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Food and Chemical Toxicology 44 (2006) 984–993
www.elsevier.com/locate/foodchemtox

# *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice

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Received 26 March 2005; accepted 2 December 2005

**Abstract**

Most pomegranate (*Punica granatum* Linn., Punicaceae) fruit parts are known to possess enormous antioxidant activity. The present study evaluated antioxidant and hepatoprotective activity of pomegranate flowers. Alcoholic (ethanolic) extract of flowers was prepared and used in the present study. The extract was found to contain a large amount of polyphenols and exhibit enormous reducing ability, both indicative of potent antioxidant ability. The extract showed 81.6% antioxidant activity in DPPH model system. The ability of extract to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) was tested and it was found to significantly scavenge superoxide ($O_2^{\bullet -}$) (by up to 53.3%), hydrogen peroxide ($H_2O_2$) (by up to 30%), hydroxyl radicals ($^{\bullet}OH$) (by up to 37%) and nitric oxide (NO) (by up to 74.5%). The extract also inhibited $^{\bullet}OH$ induced oxidation of lipids and proteins in vitro. These results indicated pomegranate flower extract to exert a significant antioxidant activity in vitro. The efficacy of extract was tested in vivo and it was found to exhibit a potent protective activity in acute oxidative tissue injury animal model: ferric nitrilotriacetate (Fe-NTA) induced hepatotoxicity in mice. Intraperitoneal administration of 9 mg/kg body wt. Fe-NTA to mice induced oxidative stress and liver injury. Pretreatment with pomegranate flower extract at a dose regimen of 50–150 mg/kg body wt. for a week significantly and dose dependently protected against Fe-NTA induced oxidative stress as well as hepatic injury. The extract afforded up to 60% protection against hepatic lipid peroxidation and preserved glutathione (GSH) levels and activities of antioxidant enzymes viz., catalase (CAT), glutathione peroxidase (GPX) glutathione reductase (GR) and glutathione-S-transferase (GST) by up to 36%, 28.5%, 28.7%, 40.2% and 42.5% respectively. A protection against Fe-NTA induced liver injury was apparent as inhibition in the modulation of liver markers viz., aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and albumin in serum. The histopathological changes produced by Fe-NTA, such as ballooning degeneration, fatty changes, necrosis were also alleviated by the extract. These results indicate pomegranate flowers to possess potent antioxidant and hepatoprotective property, the former being probably responsible for the latter.


*Keywords:* *Punica granatum*; Pomegranate; Antioxidant; Hepatoprotective; Fe-NTA

## 1. Introduction

The reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($^{\bullet}OH$) are implicated in oxidative damage to various cellular macromolecules (Farber, 1994). Increasing number of evidences suggest that oxidative stress induced biochemical changes are crucial etiological factors in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in ageing process (Hogg, 1998). Based on growing interest in free radical biology and lack of effective

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doi:10.1016/j.fct.2005.12.001

therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is warranted (Jacob and Sotoudeh, 2002). Several synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are available, but are quite unsafe and their toxicity is a problem of concern (Madhavi and Salunkhe, 1995). Therefore, in recent years, considerable attention has been directed towards identification of natural antioxidants (plant derived) that may be used for human consumption.

*Punica granatum* Linn. (Punicaceae), commonly known as pomegranate, is a shrub or a small tree, native to the Mediterranean region. The plant possesses an immense therapeutic value. A number of biological activities such as antitumour (Afaq et al., 2005), antibacterial (Prashanth et al., 2001), antidiarrhoeal (Das et al., 1999), antifungal (Dutta et al., 1998), antiulcer (Gharzouli et al., 1999) have been reported with various extracts/constituents of different parts of this plant. Pomegranate, especially gulnar (flowers of pomegranate), has extensively been used in Unani and Ayurvedic systems of medicine (Sivarajan and Balachandran, 1994). The flowers are antidiabetic (Jafri et al., 2000) and strongly astringent; their decoction stops bleeding and purging. The infusion prepared by boiling the buds is used to cure chronic diarrhea, especially in children.

Pomegranate is now gaining importance because of its potent antioxidant activity. Pomegranate fruit juice, fruit and peel extracts have been found to possess a tremendous antioxidant activity (Gil et al., 2000; Noda et al., 2002; Chidambara Murthy et al., 2002; Singh et al., 2002). Some potent antioxidants have been isolated from the fruit juice and have been found to be bioavailable, effective and safe (Cerda et al., 2003a,b). Certain fruit juice flavonoids have also been found to prevent low-density lipoprotein oxidation and hence are antiarthrogenic (Aviram et al., 2002; Wang et al., 2004). Recently fruit juice has also been shown to modulate the expression of oxidation-sensitive genes in cultured EC and in atherosclerosis-prone areas of hypercholesterolemic mice (de Nigris et al., 2005). Pomegranate wine (Schubert et al., 2002) and fruit constituents (Afaq et al., 2005) inhibit nuclear factor k B (NFkB), a transcription factor activated by ROS and hence implicated in pathophysiology of numerous diseases. The seed oil (Schubert et al., 1999), seed extract and peel extract (Singh et al., 2002) of pomegranate also have a potent antioxidant activity. However, no studies have so far been reported on antioxidant activity of pomegranate flowers. Thus, in the present study, we have extensively studied the antioxidant activity of alcoholic extract of pomegranate flowers using both in vitro and in vivo models.

In vitro, the ability of extract to scavenge various reactive oxygen species (ROS) and reactive nitrogen species (RNS) and inhibit oxidation of biomolecules was determined. In vivo, the protective activity of pomegranate flower extract was determined against ferric nitrilotriacetate (Fe-NTA) induced hepatoxicity in mice. Fe-NTA is known to generate ROS and induce oxidative stress in liver and kidney. Its long-term administration causes severe hepatic and renal tumours (Toyokuni, 1996). We have previously shown that Fe-NTA is a hepatic tumour promoter that mediates its effect by inducing oxidative stress in the liver (Iqbal et al., 1995). Lipid peroxidation is known to be a crucial mechanism of Fe-NTA toxicity, with 4-hydroxynonenal, a product of lipid peroxidation, having a chief involvement (Iqbal et al., 1999a). Fe-NTA also depletes GSH, GSH metabolizing enzymes and other antioxidant enzymes causing oxidative injury to the tissue. Therefore, potent antioxidants are expected to suppress its toxicity. We have earlier shown the inhibition of Fe-NTA induced oxidative stress and carcinogenesis by a number of substances (Ansar et al., 1999; Iqbal and Athar, 1998). In this study, we investigated the inhibition of Fe-NTA induced oxidative injury in mice liver by alcoholic extract of pomegranate flowers.

The present paper reports and discusses antioxidant potential and hepatoprotective activity of alcoholic extract of pomegranate flowers.

## 2. Materials and methods

### 2.1. Materials

Napthylethylenediamide dihydrochloride, sodium nitroprusside (SNP), DPPH, oxidized and reduced glutathione, Folin–Ciocalteau reagent, NADPH, butylated hydroxy anisole (BHA), 2-mercaptoethanol, phenylmethylsulphonyl fluoride (PMSF), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and nitrilotriacetic acid (NTA) were purchased from Sigma Chemicals, St. Louis, MO, USA. Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), ethylene diamine tetraacetic acid (EDTA) disodium salt and sulphosalicylic acid were procured from Amresco. Thiobarbituric acid (TBA), $K_3Fe(CN)_6$ and hydrogen peroxide were purchased from E. Merck. All other chemicals and reagents used were of the highest commercially available purity.

### 2.2. Preparation of the extract

The air-dried flowers of pomegranate were purchased from Khari Baoli, New Delhi, India and authenticated by Dr. M.P. Sharma, Department of Botany, Hamdard University. The powdered flowers (1.27 kg) were Soxhlet extracted exhaustively with (95%) ethanol. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried ethanolic extract, which was 23.51% of the starting material.

### 2.3. Total phenolics

Total phenolics in the alcoholic extract of pomegranate flowers were determined by the method of Taga et al. (1984). One hundred milligrams of the extract was extracted with 250 ml of methanol/water (60:40, v/v, 0.3% HCl) and filtered through a 0.45 μm Millipore filter. To 100 μl filtrate, 100 μl of Folin–Ciocalteau reagent (50%, v/v) and 2.0 ml sodium carbonate (2%, w/v) were added and mixed completely. After 2 h, the absorbance of the solution was measured at 750 nm. Quantitation was based on the standard curve of gallic acid (0–1.0 mg/ml), dissolved in methanol/water (60:40, v/v; 0.3% HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent (GAE). To determine polyphenolics level in serum, the proteins were first removed by precipitation with 10% TCA and then polyphenolics level measured by Folin–Ciocalteau reagent.