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Protective effect of the fruits of *Terminalia arjuna* against cadmium-induced oxidant stress and hepatic cell injury via MAPK activation and mitochondria dependent pathway

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ABSTRACT

Cadmium (Cd), a highly toxic environmental pollutant, induces hepatic disorders. The present study has been undertaken to investigate the protective role of the fruit extract of *Terminalia arjuna* (AE) against Cd-induced oxidative liver impairment using a murine model. Cadmium reduced hepatocytes viability, activated MAPKs, disturbed Bcl-2 family protein balance, increased reactive oxygen species (ROS) production and induced apoptotic cell death by mitochondria dependent caspases-3 activation. AE treatment, however, suppressed all the apoptotic actions of cadmium. Similarly, mice treated with cadmium altered a number biomarkers related to hepatic oxidative stress and other apoptotic indices. Oral administration of AE both pre and post prevented all the Cd-induced hepatic damages.

Results suggest that Cd-induced hepatic dysfunction and apoptosis might be triggered by the ROS formation and mediated via the activation of MAPK family proteins. AE treatment however, reduced Cd-induced oxidative stress, attenuated the changes in MAPK families and mitochondrion-dependent apoptotic signaling pathways.

1. Introduction

Cadmium (Cd), a highly toxic heavy metal, is mainly released from the smelting, burning of fossil fuels and municipal wastes, refining of metals, and cigarette smoking, resulting in the pollution of water, air, and soil. After entering the body, it is transported by the blood, especially by red blood cells and high molecular weight protein, albumin (Bauman, Liu, & Klaassen, 1993). Although Cd is widely distributed in the body, most of it accumulates in the liver and kidneys and has varying degrees of toxicity (Pope and Rall, 1995). Earlier reports suggest that Cd is a hepatotoxin (Casalino, Calzaretti, Sblano, & Landriscina, 2002). However, the mechanisms of Cd-induced hepatotoxicity at molecular level are not very clear. Cd-intoxication induces the expression of a number of stress genes and that might affect the activities of some anti-oxidant enzymes (Watjen et al., 2001). Cd-induced oxidative stress has been associated with the production of excessive reactive oxygen species (ROS) interaction of which with the cellular macromolecules caused lipid peroxidation, DNA damage and membrane protein degradation (Szuster-Ciesielska et al., 2000). Besides lipid peroxidation, cadmium toxicity is reported to be associated with modification in thiol-containing proteins, inhibition of energy metabolism, membrane damage and altered gene expression (Li, Zhao, & Chou, 1993).

In recent years, numerous studies describing the therapeutic properties of extracts from different parts of various medicinal plants have been developed. Indeed, the use of such extracts as complementary and alternative medicine has lately increased, and also serves as an interesting source of drug candidates for the pharmaceutical industrial research (Newman and Cragg, 2007). *Terminalia arjuna* (TA), belonging to the family Combretaceae, holds a reputed position in Ayurvedic medicine since ancient times (Scassellati-Sforzolini, Villarini, Moretti, et al., 1999). Different parts, particularly its fruit and bark are used as a human consumable component in water, milk and other drinks to maintain good health (Kiritiker and Basu, 1987). Chemical analyses showed that the entire plant is full of compounds like tannin, saponin, ester, sugar, steroids, acids and minerals etc. (Kiritiker and Basu, 1935). Experimental and clinical studies revealed the beneficial effects of this plant against various diseases by exerting its effect as gastroprotective (Devi, Narayan, Vani, & Devi, 2007) as well as antimutagenic (Kaur, Kumar, & Nagpal, 2002) activities. The beneficial role of the bark of the plant *T. arjuna* (TA) in organ pathophysiology

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has been extensively studied. However, little is known about the role of its fruit in toxin and drug-induced hepatic pathophysiology. The active constituent of this extract has already been defined by Haque, Saki, Ali, Ali, and Maruf (2008b). However, there have been no studies carried out on the protective action of these active constituents in organ pathophysiology. It is therefore, planned to extend our knowledge on the beneficial role of this fruit extract and conducted the present study to evaluate its preventive as well as curative role against Cd-induced hepatic damage and cellular death using both *in vivo* and *in vitro* working models.

2. Materials and methods

2.1. Animals

Swiss albino male mice of weighing approximately 24–27 g were acclimatised under laboratory conditions for two weeks prior to the experiments. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee.

2.2. Plant

T. arjuna (TA), belonging to the family Combretaceae, holds a reputed position in both Ayurvedic and Yunani systems of medicine. For this particular study, the fruits of TA were collected in the months of June–July of the year 2009 from the local botanical garden, Calcutta, India. Mr. Pulok Roy, the supervisor of the Bose Institute Experimental Farm and Dr. Swathi Gupta Bhattacharyya, one scientist of the botany department of Bose Institute, identified the plant and its fruits.

2.3. Preparation of the fruit extract of T. arjuna

The phytochemical constituents present in the fruit of TA were extracted using the method described by Haque, Saki, Ali, Ali, and Maruf (2008a). Authenticated fruits of TA were collected from local botanical garden, cut into small pieces and ground into powder. The powder (1 kg) was immersed in 2 l. of 50% aqueous ethanol (1 l ethanol and 1 l distiled water) at room temperature and refluxed for 48 h using Soxhlett's apparatus. Then the extract was collected and the process of extraction was repeated five times with 50% aqueous ethanol until the extract became almost colourless. The extracts were then combined and filtered. The filtrate was concentrated by removal of ethanol in a rotary evaporator under reduced pressure at a temperature below 45 °C. A greenish mass was obtained.

2.4. Extraction of arjunolic acid (AA)

The extraction of arjunolic acid (AA) was carried out by following the method of Ghosh, Das, Manna, and Sil (2010b). Briefly, the bark of *T. arjuna* was cut into small pieces, dried, ground into powder and extracted with petroleum followed by diethyl ether. The crude material was filtered off and the mother liquor was concentrated under reduced pressure to yield a white amorphous solid which showed two spots on thin layer chromatography (TLC). The material was then subjected to separation on silica gel column. Elution with chloroform:methanol yielded a compound later identified as arjunolic acid (m.p. 325–328 °C). Approximately 250 mg of AA was obtained from 1 kg of the bark.

Pure standard mixture of arjunetin, arjungenin, arjunolic acid, and arjunic acid has been isolated from the bark of *T. arjuna* according to the method of Sing, Verma, Sing, and Gupta (2002). HPLC analysis was carried out using a C_{18} column (8 × 10 cm). The column was eluted with a mobile phase 70:30 acetonitrile/water, flow rate 1 ml/min. UV detection was done at 220 nm.

2.5. Qualitative analysis of the fruit extract of T. arjuna

2.5.1. Test for flavonoids

A portion of crude mass was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow colouration (Edeoga, Okwu, & Mbaebie, 2005).

2.5.2. Test for saponins

Crude mass (0.5 g) was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins (Kapoor, Singh, Kapoor, & Shrivastava, 1969).

2.5.3. Test for tannins

Crude mass (0.5 g) was stirred with 10 ml of distiled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of tannin (Harborne, 1973).

2.5.4. Test for terpenoids

Crude mass (0.5 g) was dissolved in 5 ml of methanol. 2 ml of the extract was treated with 1 ml of 2,4-dinitrophenyl hydrazine dissolved in 100 ml of 2 M HCl. A yellow–orange colouration was observed as an indication of terpenoids (Kolawole, Oguntoye, Agbede, & Olayemi, 2006).

2.5.5. Test for glycosides

Crude powder mass (0.5 g) dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. The mixture was heated in a boiling water bath for 30 min. Five millilitres of Fehling's solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides (Harborne, 1973).

2.6. Quantitative analysis of the fruit extract of T. arjuna

2.6.1. Flavonoid determination

Crude mass (5 g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 m). The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight (Boham and Kocipal-Abyazan, 1974).

2.6.2. Saponin determination

Total saponin content was determined according to the method of Obadoni and Ochuko (2001).

2.6.3. Total phenol determination

Total phenol content was determined according to McDonald's method using Folin–Ciocalteau reagent (Gallic acid as a standard) (McDonald, Prenzler, Autolovich, & Robards, 2001).

2.6.4. Carbohydrate estimation

Carbohydrate content was estimated using anthrone reagent (Yemm & Willis, 1954).