The Antioxidative Effect of Chamomile, Anthocyanoside and their Combination on Bleomycin-induced Pulmonary Fibrosis in Rat

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ABSTRACT

Introduction: Bleomycin is a small peptide with 1500 Dalton of molecular weight which has two junction areas in two molecule's opposite sides, one of them to relate to the DNA and the other to relate to the iron. Iron is a crucially important factor in free radical production and cytotoxic activity of bleomycin. Material and methods: The study attempts to study, and compare, the effect of using Chamomile, Anthocyanoside and their combination, as anti-inflammatory agent to ameliorates, to prevent or control the development of fibrosis due to Bleomycin (BLM). to prepare pulmonary fibrosis model, male Wistar rats weighting 180-220g were assigned to specific groups. Rats of each group received intratracheally 1U/100 g of BLM. 20 rats were divided to five comparable groups, as (1) BLM group, (2) saline group, (3) Chamomile group, (4) Anthocyanoside group, (5) combination of Anthocyanoside and Chamomile group. Antioxidative combinations were given as pretreatment and treatment after the rats received Bleomycine. Results: After 3 week, Malondialdehyde (MDA) was measured for each rat's lung. After three weeks, MDA was reduced, compared to BLM group, to 44.27%, 37.80% and 46.07% in Anthocyanoside, Chamomile and combination group, respectively. It was concluded from the present study that administration of combination of Chamomile and Anthocyanoside lead to a significant reduction in Bleomycin-induced MDA.

Conclusion: The mechanism of the effect of these combinations is possibly the result of phenolic combinations as antioxidant and oxy free radical scavenger and inhibitor of lipid peroxidation.

Key words: Bleomycin, pulmonary fibrosis, Chamomile, Anthocyanoside.

1. INTRODUCTION

Bleomycin is a small peptide with 1500 Dalton of molecular weight which has two junction areas in two molecule's opposite sides, one of them to relate to the DNA and the other to relate to the iron. Iron is a crucially important factor in free radical production and cytotoxic activity of bleomycin. Bleomycin forms a complex with Fe 2+ which, then, oxidizes to Fe3+ and results in Oxygen reduction and free radical production. These free radicals cause one or two DNA chain(s) to break and cell death happen (1). Moreover, Bleomycin plays a role in oxidative damage in all kinds of cellular RNA. The function of Bleomycin is specific to a particular stage in cell cycle so that its main effects appear in M and G2 phases (2). It is not fully clear what the mechanism of Bleomycin-induced pulmonary damages is, but it seems to include an oxidative damage, a relative Bleomycin hydrolyzate loss, genetic predisposition and an increase in inflammatory cytokines (3). With Fe3+, Bleomycin causes reactive radicals of Oxygen to be produced. Taking the direct role of this mechanism in pathogens of this disease into account, iron chelators can reduce Bleomycin-induced pulmonary toxicity in animal models (4). Taking a direct part in oxidation reactions; i.e. reduction and oxidation of fatty acid, active variants of Oxygen can cause toxicity leading to membrane instability. Oxidants can cause inflammatory reactions in the lung. Damaging and activating epithelial alveoli can result in the release of the cytokines and the growth factors which evokes division of the cell in myofibroblast and the pathological extracellular matrix secretes; then, the fibrosis will be finally produced (5). German Chamomile from the compositae family, is a kind of plant whose flowers, having Kamazolen and bisabolol, have strong anti-inflammatory, anti-mutagene, anti-spasmodic and anti-stress effects and can decrease blood cholesterol (6). The most important combinations are flavonoids, Alpha-bisabolol, Sesquiterpenes, Kamazolen, Farnesene, and Cis-Trans-N-di-cyclo-eter isomers (7). The research shows the anti-inflammatory effect of the Chamomile is mainly due to the volatile oil and flavonoid, specially Kamazolenand, Al-
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Inflammation and development of inflammatory reactions in lungs. As the inflammatory disease in the lungs appear, some of the cell membrane and join the blood circulation; then, it leads to oxidation of unsaturated fat. As antioxidants, Chamomile and Anthocyanoside inhibit lipid peroxidation in lung tissues, and the results showed MDA decreased in the experimental groups, Duncan test shows rats have significant different amount of MDA in the groups with different letter (p-value<0.05).

As it can be seen in Table 1, after treatment, MDA was reduced 44.27%, 37.80%, and 46.07% in groups # 4, 3 and 5, respectively. Moreover, a comparison between each two experimental groups using Duncan test showed there is no significant difference between the experimental groups #4 and 5 (p-value> 0.05), but the differences between the other couple groups were significant (P-value<0.05). The results also revealed the more effect Anthocyanoside had on MDA reduction than Chamomile, and even the more effect the combination of Anthocyanoside and Chamomile had. Besides, each one on of these inhibitors had a significant difference with the positive control group (p-value<0.05).

Figure 1 demonstrated that, comparing to the negative control group, the inhibitors mentioned above reduced the amount of MDA produced in the lung approaching the MDA level in that control group; however, there was still a significant difference with the negative control group (p-value<0.05).

4. DISCUSSION

MDA test was used to measure the amount of bleomycin-induced increase in lipid peroxidation in lung tissues, and the results showed MDA decreased in the rats in groups # 3, 4 and 5 due to the antioxidative activity of antioxidant combinations, and that this amount of decrease was the most for rats in group #5, the least for those in group #3 and for group #4 in between. The previous studies in the domain just focused on the positive effect of either Chamomile (17, 18) or Anthocyanoside (19) by themselves. The increase in the level of MDA in rats in positive control group (BLM) is due to the formation and development of inflammatory reactions in lungs. As the inflammatory disease in the lungs appear, some of

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average</th>
<th>Standard deviation (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<tr>
<td>Negative control</td>
<td>8.8 ± 0.163</td>
<td>2.34</td>
<td>9.23</td>
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<tr>
<td>Positive control</td>
<td>16.69 ± 0.243</td>
<td>8.68</td>
<td>20.30</td>
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<tr>
<td>Anthocyanoside</td>
<td>9.30 ± 0.173</td>
<td>2.01</td>
<td>10.15</td>
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<tr>
<td>Chamomile</td>
<td>10.38 ± 0.214</td>
<td>3.11</td>
<td>11.21</td>
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<tr>
<td>Combination of Anthocyanoside and Chamomile</td>
<td>9 ± 0.217</td>
<td>1.67</td>
<td>10.06</td>
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</tr>
</tbody>
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Table 1. Comparing the MDA (μg per tissue) for rats’ lungs in the groups under study

Figure 1: Comparing the MDA concentration (μg per tissue) for rats’ lungs in the experimental groups, Duncan test shows rats have significant different amount of MDA in the groups with different letter (p-value<0.05)
the produced oxidants in the lung pass through the cell membrane and join the blood circulation; then, it leads to oxidation of unsaturated fat. As antioxidants, Chamomile and Anthocyanoside inhibit lipid peroxidation which results in inflammation as well as fibrosis reduction. Then, the amount of metabolites as MDA production would decrease (20, 21).

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CONFLICT OF INTEREST: NONE DECLARED.

REFERENCES