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Red onion extract (*Allium cepa* L.) supplementation improves redox balance in oxidatively stressed rats

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Abstract

Onions, consumed worldwide, are a good source of dietary phytochemicals with proven antioxidant properties. Catechin and quercetin are the most common and widely consumed flavonoids. The present study aimed to investigate the possible protective effect of onion extract as well as flavonoids (catechin and quercetin) on rats subjected to oxidative stress by mercuric chloride ($HgCl_2$) treatment. Experiments were conducted on rat erythrocytes, which are a good model system to study oxidative stress. Results show that the oxidative stress induced by $HgCl_2$ in Wistar rats resulted in substantially increased erythrocyte lipid peroxidation and higher activity of red cell plasma membrane redox system (PMRS) along with corresponding decrease in the intracellular reduced glutathione and antioxidant activity. Onion extract supplementation significantly ($P < 0.05$) attenuated these adverse effects of $HgCl_2$. Flavonoid supplementation resulted in a slightly higher antioxidant response compared to onion extract. We conclude that supplementation of these flavonoids results in normalization of erythrocyte PMRS activity which provides onion (rich in quercetin), a novel mechanism to exert its antioxidant effect against $HgCl_2$-induced oxidative stress in rat erythrocytes *in vivo*.

Keywords: Onion; Catechin and quercetin; Mercuric chloride; Oxidative stress; Antioxidant activity; Plasma membrane redox status (PMRS)

1. Introduction

Flavonoids are a large group of naturally occurring phenolic constituents, ubiquitously present in edible plants, vegetables and fruits. Epidemiological evidence suggests that consumption of foods and beverages rich in flavonoids correlate with lower risk of various diseases, including certain cancers, cardiovascular diseases and oxidative stress-related diseases [1]. More than 6000 varieties of flavonoids have been identified, among them quercetin (abundant in onion, apple, broccoli and berries) and catechin (abundant in tea) are the most common and widely consumed flavonoids [2]. Onion (*Allium cepa* L.), a widely consumed vegetable, is a good source of dietary phytochemical (organosulphur compounds and flavonoid especially quercetin) with proven antioxidant properties and ability to modulate the detoxification systems [3,4]. Various scientific reports have confirmed its functional properties which include free radical scavenging activities, immune stimulation, cardioprotective effects (by lowering serum cholesterol and blood pressure), anti-cancer, and anti-infectious properties [5].

Various degenerative and metabolic diseases such as diabetes, atherosclerosis, cancer and aging are known to lead toward oxidative stress [6]. In recent years, there is renewed interest toward study of plants and their isolated compounds for the prevention of diseases and diverse pathological conditions by offering protection against cellular damage and oxidative stress [7].

The present study reports the antioxidant effect of onion extract, quercetin and catechin on markers of oxidative stress in blood (plasma membrane redox system, antioxidant capacity of plasma, erythrocyte lipid peroxidation and intracellular reduced glutathione) in a rat model of experimental oxidative stress.

2. Materials and methods

2.1. Chemicals

Quercetin, (+)-catechin, 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ). 4,7-Diphenyl-1, 10-phenanthroline disulfonic acid

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disodium salt (DPI), reduced glutathione and dithiobis nitro benzoic acid (DTNB) were purchased from Sigma Aldrich, St. Louis, USA. All other analytical grade chemicals were procured from Merck, India and Himedia Labs, India.

2.2. Preparation of extract

Fresh bulbs of onions were purchased from a local market in Allahabad, India. Among the various local varieties of onion in India, the red onion (Pusa cultivar) was preferably selected because of its reported high antioxidant potential [3]. The plant was collected, its botanical identification and authentication was confirmed and the herbarium sheets were sent to the herbarium of the Department of Botany, University of Allahabad. The voucher specimen number (reference number 25840) was obtained. The bulbs were carefully dressed and frozen (4 °C). About 100 g of onion was crushed in a grinding machine with 100 mL of chilled, distilled water. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen (4 °C) until used.

2.3. Animal model and study protocol

The experiment was carried out with 48 male Wistar rats (5 ± 0.5 months and body weight 150 ± 20 g). They were housed in a temperature controlled room (25 ± 5 °C) with 12-h light–dark cycles for at least 1 week. After the stabilization period of one week, the rats were randomly divided into eight groups, containing six animals each. Onion extracts and flavonoids (catechin and quercetin) were administered by gavage for 30 days. Flavonoids under certain reaction conditions, can display prooxidant activity, thus, we selected optimal dosages for the flavonoid treatments as reported in previous studies [8]. *Group I*: Control, receiving no treatment/supplementation. *Group II*: Onion extract treated group (1 mL/100 g bw/day) for 30 days. *Group III*: Quercetin-only group, quercetin was dissolved in 0.5% DMSO and rats were treated per day *via* gavage (20 mg/kg bw) for 30 days. *Group IV*: Catechin-only group, catechin was dissolved in 0.5% DMSO and rats were treated per day *via* gavage (20 mg/kg bw) for 30 days. *Group V* (negative control): Rats were injected intra peritonealy $HgCl_2$ 5 mg/kg body weight in 0.9% NaCl [9]. *Group VI*: Onion extract plus $HgCl_2$ treated group, onion extract (1 mL/100 g bw/day) was given *via* gavage 10 days before $HgCl_2$ injection and onion extract continued for next 30 days. *Group VII*: Quercetin plus $HgCl_2$ treated group, quercetin dissolved in 0.5% DMSO (20 mg/kg bw per day) was given *via* gavage 10 days before $HgCl_2$ injection and quercetin continued up to next 30 days. *Group VIII*: Catechin plus $HgCl_2$treated group, catechin dissolved in 0.5% DMSO (20 mg/kg bw• d) was given *via* gavage 10 days before $HgCl_2$ injection and catechin continued up to 30 days.

2.4. Collection of blood, isolation of red blood cells and plasma

Rats were sacrificed under light anesthesia. Blood samples were collected by cardiac puncture into 10 unit/mL heparin. Red cells were pelleted by centrifugation at $800 \times g$ for 10 min at 4 °C. After the removal of plasma (immediately frozen at −80 °C until use for biochemical assays), buffy coat, and the upper 15% of packed RBC, the packed RBCs were washed twice with cold phosphate buffered saline (PBS) (0.9% NaCl and 10 mmoL/L Na_2HPO_4; pH 7.4) and then used for experiment. All protocols for experiments were approved by the Animal Care and Ethics Committee of University of Allahabad.

2.5. Measurement of total antioxidant activity by FRAP

The total antioxidant potential of the plasma samples was determined using a modification of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain [10]. FRAP reagent was prepared from 300 mmoL/L acetate buffer, pH 3.6, 20 mmoL/L ferric chloride and 10 mmoL/L TPTZ made up in 40 mmoL/L hydrochloric acid. All three solutions were mixed together in the ratio 10:1:1 (v:v:v) respectively, 3 mL of FRAP reagent was mixed with 100 μL of plasma and the contents were mixed thoroughly. The absorbance was read at 593 nm at 30 s intervals for 4 min. Aqueous solution of known Fe (II) concentration in the range of 100–1000 μmoL/L was used for calibration. Regression equation of the FRAP values (μmoL Fe (II)/L) of the plasma was used for calculation.

2.6. Determination of erythrocyte malondialdehyde (MDA) content

Erythrocyte MDA was measured according to the method of Esterbauer and Cheeseman [11] with slight modification. Packed RBC (0.2 mL) were suspended in 3 mL PBS containing 0.5 mmoL/L glucose, pH 7.4. The lysate (0.2 mL) was added to 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.67% thiobarbituric acid (TBA) boiled for 20 min at 90–100 °C, cooled then the mixture was centrifuged at $1000 \times g$ for 5 min and the absorbance of supernatant was read at 532 nm. The concentration of MDA in erythrocytes was calculated using extinction coefficient ($\varepsilon = 31{,}500$) and is expressed as nmoL/mL of packed RBC.

2.7. Determination of erythrocyte GSH

Erythrocyte GSH was measured following the method of Beutler [12]. The method is based on the ability of the −SH group to reduce DTNB and form a yellow colored anionic product whose optical density is measured at 412 nm. Concentration of GSH is expressed in mg/mL packed RBCs.

2.8. Measurement of erythrocyte PMRS

The activity of the erythrocyte PMRS was estimated by the method of Avron and Shavit [13]. Briefly, PRBCs (0.2 mL) were suspended in PBS containing 5 mmoL/L glucose and 1 mmoL/L freshly prepared potassium ferricyanide to a final volume of 2.0 mL. The suspensions were incubated for 30 min at 37 °C and then centrifuged at $1800 \times g$ at 4 °C. The supernatant collected was assayed for ferrocyanide content using DPI and