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Extended Abstract

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Non-cytotoxic, non-genotoxic and non-mutagenic phenolic extract of cyanobacteria Oscillatoria sp. SI-SA with chemotherapeutic properties against breast cancer cell-lines

Saadia Ijaz

University of the Punjab, Pakistan

E-mail: saadiaijaz786@gmail.com

The 70% methanolic extract of Cyanobacterial strain; Oscillatoria sp. SI-SA containing phenolic compounds; Tannic acid, Orcinol, Pholoroglucinol, Salicylic acid, Acetyl Salicylic acid and Protocatechuic acid, was evaluated for its non-cytotoxic, non-genotoxic and non-mutagenic activities along with its anticancer potential against two (MCF-7 and MDA-MB-231) breast cancer celllines in order to study their chemotherapeutic effects without causing damage to normal cells. The strain was isolated from Kallar Kahar Salt Lake, Pakistan. It was identified by polyphasic approach including both morphological and molecular methodologies. The extract showed very low cytotoxicity against normal lymphocytes only at highest concentration of 1000 µg/ml with high IC50 value of 1088 µg/ml. Comet assay also showed low genotoxicity only at highest non-cytotoxic concentration of 1000 µg/ml with 15.37% DNA damage. Ames fluctuation Salmonella typhimurium test against four mutant strains TA100, TA98, TA97a and TA102 also showed significant non-mutagenic effects of the extract with and without metabolic activation. Finally, the extract was evaluated for its anticancer potential which showed significant anticancer activities in dose-dependent manner against MCF-7 and MDA-MB-231 cell-lines giving low IC50 values of 61.75 and 82.75 µg/ml respectively. It was further observed that after three days of treatment with the extract, 19.29 and 26.81 % cell viability remained of MCF-7 and MDA-MB-231 cell-lines respectively at highest concentration of 250 µg/ml. The phenolic extract of Oscillatoria sp. SI-SA indeed showed promising anticancer activities without causing severe damage to normal cells and thus could be used as an alternative bioresource for anticancer therapeutics against breast cancers. The cellular reducing environment is provided by two mutually interconnected systems; the TRX system and the glutathione (GSH) system. Under physiological conditions, the intracellular reducing environment is maintained by the disulfide/dithiol-reducing activity of the GSH and TRX systems. GSH is a cysteine-containing tripeptide (γ-glutamyl-cysteinyl-glycine), which is a major component of cytosolic antioxidant. TRX is a small protein with two redox-active cysteine residues in its active center (–Cys–Gly–Pro–Cys–) and operates together with NADPH and thioredoxin reductase as an efficient reducing system for exposed protein disulfides. While the amount of TRX (micromolar concentration) is much less than that of GSH (millimolar concentration), TRX and GSH play distinct roles in maintaining cellular environment. TRX enhances the binding of transcription factors to the target DNA more efficiently than GSH. TRX can directly associate in the nucleus with redox factor 1 (Ref-1), which is identical to a DNA repair enzyme, AP endonuclease, and both molecules through their redox-active cysteine residues augment the DNA-binding activity of transcription factors, such as activator protein 1 (AP-1) and p53. The components of the TRX system not only scavenge reactive oxygen species (ROS) but also play regulatory roles in a variety of cellular function through protein–protein interaction. Mice carrying the homozygously deleted *TRX* gene died shortly after implantation, suggesting that TRX is essential for cell survival and early development. TRX transgenic mice, which ubiquitously overexpress human TRX under the control of β-actin promoter, display various phenotypes, such as an elongated lifespan and protection against ischemic injury, acute lung failure, diabetes mellitus, and the toxicity caused by environmental stressors. Since oxidative stress has been implicated in these conditions, TRX seems to play an important role in protection against oxidative stress-associated diseases. In cooperation with peroxiredoxins (described below), TRX has an antiapoptotic effect by scavenging intracellular ROS through the dithiol at its active site. Intriguingly, S-nitrosylation at cysteine 69 is required for the reducing activity and antiapoptotic function. Proapoptotic signal including oxidative stress converges on mitochondria to induce mitochondrial outer membrane permeabilization, which is lethal because it results in the release of proapoptotic caspases-activating molecules and caspases-independent death effectors and metabolic failure in mitochondria. Permeability transition pore complex consists of voltage-dependent anion channel (VDAC) localized in outer membrane, adenine nucleotide translocator (ANT) in inner membrane, and cyclophilin D in the matrix, and opening of the pore leads to loss of the mitochondrial transmembrane potential and swelling of the matrix. Since diamide-induced crosslinking of ANT mediates the membrane permeabilization, the function of ANT is regulated by the redox. Oxidative stress seems to decrease the threshold of the pore opening to induce mitochondria-mediated apoptosis. The members of the TRX system form a network and maintain the cellular reducing environment. TRX scavenges intracellular hydrogen peroxide in collaboration with a family of thioredoxin-dependent peroxidases, peroxiredoxins. The mammalian peroxiredoxin family has six members expressed in several subcellular compartments, including peroxisomes and mitochondria, while catalase is present only in peroxisomes. Peroxiredoxins I and II are cytosolic proteins, whereas peroxiredoxin III is specifically expressed in mitochondria. Mammalian peroxiredoxin IV is found in the endoplasmic reticulum and lysosomes and also secreted into the extracellular space. Peroxiredoxin V is located in peroxisomes and mitochondria, while peroxiredoxin VI is in cytoplasm and mitochondria. All peroxiredoxins except type III also exsist in the nucleus. Peroxiredoxins I–V contain two cysteines, whereas peroxiredoxin VI has one cysteine for the catalytic activity. Peroxiredoxins protect cells against apoptotic stimuli. Mice deficient in peroxiredoxin I or II have hemolytic anemia, showing that erythrocytes are susceptible to oxidative stress.

Peroxiredoxin knockout mice thus display milder phenotypes than TRX knockout mice, suggesting that the function of peroxiredoxins is redundant and can be partly compensated. Two independent pathways of apoptosis converge in mitochondria. Stress as well as death receptor-mediated activation of caspase-8 triggers the release of proapoptotic proteins, such as cytochrome *c* and apoptosis-inducing factor (AIF), from mitochondria, leading to the activation of downstream caspases (ex. caspase-3) and subsequent execution of apoptosis. There is also a link between nuclei and mitochondria. In response to DNA double-strand breaks, histone H1.2 is released from the nucleus into the cytosol and induces cytochrome *c* release. Smac/DIABLO and HtrA2/Omi are also released from mitochondria upon receiving apoptotic stimuli and inhibit the functions of inhibitor of apoptosis proteins (IAPs; endogenous inhibitors of caspases) by direct binding, leading to the activation of caspases. HtrA2/Omi has the serine protease activity, which seems to be required for the execution of cell death and could have other targets than IAPs. Since mitochondria are at the center of several stress-induced apoptotic signaling pathways, mitochondria have several protective mechanisms. Mitochondria contain large amount of GSH which may serve as a buffer against oxidative stress. Mammalian TRX2 is specifically expressed in mitochondria and essential for cell survival. TRX2 has a mitochondrial translocation signal peptide at the N-terminus and a conserved active disulfide/dithiol-like cytosolic TRX. In mammals, there are three thioredoxin reductases (TRXRs): cytosolic TRXR (TRXR1), mitochondrial TRXR (TRXR2), and testis-specific TRX glutathione reductase (TGR). In exquisite contrast to the cytosolic TRX system, which is composed of cytosolic peroxiredoxin (mainly peroxiredoxins I and II)–TRX–TRXR1, the TRX system in mitochondria consists of mitochondrial peroxiredoxin III–TRX2–TRXR2. In a conditional TRX2-deficient chicken B-cell line, DT-40, suppression of TRX2 expression caused the accumulation of intracellular ROS and induced cytochrome *c* release and subsequent apoptosis. TRX2 prevents mitochondria-mediated cell death by scavenging ROS generated in mitochondria, which are a major physiological source of ROS during respiration and pathological conditions. In addition, TRX2 might inhibit apoptotic signaling by anchoring cytochrome *c* in mitochondria, given that TRX2 associates directly with cytochrome *c in vitro* and *in vivo*, or interacting with another molecule in mitochondria (Wang *et al.*, unpublished observation). Other reports also indicate the regulatory role of TRX2 in mitochondrial cell death. Overexpression of TRX2 confers an increase in mitochondrial membrane potential and resistance to etoposide-induced cell death. The knockout of *TRX2* gene in mice is embryonic lethal, further indicating that TRX2 is indispensable for cell survival and that TRX and TRX2 cannot compensate for each other. Recently, it was reported that mice lacking mitochondrial thioredoxin reductase (TRXR2) also die in the embryonic stage because of reduced myocardial function and perturbed hematopoiesis in the liver. The proapoptotic activity of another redox-active inducer of apoptosis, AIF, might be regulated by TRX or TRX2, although its function is reported to be independent of its NADH oxidase activity.

Bottom Note: *This work is partly presented at* EuroSciCon Joint Event on Biotechnology, Stem Cell and Molecular Diagnostics
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