Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Antioxidant and antimutagenic activities of bark extract of *Terminalia arjuna*

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ARTICLE INFO

Article history:
Received 29 September 2010
Received in revised form 4 October 2010
Accepted 15 December 2010
Available online 20 December 2010

Keywords:
Terminalia arjuna
Antioxidant activity
Antimutagenic activity
Micronuclease test

ABSTRACT

Objective: The alcoholic extract of stem bark of *Terminalia arjuna* (ALTA) was screened for antioxidant and antimutagenic (anticlastogenic) activity. **Methods:** Antioxidant property was determined by 1,1,Diphynyl,2-Picryl hydrazyl (DPPH) assay, super oxide radical scavenging activity, lipid peroxidation assay and total polyphenolic content was determined by Folin-Ciocalteau's reagent. Antimutagenic activity was evaluated using micronucleus test in mice. **Results:** The ALTA has shown potent antioxidant activity with EC_{50} of 2.491±0.160, 50.110±0.150 & 71.000±0.250 in DPPH assay, superoxide radical scavenging activity and lipid peroxidation assay, which is comparable with ascorbic acid with EC_{50} of 2.471±0.140, 40.500±0.390 and 63.000±0.360 respectively. In micronucleus test, ALTA (100 & 200 mg/kg, p.o.) showed significant reduction in percentage of micronucleus in both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) and also shown significant reduction in P/N ratio. **Conclusions:** These results suggested that ALTA possess significant antioxidant and antimutagenic activity.

1. Introduction

Free radicals are the highly reactive species capable of wide spread, indiscriminate oxidation and peroxidation of proteins, lipids and DNA which can lead to significant cellular damage, the involvement of free radicals in pathological process such as aging, behavioural and psychiatric disorders, cancer, atherosclerosis and rheumatoid arthritis is well recognized[1]. Antioxidant reacts with reactive oxygen species (ROS) to quench the radicals and to produce less aggressive chemicals species likely to cause tissue damage. Much attention has been focused on the use of antioxidants because of their protective effect against damage from reactive oxygen species, on this basis the beneficial effect of antioxidants are being increased[2]. It is now widely recognized that the antioxidants are also useful in treatment of cancer. The changes in the base pair sequence of genetic material (either DNA or RNA) is called mutations, mutations can be caused by copying errors in the genetic material during cell division and/or by exposure to ultraviolet and/or ionizing radiation, chemical mutagens, viruses or it can also occur deliberately under cellular control during processes such as meiosis or DNA replication[3-5]. One of the best ways to minimize the effect of a mutagen is by the use of anticlastogens; they act by interfering with DNA repair and/or with mutagen metabolites and/or with free radicals.

Naturally occurring substances from plant origin and dietary components have been widely studied for the antimutagenic activity, which includes phenolics, pigments, allylsulphides, glucosinolytes, anthocyans, phytosterols, protease inhibitors, phytoestrogens, carotenoids, flavonoids, tea-polyphenols, vitamins, cucuminoids, tannins, coumarins, chlorophyllin, porphyrins and alkylresorcinols from cereal grains[6]. The anticlastogenic phytochemicals also play an important role in prevention of cancer. Hence there is a need to establish the relations between antioxidants and anticlastogenic agents[7].

Terminalia arjuna (Combretaceae) (*T. arjuna*) is an

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evergreen large decidous tree; the plant has been reported to be used in ayurvedic system of medicine for derangement of all the three humours kafa, pitta, vayu and all types of cardiac failure[8], dropsy, diuretics, antiinfective[9], antiasthamatic, treatment of rheumatoid arthritis and treatment of cancer[10]. Keeping in mind the great medicinal value of *T. arjuna* and its high content of polyphenols, flavones and flavonoids, present study was planned to investigate the antimutagenic effect of ALTA.

2. Materials and methods

2.1. Drugs and chemicals

Analytical grade petroleum ether, 95% ethanol, distilled water, methanol (AR Grade, Rankem, S.A.S nagar), cyclophosphamide (Sigma, Germany), bovine albumin fraction-V (Otto kemi, Mumbai), geimsa's stain (Loba Chemi, Mumbai), May-Gruenwald's stain (Loba Chemi, Mumbai) and sodium azide (Loba Chemi, Mumbai) were used for the study.

2.2. Plant material

2.2.1. Collection and identification of plant material

The dried bark of *T. arjuna* was obtained from the Natural Remedies Ltd. Bangalore and it was authenticated by Dr. H.B. Singh, Head, Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources, New Delhi.

2.2.2. Extraction of plant material

The shade dried plant material was powdered. The coarse powder was subjected to successive extraction with petroleum ether, alcohol (95%) in soxhlet apparatus (at 60-80 ℃) and the marc obtained after alcoholic extraction was macerated with distilled water to obtain an aqueous extract.

2.2.3. Phytochemical investigation

The alcoholic (ALTA) and aqueous extracts (AQTA) of *T. arjuna* bark obtained were subjected to various phytochemical tests for identification of secondary metabolites present in them[11].

2.2.4. Determination of total polyphenols

The total polyphenol content (TPC) was determined by spectrophotometry, using tannic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The concentration of polyphenols in samples was derived from a standard curve of tannic acid ranging from 10 to 50 μg/mL and expressed in terms percentage[10].

2.3. Experimental animals

Wistar rats (200 to 250g) and Swiss albino mice (18-22) purchased from Bioneeds, Tumkur for experimental purpose were all acclimatized for 7 days under standard husbandry conditions, *i.e.*; room temperature of (25±1) ℃; relative humidity of 45%-55% and a 12:12 h light/ dark cycle.

All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of P.E.S College of Pharmacy, Bangalore (Karnataka) and were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.4. Acute toxicity studies

Acute oral toxicity of the ALTA was determined by using female, nulliparous and non pregnant mice weighing 18-22 g. The animals were fasted for 3 h prior to the experiment. Up and down procedure OECD guideline No. 425 was adopted for toxicity studies (http://www.epa.gov/oppfead1/harmonization/). Animals were administered with single dose of extract and observed for their mortality during 48 h (acute) and 14 days (chronic). LD_{50} was calculated as per OECD 425 using AOT 425 stat program.

2.5. Antioxidant activity

2.5.1. DPPH assay[12]

A working solution of methanolic DPPH (Sigma, Germany) having an absorbance of 0.9 at 516 nm was used. This was prepared by taking 150 μL of stock solution (12.9 mg of DPPH in 10 mL of methanol) in 3 mL of methanol. To 150 μL of DPPH solution in methanol, different concentrations of ascorbic acid were added and the total volume was made up to 3 mL with methanol. DPPH diluted to 3 mL was taken as blank. Decrease in absorbance in the presence of ascorbic acid was noted down at 516 nm after 15 min. A standard graph was plotted between concentration vs absorbance and EC_{50} values were calculated. The test solutions were treated in the similar manner and the EC_{50} values were calculated.

2.5.2. Superoxide radical scavenging activity[13]

It was carried out by using Nitro blue tetrazolium (NBT) reagent, the method is based on generation of super oxide radical (O_2^-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, during the reaction the NBT is reduced to nitrite.

In brief, aliquots of 0.1 to 1.0 mL to ascorbic acid solution were taken in a test tube, to which 1 mL of sodium carbonate, 0.4 mL of NBT and 0.2 mL of EDTA were added and zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 mL of hydroxylamine