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Arjunolic acid, a triterpenoid saponin, ameliorates arsenic-induced cyto-toxicity in hepatocytes

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Abstract

Arsenic is a well-established environmental toxin, which damages various organs of the body. A triterpenoid saponin, arjunolic acid (AA) has been isolated from the bark of *Terminalia arjuna*. The present study was conducted to investigate the preventive role of AA against arsenic-induced cytotoxicity in isolated murine hepatocytes. Sodium arsenite ($NaAsO_2$) was chosen as the source of arsenic. Incubation of the hepatocytes with $NaAsO_2$ (1mM) for 2 h caused reduction in the cell viability and activities of the intracellular enzymatic as well as non-enzymatic antioxidants. Treatment of $NaAsO_2$ enhanced lipid peroxidation and also increased the activities of the membrane leakage enzymes. Administration of AA (100 μg/ml) before and with the toxin almost normalized the altered activities of antioxidant indices. AA possesses free radical scavenging activity and could enhance the cellular anti-oxidant capability against $NaAsO_2$-induced cyto-toxicity. The cytoprotective activity of AA was found to be comparable to that of a known antioxidant, vitamin C. Experimental results, therefore, suggest that AA protects arsenic-induced cytotoxicity in murine hepatocytes.

Keywords: Arsenic poisoning; Oxidative stress; Hepatocytes; Cyto-toxicity; Arjunolic acid; Cyto-protection

1. Introduction

Arsenic is a widespread environmental toxin. It occurs naturally as an element of the earth's crust. Exposure to higher than average level of arsenic occurs either in workplaces, e.g. in smelting industries, coal fired power plants, cosmetic industries, agriculture, etc. or through arsenic contaminated food and drinking water. According to World Health Organization (WHO), exposure of high levels of arsenic in drinking water has been recognized for many decades in some regions of the world, notably in China (Taiwan), Bangladesh, India and some countries in Central and South America. Arsenic occurs in drinking water primarily as arsenate, As(V), although in reducing environments significant concentrations of arsenite, As(III), have also been reported. Toxicity of arsenic depends on its valence state; As(III) is more toxic than As(V) [1,2].

Because arsenic targets ubiquitous enzyme reactions, it affects nearly all organ systems in humans and other animals. Acute arsenic exposure causes nausea, vomiting, colicky abdominal pain and profuse, watery diarrhea [3]; whereas its chronic exposure arises mainly from the environment and is associated with different types of cancers as well as cardiovascular diseases and neurological disorders, etc. [4–8]. After ingestion, the dissolved arsenic compounds are readily absorbed through the gastrointestinal tract and distributed in the blood to various

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organs of the body. Liver is an important target organ for arsenic toxicity [9,10]. During its cycles between different oxidation states, arsenic generates reactive oxygen species (ROS) and causes organ-toxicity [11,12]. The ROS directly react with cellular biomolecules; damage lipids, proteins and DNA in cells and that can ultimately lead to cell death [13].

Although herbal medicines stepped in folklore, methodical research has borne out many of their traditional health benefits in recent years. Some examples are aspirin (originally came from willow bark), quinine (from the bark of a Peruvian tree), vinblastine (a drug used to treat leukemia, comes from periwinkle plants), digitalis (a heart stimulant, is still made from pressed foxglove leaves). India is well established for the huge source of medicinal plants like *Silybum marianum* [14,15], *Picrorrhiza kurroa* [16], *Phyllanthus niruri* [17], *Terminalia arjuna* [18], *Andrographis paniculata* [19], *Cajanus indicus* [20], etc. *T. arjuna* (TA) holds a reputed position in both Ayurvedic and Yunani Systems of medicine. The bark of TA is used since ancient times for the treatment of cardiac disorders [21,22]. We have already established that aqueous extract of the bark of TA protects liver and kidney tissues against carbon tetrachloride and sodium fluoride induced oxidative stress [23,24]. Many active constituents like tannins, triterpenoid saponins (arjunic acid, arjunolic acid arjungenin, arjunglycosides), flavonoids, ellagic acid, gallic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, copper, etc. are present in the bark of this plant [25]. Among them some biological properties of arjunolic acid (AA) has been reported [26]. To the best of our knowledge, however, there is no report in the literature describing its cytoprotective role against arsenic-induced toxicity in hepatocytes.

The aim of the present study was to investigate the protective role of arjunolic acid against arsenic-induced cytotoxicity in murine hepatocytes. The purity of AA has been checked by using standard tools like NMR (^{1}H, ^{13}C), IR, mass spectroscopy and optical rotation studies. The radical scavenging activity of AA was determined from its 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical quenching ability in cell free system. Cell viability was measured by Trypan Blue Dye Exclusion method. The optimum dose of AA needed for cytoprotection has been determined from the dose-dependent studies. Cellular damage was evaluated by measuring alanine transaminase (ALT) and alkaline phosphatase (ALP) leakage. The antioxidant power of hepatocytes was determined by ferric reducing/antioxidant power (FRAP) assay. The prooxidant–antioxidant status of hepatocytes was determined by measuring (a) the activity of the intracellular antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx); (b) the levels of cellular metabolites, such as reduced glutathione (GSH) as well as oxidized glutathione (GSSG), total thiols; (c) the extent of lipid peroxidation.

2. Materials and methods

2.1. Plant

T. arjuna (TA) is an ornamental tree, belonging to the family of Combretaceae. The bark of TA was collected in winter season of 2006–2007 and one voucher specimen of the plant was deposited in the Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, West Bengal, India.

2.2. Animals

Healthy adult male albino mice of Swiss strain, weighing between 20–25 g were purchased from Ghosh Enterprises, Kolkata, India. The animals were acclimatized under laboratory condition for a fortnight before starting experiments. The animals were maintained on a standard diet and water ad libitum. They were housed in polypropylene cages and exposed to 10–12 h of daylight under standard conditions of temperature (30 °C) and humidity (50%). All the studies were performed in conformity with the guidance for care and standard experimental animals study ethical protocols.

2.3. Chemicals

Bovine serum albumin (BSA), Bradford reagent, Collagenase type I, 2,2-diphenyl-1-picryl hydrazyl (DPPH), Dulbecco's modified Eagle's medium (DMEM), Fetal bovine sera (FBS) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO) USA. Kits for measurement of ALT and ALP were purchased from Span diagnostic Ltd., India. Calcium chloride ($CaCl_2$), 1-chloro-2,4-dinitrobenzene (CDNB), dimethyl sulphoxide (DMSO), 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB (Ellman's reagent)], ethylene diamine tetraacetic acid (EDTA), glacial acetic acid, hydrogen peroxide (H_2O_2), *N*-ethylmaleimide (NEM), nicotinamide adenine dinucleotide reduced disodium salt (NADH), nitro blue tetrazolium chloride (NBT), oxidized glutathione (GSSG), phenazine methosulphate (PMT), potassium dihydrogen phosphate (KH_2PO_4), reduced glutathione (GSH), sodium arsenite ($NaAsO_2$), sodium azide