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Original article

Therapeutic potential of quercetin against acrylamide induced toxicity in rats

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ABSTRACT

Acrylamide (AA) is found in foods containing carbohydrates and proteins, where it is formed during the heating process. It is classified as neurotoxic and probably carcinogenic to humans. The present investigation was aimed to determine the lethal Dose (LD_{50}) of AA and to evaluate the protective effects of quercetin (QE) against AA induced adverse effects in rats. For the determination of LD_{50}, AA was administered orally at four different doses (46.4 mg/kg, 100 mg/kg, 215 mg/kg and 464 mg/kg) to experimental animals for seven days. After 7 days LD_{50} of AA was determined using graphical method of Miller and Tainter. Then AA was administered at 1/3rd dose of LD_{50} (38.27 mg kg^{-1} body weight; p.o. for 10 days) followed by the therapy of QE (5, 10, 20 and 40 mg kg^{-1} orally), for 3 consecutive days for the determination of protective effect of QE against AA. The estimated LD_{50} of AA was 114.81 mg/kg with 95% confidence interval. Exposure to AA 1/3rd dose of LD_{50} for 10 days induced neurotoxicity which was confirmed by decreased acetylcholinesterase (AChE) activity. AA substantially increased lipid peroxidation (LPO), decreased the level of reduced glutathione (GSH) and antioxidant enzymes (SOD and CAT) in liver, kidney and brain. It also increased the activities of serum transaminases, urea, uric acid, creatinine, lipid profile, bilirubin in serum. Treatment with QE restored tissue and serological indices concomitantly towards normal levels. These results revealed that QE is able to significantly alleviate the toxicity induced by AA in rats.

1. Introduction

Acrylamide (AA, CH2 = CHCONH2) is an industrial chemical that is usually used as a monomer to produce polyacrylamides for wastewater treatment.

AA is formed in carbohydrate rich food during preparation at high temperatures raised concern about cancer risks associated with the dietary intake of fried or baked carbohydrate food. It is formed when frying, roasting, grilling or baking of carbohydrate rich foods like bread, potato crisps, crackers, and french fries at temperatures above 120 °C through interactions of amino acids with reducing sugar (maillard reaction) [1,2].

AA and its metabolite, glycidamide (2,3- epoxy1propanamide) form adducts with glutathione, proteins, and DNA and produces neurotoxicity [3]. AA form adducts with haemoglobin which results in hypoxia, thus causes vascular disturbance [4]. AA is capable of inducing genotoxic, carcinogenic, developmental, and reproductive effects in tested animals. Reproductive toxicity caused by AA was evident by dominant lethal effects, degeneration of testicular epithelial tissue, and sperm-head abnormalities [5].

Thus, it is necessary to develop novel therapeutic agent from substances possessing antioxidant activity has been considered for combating the toxicity caused by AA. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes.

The most important polyphenol compounds are the flavonoids, which are abundant components of the human diet. Flavonoids (C6-C3-C6) are compounds in which each C6 component is a benzene ring, the variation in the state of oxidation of the connecting C3 component demonstrates the properties and class of compounds. [6].

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone, Fig. 1) [7], a key representative flavonoid molecule, is found in common foods (fruits and vegetables), such as tea, berries, apples, nuts, tea, onions, cauliflower, cabbage and apples with a estimated daily intake of up to 25 mg/day in a United States [7]. It exerts numerous

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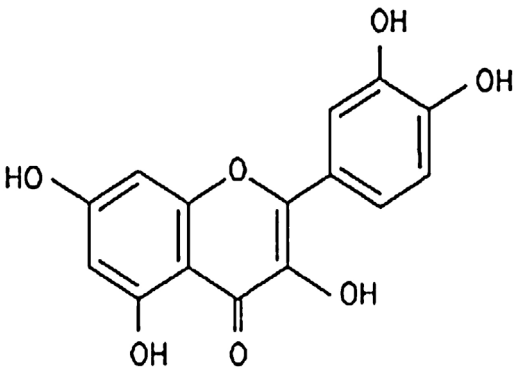

Fig. 1. Structure of quercetin.

beneficial effects on human health, including anti-ischemia and cardiovascular protection [8,9]. It's known to be an antioxidant agent [10] and it possess free radical scavenging properties and neuroprotection [11], from oxidative injury by their ability to modulate intracellular signals promoting cellular survival and by protecting brain cells against oxidative stress, a tissue damaging process associated with Alzheimer and other neurodegenerative disorders [12]. Quercetin has been reported to slow down the progression of degenerative diseases by passing through the BBB. In addition, oral administration of quercetin was also able to improve memory ability and learning [13]. It was reported to exert antitumoral, anti-allergy, antithrombotic, anti-inflammatory [14], antiapoptotic ones, as well as inhibiting platelet aggregation and protect against oxidative injury and cytotoxicity [15].

Thus, the main aim of this study is to determine the LD_{50} of AA and protective effect of QE against AA induced toxicity in rats.

2. Materials and methods

2.1. Animals and chemicals

Female albino rats of *Wistar strain* (160 ± 10 g b.w.) were used in this study. Animals were housed under standard husbandry conditions (25 ± 2 °C temp, 60–70% relative humidity, 14 h light and 10 h dark). The rats were fed on standard pellet diet and water ad libitum. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

AA was procured from Sigma Aldrich Co., USA. QE was procured from HiMedia. All the other chemicals used in this study were of analytical grade and procured from Sigma Aldrich Company (USA), E-Merck (Germany), Loba, Ranbaxy and BDH etc. All diagnostic kits used in the experiments were procured from E-Merck.

2.2. Experimental protocol

2.2.1. Experiment 1

Animals were divided into four groups of five animals each and were administered AA at different doses. All the rats were observed for a period of 24 h and thereafter for 7 days for toxicity signs including mortality. The LD50 of the AA was estimated using graphical method of Miller and Tainter [16,17].

GROUP I: AA (46.4 mg/kg p.o,)
GROUP II: AA (100 mg/kg p.o,)
GROUP III: AA (215 mg/kg p.o,)
GROUP IV: AA (464 mg/kg p.o,)

2.2.2. Experiment 2

Rats were divided into seven groups of six animals each. Group I served as the healthy control. Group II was administered QE treatment control at a dose of 40 mg/kg orally. Groups III–VII were administered AA at the dose of 38.27 mg/kg, p.o. for 10 days and group III was served as experimental control. Animals of groups IV–VII were given QE at the doses of 5, 10, 20 and 40 mg/kg p.o, for 3 consecutive days. All animals were sacrificed after 24 h of last treatment.

Group I: Control
Group II: Treatment control (QE at 40 mg/kg p.o.)
Group III: AA at the dose of 38.27 mg/kg p.o. (1/3rd of LD_{50})
Group IV–VII: AA (as in group III) + QE (5, 10, 20 and 40 mg/kg p.o.)

2.3. Serological biochemical assays

Blood samples were collected from retro-orbital venous sinus [18]. Blood samples were allowed to stand at room temperature for 1 h and then centrifuged at 3000 rpm for 10 min to isolate serum. The obtained serum was stored at −20 °C.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were estimated using the method described by Reitman and Frankel [19]: 0.5 ml of AST and ALT substrate were incubated for 5 min, after which 0.1 ml of serum was added, followed by incubation for 60 min and 30 min for AST and ALT, respectively, at 37 °C. Equal amounts of serum, working standard, and distilled water were added in the control, standard, and blank tubes, respectively. 2,4-Dinitrophenylhydrazine was allowed to react at room temperature in all the tubes for 20 min; then, 5 ml of 0.4 N sodium hydroxide was added and mixed well. The contents were incubated for 10 min at room temperature, and optical density (OD) was read at λ 510 nm against blank.

Serum urea, uric acid, creatinine, triglycerides, cholesterol, and bilirubin were measured by the kit protocol method using a Merck auto-analyser (Micro Lab 200) according to the directions given in the manual of the diagnostic kit (E-Merck India Ltd.).

2.4. Tissue biochemical assays

Immediately after necropsy, liver, kidney and brain tissues were excised, rinsed in ice cold normal saline and blotted to dry for tissue biochemical estimations. Tissues were homogenized with a Remi Motor Homogenizer (RQ-122) using glass tube and Teflon pistle. were immediately processed to determine lipid peroxidation (LPO) [20], reduced glutathione (GSH) [21], superoxide dismutase (SOD) [22], catalase (CAT) [23], acetyl cholinesterase (AChE) [24].

2.5. Lipid peroxidation

Liver, kidney and brain of each rat were promptly removed out to determine the level of lipid peroxidation. The amount of TBARS formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of LPO [20]. About 1 ml of homogenate, prepared in KCl (0.15 M) were incubated at 37 °C for 30 min and proteins were precipitated by adding 1 ml chilled trichloroacetic acid (TCA) (10%) then centrifuged at 450 × *g* for 15 min. Supernatant and TBA solution (0.67%) of 1 ml each were kept in boiling water bath for 10 min and after cooling, optical density was noticed at 535 nm.

2.6. Reduced glutathione

Reduced glutathione (GSH) was estimated using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) [21]: Hepatic, renal and neuronal