Original Article

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Protective Effects of Celery (Apium graveloens) Seed Extract on Bleomycin-Induced Pulmonary Fibrosis in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Pulmonary fibrosis is one of the most common side effects of bleomycin which is used as a chemotherapeutic agent. Reactive oxygen species play a key role in the development of pulmonary fibrosis. Celery seed contains a variety of flavonoids which are considered as asantioxidants. This study investigated the effects of celery seed hydroalcoholic extract on bleomycin-induced pulmonary fibrosis in rats.

METHODS: This empirical study was conducted on 20 Sprague-Dawley rats weighing from 180 to 150 g. The animals were divided randomly into 5 groups of 4 rats. Groups 1 and 2, respectively, received a single dose of saline or bleomycin (7.5 units per kg) endotracheally. Group 3-5 received different daily doses of celery seed extract (100, 200 and 400 mg/kg) intraperitoneally for one week before and two weeks after the bleomycin. The animals were killed after 21 days and their blood and lungs were collected and tested so as to measure the plasma malondialdehyde, lung hydroxyproline and histopathology test.

FINDINGS: The results showed that the index of lung, hydroxyproline and malondialdehyde in the saline normal group were respectively, 2.02±7.27 milligrams of lung per gram of body weight, 0.24±1.78 mg per gram of lung tissue and 0.17±1.48 micromol per liter of plasma. On the other hand, in the group receiving bleomycin, the figures were 0.99±10.1, 1.5±5.75 and 0.23±3.27, respectively. Treatment with extract, especially in groups 4 and 5, significantly reduced these factors compared to the bleomycin group ($p<0.05$). Furthermore, the results of histology revealed that bleomycin could lead to lung damage and the thickening of the alveolar.

CONCLUSION: The results showed that celery seed hydroalcoholic extract has a protective effect on bleomycin-induced pulmonary fibrosis.

KEY WORDS: Pulmonary Fibrosis, Celery Seed Extract, Bleomycin, Rat.

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Introduction

Pulmonary fibrosis is a fatal and chronic lung disease whose prevalence increases with aging, smoking, viral infections, genetic factors (male), environmental exposure to paraquat, toluene and oxygen and the consumption of synthetic drugs such as amiodarone and bleomycin (1-4).The true cause of this disease still remains unknown. However, the main theory about how the disease appears points out the chronic pulmonary inflammation and the subsequent lack of healing (2, 4 and 5).

Oxygen-free radicals are the key mediators of acute lung inflammation processes. Pulmonary fibrosis are identified with hyperplasia and alveolar epithelial cell injury, accumulation of inflammatory cells and macrophages, fibroblasts hyperplasia and excessive synthesis and deposition of extracellular matrix (especially Collagen and Fibronectin) with ulcer formations (1, 4 and 5). Bleomycin is an anti cancer antibiotic drug which is used to treat the cytostatic of a number of malignant tumors (4).

The genotoxic effects of this drug on normal tissues often cause secondary malignancies. One of the most dangerous and fatal side effects of bleomycin pulmonary fibrosis, which is dependent on time and dosage, is irreversible pulmonary fibrosis induction whose mechanism is still unknown. However, it is presumed that bleomycin could expand the production of oxygen free radicals through the Fenton reaction which might lead to DNA damage, lipid peroxidation as well as certain changes in the construction and destruction of prostaglandins.

It also causes acute inflammation of eicosanoids and the pulmonary collagen synthesis to soar. The side effects of fibrotic bleomycin are very common. Thus, the drug is used in order to create animal models of inflammation and pulmonary fibrosis (4 and 6). So far, no medications have been found to be effective for pulmonary fibrosis (2, 4 and 5). One of the approaches to the treatment of this disease is using antioxidants compounds so as to eliminate the free radicals and thus prevent inflammatory processes (7). Today, the use of herbal medications have come to great attention. In this regard, there are certain compounds extracted from the celery seed which can inhibit cyclooxygenase as well as have antioxidant properties (8). Celery, scientifically termed *Apium Graveloens*, belongs to the Apiaceae family (9). Celery seed extract has been known to have anti-inflammatory and analgesic properties (10). The seed extract contains volatile oils, flavonoids and resins and the anti-inflammatory effects of these compounds have been proven. Flavonoids are the natural polyphenol compounds in plants which have anti-inflammatory and analgesic properties. The phytochemistry examination of celery seeds revealed the presence of apigenin as the main ingredient (11). This study was conducted in order to investigate the protective effects of hydroalcoholic extracts of celery seeds in bleomycin-induced pulmonary fibrosis.

Methods

Extraction: In order to provide the extract, we used the soaking method (12, 13). After cleaning and drying in the shade, the celery seeds were grinded and then were soaked for 3 days in 70% ethanol (30% Water and 70% ethanol). After three days, the extract was filtered and the remaining 70% ethanol was poured on the residue and then it was added to the first extract. In the next step, the obtained extract was passed through a paper filter and the filtrate was concentrated by a rotary machine. After it was placed in 40-30 ° C dry heat, the dry extract was obtained (15-12).

Animal Study: This empirical study was conducted on 20 Sprague-Dawley rats weighing form 180 to 150 g. The animals were purchased from the Laboratory of the Animal Production Center of Jondi Shapur Medical University of Ahvaz, Iran. They were kept in polycarbonate cages at 24 ± 4 ° C in the light cycle of 12 hours of light and 12 hours of darkness and were fed special compact food which had been purchased from the Isfahan company of animal feed and urban piped water.

For more compatibility with the laboratory environment, the animals were placed in the aforementioned condition a week before the start of the

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study. Also, they were divided into 5 groups of 4 members:

- Group 1 (normal saline) received physiological saline solution intraperitoneally for 21 days.
- Group 2 (bleomycin) received drug carrier (normal saline) with the same amount for 7 days before and 14 days after the administration of a single dose of bleomycin (7.5 IU/Kg).
- Groups 3, 4 and 5 received doses of 100, 200 and 400 mg/kg of celery seed extract intraperitoneally for 7 consecutive days before and 14 consecutive days after the endotracheal administration of a single dose (7.5 IU/Kg) of bleomycin by insulin syringe (12, 16, 17).

At the end, after examining the weight and blood of animals, they were killed with ether. The animals' chests were split then and the lungs were carefully removed and the lungs weight of the mice were measured.

Measurement of lipid peroxidation (malondialdehyde): With slight modifications, Satoh method was used for measuring the lipid peroxidation (18). Accordingly, 10% trichloroacetic acid was added to 500 microliter of 5.1 ml plasma and it was centrifuged at 4000 rpm for 10 minutes. Moreover, 1.5 ml of the supernatant was removed and 2 ml of thiobarbituric acid thiourea 0.67% was added and then it was placed for 30 minutes in a boiling water bath. Upon cooling, 2 ml of n-butanol was added to the compound and it was thoroughly mixed and centrifuged at 4000 rpm for 15 minutes. The supernatant, which is pink, was isolated and its absorption was read in 532 nm by a spectrophotometer. In order to plot malone aldehyde standard curve, various concentrations of 3, 3, 1, 1-Tetratoxy propane were built in the nanomoles.

Hydroxyproline Measurement: About 1% of the homogenates of the right lung tissue was prepared in normal hydrochloric acid 6. The measurement of hydroxyproline was made by the colorimetric method of O'Brien and Edwards using Ehrlich reagent with Chloramine T and standard hydroxyproline (19-22).

Histopathological Study: After blood sampling, the animals' chests were cut open with surgical scissors, the left lung was removed for histological studies and a part of it was placed in 10% formalin solution. The tissue samples were processed routinely and after molding with paraffin, sections with a thickness of 4 to 6 microns were prepared. The provided slides were stained and examined by hematoxylin and the Eosin method. For histological evaluations, eight slides were prepared for each group (two slides per each animal) and five fields per slide were examined.

Statistical Analysis Method: For statistical comparison, one-way ANOVA was used and for the evaluation and determination of the mean differences and the significance of variance analysis, Duncan test was applied and $p<0.05$ was considered significant.

Results

The results of this study showed that the malondialdehyde levels in the normal saline group and the group receiving bleomycin were respectively 0.17±1.48 and 0.23±3.27 micromoles per liter of plasma ($p<0.05$). Moreover, malondialdehyde levels in the groups treated by 100, 200 and 400 mg/kg of celery seed extract were 0.12±2.96, 0.27±2.43 and 0.28±1.84, respectively (fig 1). The hydroxyproline levels in the normal saline group and the group receiving bleomycin were respectively 1.78±0.24 and 5.75±1.5 milligrams per lung tissue ($p<0.05$). Hydroxyproline levels in the groups treated by 100, 200 and 400 mg/kg of celery seed extract were 5.28±0.62, 4.86±0.15 and 3.53±0.95, respectively(fig 2).

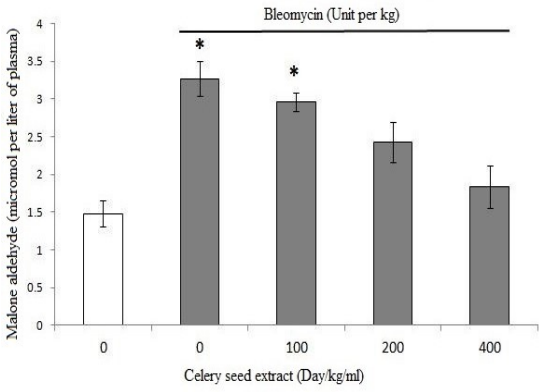

Figure 1. The effects of ethanol extracts of celery seeds on the Malondialdehyde in the study groups * significant difference from saline group ($p<0.05$)

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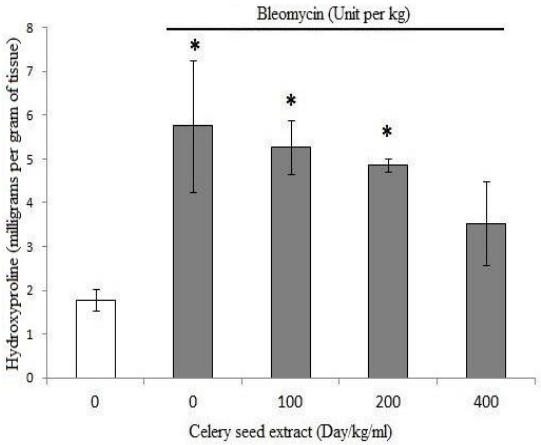

Figure 2. Effect of celery seed extract on lung hydroxyproline content in the study groups * significant difference from saline group (p<0.05)

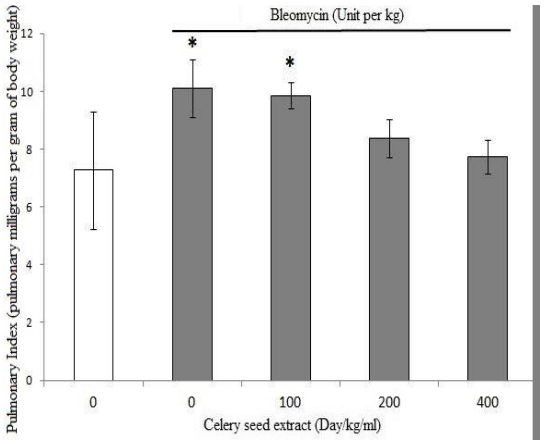

Figure 3. Effects of celery seed extract on pulmonary Index in the study groups * significant difference from physiological serum group (p<0.05)

The pulmonary index in the normal saline group and the group receiving bleomycin were respectively, 2.02±7.27 and 0.99±10.1 milligrams per gram of body weight (p<0.05). Furthermore, the pulmonary index in the groups treated by 100, 200 and 400 mg/kg of celery seed extract were 9.86±0.46, 8.38±0.67 and 7.75±0.58, respectively (fig 3).

The histopathologic examination of the present study reveals the normal and unchangeble pulmonary pathological structure in the normal saline group (fig A4). In the bleomycin group, however, severe pulmonary fibrosis with extensive infiltration of the inflammatory cells, collagen accumulation and destruction of alveolar walls were observed (fig B4). In addition, pulmonary fibrosis and inflammatory lesions were observed in group 3. However, compared to the positive control group, the intensity of the waste was less severe (fig C4). On the other hand, in group 4, compared with the previous two groups, a relative reduction in the pulmonary injury with inhibiting inflammatory cell infiltration and loss of alveolar septal thickness were observed (fig D4). Also, in groups 5 and 1, the level of fibrosis and pulmonary tissue damage were noticeably reduced and the pulmonary structure in these groups was similar to that of the control group. Moreover, the lowest rate of spindle cell proliferation and fibrosis were observed in these levels (fig E4).

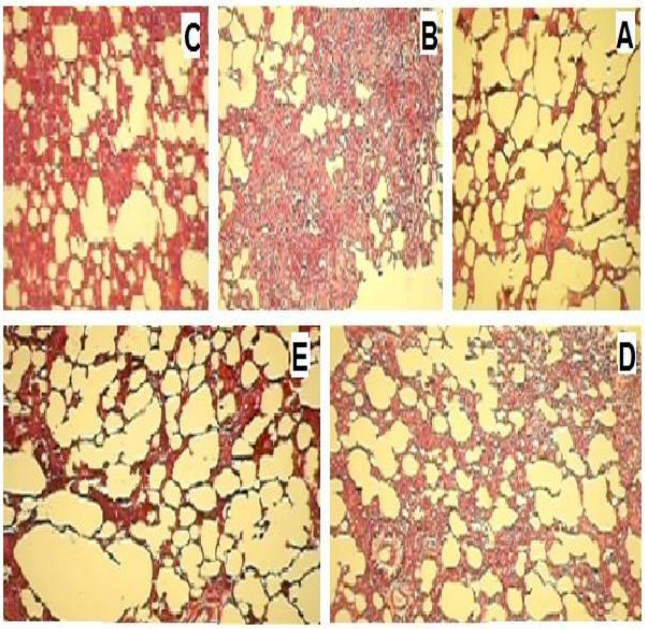

Figure 4. The tissue's cross-sectional images of rats' lungs in different tested groups from A to E. Hematoxylin and eosin staining with 400 magnification

Discussion

The results of the current study indicated that the highest plasma malondialdehyde mean, the pulmonary tissue hydroxyproline and the pulmonary index were observed in the normal saline group receiving bleomycin. In this regard, the increased plasma malondialdehyde levels in the rats of the positive

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control group was due to the formation and progression of lung inflammatory response. As for the inflammatory pulmonary diseases, a part of the oxidants produced in the lungs crosses through cell membranes and consequently, it enters the blood flow to the tumors where they cause the oxidation of unsaturated fats. In the present study, the components of the celery seed extract were reported to have effectively reduced the oxidizers in the blood of the sick mice and the plasma malondialdehyde level in the protected groups of 1, 2 and 3 declined as much as 9.5%, 25.7% and 43.7% respectively compared to the positive control group. It seems that such flavonoids in the celery seed extract as apigenin are responsible for the reduction in the malondialdehyde level in all the therapy groups.

As was observed, flavonoids can collect reactive oxygen species as well as chelateiron ions and inhibit lipid peroxidation (11). Pulmonary fibrosis is associated with inflammatory cells' accumulation in the alveolar spaces, alveolar wall thickening and fibrotic lesions development (4,5). Thus, it is concluded that the hydroalcoholic extract of the celery seed decreases the alveolar wall fibrosis progression in the mice who received bleomycin. Pulmonary fibrosis occurs upon the penetration of extracellular matrix, collagen deposition and cell proliferation in the interstitial tissue.

A high proportion of these cells include fibroblasts and myofibroblasts, which increases the pulmonary collagen generation resulting in lung disabilities and defects (23). In our study, the amount of collagen in the lung tissue in the protection groups 1, 2 and 3, were 8.2%, 5.15% and 38.6% respectively. These amounts were lower than those of the bleomycin group and, at the highest dosage of collagen, close to those of the control group and had no significant difference. According to these findings, it seems that the administration of such antioxidants as this extract cannot inhibit bleomycin-induced pulmonary fibrosis. However, it can prevent its improvement to a large extent or delay the whole process. As a result, factors other than the formation of free radicals and reactive oxygen species are involved in the development of early inflammatory reactions in the disease. The apigenin existing in the celery extract, TNF-α (Tumor Necrosis Factor-α) which is the synthesis inducer of pro-inflammatory mediators of the prostaglandin E2, COX-2 and IL-8, reduces the signal of NF-Kβ (Nuclear Factor-kappa -β) through the deactivation of the path, and thus, decrease the amount of collagen is the fibrous tissue (24,25).

In general, by studying pulmonary hydroxyproline and collagen it can be concluded that treatment by the hydroalcoholic extract of celery seed can be effective in the treatment of pulmonary fibrosis and possibly preventing it as the effect is dependent on the dosage. Finally, the results of the present study revealed that the hydroalcoholic extract of celery seed, particularly at a dosage of 400 mg, can inhibit the collagen deposition as well as lipid peroxidation, reduce the level of plasma malondialdehyde and eventually, act as a pulmonary fibrosis inhibitor. Its positive effects on the diseases which involve inflammation during the initiation and progression, such as pulmonary turquoise, can be discovered through further study.

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