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Antioxidant Effect of Arabic Gum Against Paraquat-induced Lung Oxidative Stress in Mice

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The effect of Arabic Gum (AG) against toxicity of paraquat (PQ), an oxidative stress inducing substance, in mice was investigated. A single dose of PQ (30 mg kg^{-1}, i.p.) induced lung toxicity, manifested biochemically by significant decrease of the activity of alkaline phosphatase enzyme (ALP) in lung tissue. Lung toxicity was further evidenced by significant decrease of total sulfhydryl (-SH) content and significant increase in lipid peroxidation measured as malondialdehyde (MDA) in lung tissues. Pretreatment of mice with AG (25 g kg^{-1} p.o.) in drinking water, starting 5 days before PQ injection and continuing during the experimental period ameliorated the lung toxicity induced by PQ. This was evidenced by prevention of the significant decrease in ALP activity, the total sulfhydryl content and the significant increase in MDA in lung tissue homogenates compared to the PQ treated group. Moreover, pretreatment of mice with AG leads to an increase of the LD_{50} value of PQ. These results indicate that AG is an efficient cytoprotective agent against PQ-induced lung toxicity.

Key words: Paraquat, arabic gum, oxidative stress, lipid peroxidation, lung toxicity

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INTRODUCTION

Reactive oxygen species and free radicals have received a lot of attention in recent years as they are associated with cellular injury and aging process. Free radicals such as superoxide anion ($O^{\bullet}_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and lipid radical cause DNA damage[1], protein denaturation[2] and lipid peroxidation[3] that can lead to cell death. Lipid peroxidation is initiated by radicals attacking unsaturated free fatty acids and is propagated by chain reaction cycle. The peroxidation of unsaturated free fatty acids in biological membrane leads to disruption of the membrane structure and function[4]. Therefore, it is important to identify antioxidants, which can scavenge free radicals and stop the chain reaction. In past few years, there has been increasing interest in finding natural antioxidants[5].

Paraquat (PQ), an oxidative stress inducing substance, is a herbicide which is very toxic to all animals and to man as it is well-characterized pneumotoxicant[6]. It generates free radicals and leads to multi-organ toxicity with necrotic damage to the lung, myocardial muscles, liver and kidney. PQ also induces extensive haemorrhagic incidents throughout the body and consequently leads to death[7,8]. PQ accepts electron by reductases and from PQ radicals. These radicals transfer the extra electron to molecular oxygen forming superoxide radicals and regenerating the parent compound that is ready to gain a new electron. Superoxide radical changes to H_2O_2 by superoxide dismutase enzyme, in turn changes into OH radicals or detoxified by catalase or glutathione peroxidase in the presence of reduced glutathione (GSH) to form water and oxidized glutathione (GSSG)[9].

Arabic Gum (AG) is a dried gummy exudate from the stems and branches of Acacia Senegal (Leguminosae), constitutes of calcium, magnesium and potassium salts of the polysaccharide arabic gum acid[10]. Arabic gum has been used in Arabs folk medicine to reduce both the frequency and the need for haemodialysis in chronic renal failure patients[11]. Arabic gum also has been shown to reduce urinary nitrogen excretion by increasing urea disposal in the cecum and lowering serum urea concentration in rat and human[12,13]. Additionally, it has been recently reported that AG prevented gentamycin- induced nephrotoxicity. Co-treatment of AG significantly prevented gentamycin-induced lipid peroxidation in the kidney tissue, which was closely associated with protection of renal function and histological changes[11].

MATERIALS AND METHODS

Chemicals: Paraquat (Methyl viologen) was purchased from Sigma-Aldrich (St. Louis, MO,USA). Arabic gum was purchased from Riedel-deHaen, D3010Seelze, Germany. Thiobarbituric acid (TBA) was a product of Fluka (Buchs, Switzerland). N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), sulfanilamide (SULF), vanadium trichloride, sodium nitrate and absolute methanol were all purchased from Sigma-Aldrich and were used without further purification. Vanadium trichloride was stored in the dark under vacuum. All other chemicals were of the highest analytical grade and obtained from commercial suppliers. PQ was dissolved in normal saline just before use and was injected to the animals in a volume not exceeding 0.2 mL AG was dissolved in drinking water.

Animals: Male Swiss albino mice weighing 22-25 g were used in all experiments. They were obtained from the Experimental Animal Care Center, King Saud University, Riyadh, Saudi Arabia. They were housed under conventional laboratory conditions in a room temperature maintained at 25±1°C and a relative humidity range of 40 to 75% with a regular 12 h light: 12 h dark cycle. The mice were fed a standard animal pellet diet and allowed free access to water unless otherwise indicated. Experiments were approved by a local ethical committee of College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Experimental protocol: In this experiment, mice were randomly allocated into 4 groups, each consisting of eight animals. The first group, the control group (C), received saline. The 2nd group, AG group, received AG 100 gL^{-1} in drinking water which is equivalent to 25 g kg^{-1} day^{-1} p.o. for 5 days[14]. A 3rd group, PQ group, received PQ 30 mg kg^{-1} day^{-1} i.p[15]. The 4th group, (AG+PQ) group, was given AG 100 g L^{-1} in drinking water which is equivalent to 25 g kg^{-1} day^{-1} p.o. for 5 days and one day after a single dose of PQ (30 mg kg^{-1} i.p.). The doses were calculated according to the average daily water intake of mice.

Biochemical assessment of lung-toxicity: Before sacrificing the animals, blood samples were collected from the orbital venous plexus, under light ether anesthesia, into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min at 1000 g and stored at -20°C until analysis. Animals were sacrificed by cervical

dislocation and the lungs were quickly isolated, washed with saline, blotted dry on a filter paper and weighed, then lung/body weight ratio was determined. Homogenization was carried out in ice-cold KCl (1.15%, pH 7.4) to yield a 10% (w/v) tissue homogenates using Glas-Col homogenizer (USA) then the following biochemical parameters were assessed.

Lipid peroxides (LP) level in lung homogenate was determined as thiobarbituric acid-reactive substances spectrophotometrically, the absorbance was measured at 532 nm by the method of Ohkawa[16] and the concentrations were expressed as nanomole malondialdehyde (MDA) per gram tissue (nmol MDA g^{-1} lung tissue).

Lung homogenate contents of acid soluble thiols mainly reduced glutathione were measured according to the method of Ellman[17], the absorbance of the product was measured colourimetrically at 412 nm. Homogenates were precipitated with 5% perchloric acid and after centrifugation; supernatants were used for the estimation of GSH level. The concentration of GSH was expressed as μmol g^{-1} lung tissue.

The activity of ALP enzyme was determined colourimetrically in lung homogenates at 510 nm[18]. The results were expressed as μmol $min^{-1}100\ g^{-1}$ lung tissue.

The serum level of total nitrate/nitrite (μmol mL^{-1}) was determined by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride[19]. Serum proteins were precipitated by absolute methanol and after centrifugation, supernatants were used for estimation of nitrate/nitrite level.

LD_{50} determination: In a separate experiment, mice were observed for 24 h after intraperitoneal administration of PQ and the percent mortality was recorded. The LD_{50} value was calculated using the method of Lichfield and Wilcoxon[20]. Statistical analysis: Data were expressed as mean±SEM. Statistical comparison between different groups were done using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Significance was accepted at $p<0.05$.

RESULTS

General observation: Administration of a single dose of PQ (30 mg kg^{-1} i.p.) did not significantly alter body or lung weight of the treated mice. The ratio of lung weight/body weight also did not alter after treatment. Similarly, AG or combined AG + PQ treatment did not alter the body weight, lung weight or the ratio of lung weight/body weight.

Effects of AG on the LD_{50} value of PQ: The LD_{50} value of PQ after i.p. administration was 97.7 mg kg^{-1}. Pretreatment with AG for five days before PQ injection showed significant elevation of the LD_{50} value of PQ, which reached 121.076 mg kg^{-1}.

Effect of AG on PQ-induced changes in ALP activity in lung tissues: After PQ injection, ALP activity in lung homogenates was significantly decreased (39%) as compared with the control group reaching 42±4.98 μmol min^{-1} 100 g^{-1} lung tissue while control value was 106.67+11.9 μmol min^{-1} 100 g^{-1} lung tissue. Pretreatment with AG (25 g kg^{-1} day^{-1} p.o.) for five days before PQ injection prevented the significant reduction of ALP activity in lung homogenates. On the other hand, administration of AG alone did not produce any significant change in the enzyme activity level (Fig. 1).

Effect of AG on PQ-induced changes in lipid peroxides and reduced glutathione content in lung tissues: Lipid peroxides in lung homogenates measured as

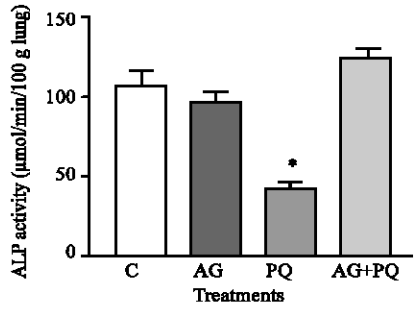

Fig. 1: Effect of AG (25 g kg^{-1} day^{-1} p.o.) on lung toxicity induced by PQ as measured by changes in ALP activity in lung tissue homogenates. AG was administered orally for 5 days. On day 5, lung injury was induced in animals by a single i.p. injection of PQ(30 mg kg^{-1} i.p.). One day thereafter, mice were sacrificed by cervical dislocation and lungs were rapidly excised and homogenized in chilled 1.15% KCl (pH 7.4) to yield 10% tissue homogenates. Data are expressed as means±SEM for all groups; n= 8. *Significantly different from control group. PQ indicates paraquat; ALP indicates alkaline phosphatase, AG indicates arabic gum

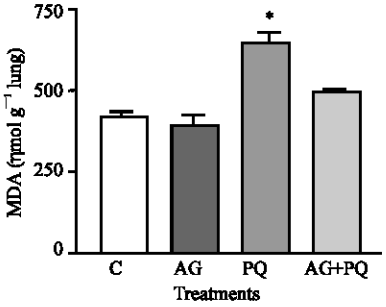

Fig. 2: Effect of AG (25 g kg^{-1} day^{-1} p.o.) on PQ induced enhancement of lung tissue MDA. *, Significantly different from control group. PQ indicates paraquat; MDA indicates malondialdehyde and AG indicates arabic gum

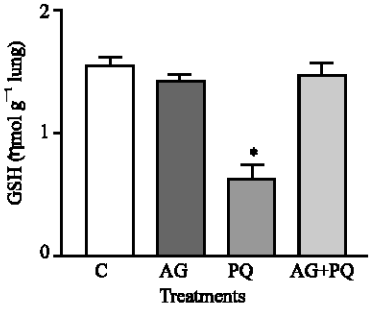

Fig. 3: Effect of AG (25 g kg^{-1} day^{-1} p.o.) on PQ-induced reduction of lung tissue GSH. *, Significantly different from control group. PQ indicates paraquat; GSH indicates reduced glutathione and AG indicates arabic gum

malondialdehyde concentrations of mice treated with PQ significantly increased (150%) to 667±36.97 nmol g^{-1} tissue as compared to control value of 443.86±18.24 nmol g^{-1} tissue (Fig. 2). Pretreatment with AG (25 g kg^{-1} day^{-1} p.o.) for 5 days before PQ injection prevented the significant increase in lung malondialdehyde concentrations compared to PQ treated group. On the other hand, PQ injection produced a significant reduction (40%) of reduced glutathione in lung homogenates (0.59±0.12 μmol g^{-1} tissue) as compared with the control value (1.49±0.07 μmol g^{-1} tissue). Pretreatment with AG (25 g kg^{-1} day^{-1} p.o.) for 5 days before PQ injection prevented the significant depletion of lung GSH content compared to PQ treated mice (Fig. 3).

Effect of PQ and/or AG on serum nitric oxide [Nitrate + Nitrite] production: Neither PQ nor AG or their combination could produce any significant change in nitric oxide production in the lung as compared to the control group (Fig. 4).

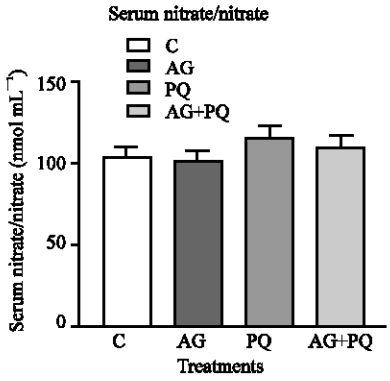

Fig. 4: Effect of AG (25 g kg^{-1} day^{-1} p.o.) on PQ-induced changes in serum nitrate + nitrite levels (nmol mL^{-1}) . PQ indicates paraquat, AG indicates arabic gum

DISCUSSION

In the present study, a single dose of PQ (30 mg kg^{-1} i.p.) induced lung toxicity manifested biochemically by a significant reduction of the ALP activity in lung tissue homogenate to about 39% of the control value. This sharp drop in ALP activity may indicate lung damage as the decrease in ALP activity has been reported as a sensitive marker of the toxicity of the alveolar epithelium[21]. Also, the marked elevation of lipid peroxides measured as MDA lung homogenate was taken as an evidence for the involvement of oxidative stress in the development of PQ-induced lung injury. This observation is in full agreement with previous investigations[22,23]. Similar finding has been reported after PQ as well as after PQ insults of pulmonary tissue by various toxicants such as bleomycin[24]. Severe PQ poisoning produces adult respiratory distress syndrome, pulmonary hypertension, edema and progressive lung fibrosis. The lungs are preferentially targeted to PQ toxicity may be due to rapid uptake and accumulation of PQ in this organ through polyamine transporters[25].

Free radicals are known to play a crucial role in PQ-induced lung toxicity[8,26]. It has been reported that the toxicity is related to redox cycling of an iron-PQ complex, which in turn catalyzes the formation of ROS with the ultimate progression of lipid peroxidation[27]. On the other hand, the observed depletion of non protein-thiols (GSH) in lung tissue could be a consequence of its consumption by the PQ-induced ROS generation. Glutathione is known as one of the endogenous antioxidants, being identified as a protector against the damaging effect of the free radicals[28].

The role of nitric oxide in PQ-dependent lung toxicity has been controversial[26]. In the present study, there was no significant difference of the NO production between the studied groups. Day *et al.*[25] concluded that PQ is actually using NOS enzyme as an electron donor to generate superoxide, which in turn changed to H_2O_2 and hydroxyl radicals leading to the lung damage. Moreover, Tomita *et al.*[26] reported that superoxide not NO played a key role in the cellular damage caused by PQ.

Present results demonstrated that AG significantly attenuated the development of PQ-induced lung toxicity. This protective effect of AG may be mediated via its reported antioxidant effect, since similar studies demonstrated that AG markedly diminish tissue injury by decreasing oxidative stress and lipid peroxidation in other models of oxidative stress induced toxicities such as nephrotoxicity induced by gentamycin and cardiotoxicity induced by doxorubicin[11,14].

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