemo-preventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. J Agric Food Chem 2002; 50:3453-7.
4. McCormack D, McFadden D. Pterostilbene and cancer: current review. J Surg Res 2012; 173:53-61.
5. Remsberg CM,Yáñez JA,Ohgami Y,Vega-Villa KR,Rimando AM,Davies NM.Pharmacometrics of pterostilbene: preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. Phytother Res 2008; 22:169-79.
6. Pan MH,Ho CT.Chemopreventive effects of natural dietary compounds on cancer development. ChemSoc Rev 2008a; 37:2558-74.

7. Pan MH,GhaiG,Ho CT. Food bioactives, apoptosis, and cancer. MolNutr. Food Res 2008b; 52:43-52.
8. Pan MH,Chang YH,Badmaev V,Nagabhushanam K,Ho CT. Pterostilbene induces apoptosis and cell cycle arrest in human gastric carcinoma cells. J Agric Food Chem 2007; 55: 7777-85.
9. Alosi JA,McDonald DE,Schneider JS,Privette AR,McFadden DW. Pterostilbene inhibits breast cancer in vitro through mitochondrial depolarization and induction of caspase-dependent apoptosis.J Surg Res 2010; 161: 195-201.
10. Schneider JG,Alosi JA,McDonald DE,McFadden DW. Pterostilbene inhibits lung cancer through induction of apoptosis. J Surg Res 2010; 161:18-22.
11. Mannal P,McDonald D,McFadden D. Pterostilbene and tamoxifen show an additive effect against breast cancer in vitro. Am J Surg 2010; 200: 577–80.
12. Chen RJ, Ho CT, Wang YJ. Pterostilbene induces autophagy and apoptosis in sensitive and chemoresistant human bladder cancer cells. MolNutr Food Res 2010; 54:1819-32.
13. Wang Y,Ding L,Wang X,Zhang J,Han W,Feng L,Sun J,Jin H,Wang XJ. Pterostilbene simultaneously induces apoptosis, cell cycle arrest and cyto-protective autophagy in breast cancer cells. Am J Transl Res 2012; 4:44-51.
14. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55-63.