Abstract

Chemotherapy is an important modality in cancer treatment. Chemotherapy drugs act by damaging highly proliferating cells and by virtue of their action they also damage the normal cells and result in toxic side effects. The side effects of chemotherapy comprise of myocardial, hepatic and renal complications, which can span from subclinical abnormalities to serious life-threatening events. Arsenic trioxide is an effective anticancer drug for the treatment of acute promyelocytic leukemia. It was approved by the Food and drug administration (FDA), U.S.A and showed a high remission rate (around 85% to 90%) in acute promyelocytic leukemia patients. But the therapeutic efficacy of As$_2$O$_3$ is burdened with serious side effects including cardiac QT interval prolongation, torsades depointes, hepatocellular damage and renal failure. Sudden death was also reported in some acute promyelocytic leukemia patients during As$_2$O$_3$ therapy, due to cardiac functional impairment. So there is a need for the identification of an effective compound, which can reduce the toxic side effects, without limiting the therapeutic potential of As$_2$O$_3$.

Omega-3 fatty acids containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have many beneficial functions, particularly on cardiovascular health, hepatic and renal functions. It is also found to exhibit anticancer properties. The existing knowledge of omega-3 fatty acid on cardiac protection enabled the study to design a combination treatment with As$_2$O$_3$ to reduce the toxicity.

The extent of toxic potential of As$_2$O$_3$ was assessed in male albino Wistar rats by supplementing As$_2$O$_3$ (2, 4 & 8 mg/kg body weight), orally for 45 days. The inductively coupled plasma optical emission spectroscopic
analysis revealed arsenic deposition in heart, liver and kidney. In serum analysis, increased concentration of cardiac, hepatic and renal marker enzymes were identified in systemic circulation. Blood glucose, serum potassium and calcium concentrations were found altered with \( \text{As}_2\text{O}_3 \) treatment. Significant depletion in the antioxidant defense mechanism and increased lipid peroxidation was observed in blood and tissues with \( \text{As}_2\text{O}_3 \) treatment. Structural abnormalities in cardiac, hepatic and renal tissues subjected to \( \text{As}_2\text{O}_3 \) treatment were noticed in histopathological examinations.

The protective effect of omega-3 fatty acid was assessed by combination treatment with \( \text{As}_2\text{O}_3 \). Two concentrations of omega-3 fatty acid (25mg and 50mg/kg body weight) were used for identifying the effective concentration against \( \text{As}_2\text{O}_3 \) (4mg/kg body weight) induced toxic effects. Omega-3 fatty acid co-treatment effectively reduced the arsenic deposition in heart, liver and kidney. A reduction in the cardiac, hepatic and renal marker enzymes was also noticed. Omega-3 fatty acid co-treatment significantly controlled the blood glucose, serum potassium and calcium concentrations. Antioxidant enzymes were significantly increased and the lipid peroxidation was found reduced. The structural aberrations of cardiac, hepatic and renal tissues were significantly decreased with omega-3 fatty acid co-treatment. From all these observations, omega-3 fatty acid at 50mg/kg body weight was found more effective than the concentration of 25mg/kg body weight in reducing the \( \text{As}_2\text{O}_3 \) toxicity.

Electrocardiographic (ECG) analysis significantly identified alterations in the cardiac conducting mechanism with \( \text{As}_2\text{O}_3 \) treatment. Omega-3 fatty acid co-treatment significantly reduced the \( \text{As}_2\text{O}_3 \) induced ECG alterations (QT and QTc intervals) by its anti-arrhythmic action. Cytokinesis block micronuclei assay revealed that the co-treatment with omega-3 fatty acid
reduced the arsenic induced micronuclei frequency, reflecting its protection against the As₂O₃ induced genotoxicity. Transmission electron microscopic analysis shows that omega-3 fatty acid improved the As₂O₃ induced cardiac ultrastructural changes-maintained cell membrane integrity, reduced interstitial edema, and the structural aberrations of mitochondria and myofilaments. The findings of the current investigation suggest that the omega-3 fatty acid is an effective cardio protective agent against As₂O₃ toxicity.

*In vitro* experiments were performed in H9c2 cardiomyocytes to identify the cellular mechanism of cardio protection. The effective concentration of docosahexaenoic acid (DHA) (100µM) against As₂O₃ toxicity was identified by cell viability assay. As₂O₃-treatment increased lactate dehydrogenase (LDH) release, lipid peroxidation, cellular calcium (Ca²⁺) levels and decreased mitochondrial membrane potential (ΔΨᵢ). Cardio protective efficacy of omega-3 fatty acid was assessed by the combination treatment with As₂O₃. Omega-3 fatty acid co-treatment significantly reduced the LDH release, lipid peroxidation, intracellular Ca²⁺ concentration and improved the ΔΨᵢ. These *in vitro* findings suggested that the omega-3 fatty acids can reduce the As₂O₃ induced toxic cellular changes in cardiomyocytes.

The effect of omega-3 fatty acid co-treatment on cancer cells was assessed with human acute promyelocytic leukemia cell line HL-60. Omega-3 fatty acid co-treatment increased the As₂O₃ induced cytotoxicity by increasing the LDH release, lipid peroxidation, intracellular Ca²⁺ level and reduced ΔΨᵢ. The reduced ΔΨᵢ may result in the activation of mitochondrial permeability transition pore opening and there by the release of apoptotic factors in to the cytoplasm, leading to cell death. The results of
the current *in vitro* investigation suggest that omega-3 fatty acid treatment during As$_2$O$_3$ therapy can increase the effectiveness of chemotherapy.

The results of the current *in vivo* and *in vitro* investigation suggest that, omega-3 fatty acid is an effective agent in reducing the serious side effects of As$_2$O$_3$. 