ORIGINAL PAPER

doi: 10.5455/medarh.2015.69.229-231

Med Arh. 2015 Aug; 69(4): 229-231
Received: May 25th 2015 | Accepted: June 30th 2015

Published online: 04/08/2015 Published print:08/2015

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

Iraj Javadi[1], SeyedAlireza Emami[2]

[1]Azad University of Shahreza, Isfahan, Iran
[2]Alzahra Hospital, Isfahan University Of Medical Sciences, Isfahan, Iran

Corresponding author: SeyedAlireza Emami, MSc Toxicology, Alzahra Hospital, Isfahan University Of Medical Sciences, Isfahan, Iran.

ABSTRACT

Introduction: Bleomycin is a small peptide with 1500Daltun 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, Chamomiland 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 Daltun 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-

pha-bisabolol, and its antioxidant effect is related to Apigenin and Quercetin (8, 9). Amongst the effective herbal combination is the phenolic combination among which is flavonoid as the biggest sub-group (10). Hemmati et. al. (2011) examined the remedial effect of Yarrow (Achilleamillefolium L.) extract due to the fibrogenic effect of Bleomycin in rat lung. The found that due to the anti-oxidative and anti-inflammatory effect it has, the plant extract can hinder fibroblast to proliferate and reduce the pulmonary fibrosis. Tang, et. al. (2012) studied the effect of flavonoid Tranilast on hindering Bleomycin induced pulmonary fibrosis, and found this flavonoid reduces the pulmonary fibrosis to a great extent and there was no significant difference between the Tranilast and control group regarding lung weight (12).Anthocyanosides are among the flavonoid group, and structurally flavonoid glucosides are recognized as anthocyanin or anthocyanosid (13). It proves fruitful in treating many diseases, antioxidative and oxy radicals activity of the anthocyanin pigments are among its effects (13, 14, 15). Agackiran, et. al. (2012) examined the anti-inflammatory and anti-fibrosis efficiency of Taurine and proanthocyanid in bleomycin-induced pulmonary fibrosis in rats. The results showed Taurine and proanthocyanidins reduce the inflammatory and histopathological symptoms in rats; also, the inflammatory reduction effect in the latter is more than the former (16). The present study is, then, an attempt to investigate and compare the effect of a combination of BLM and Chamomile as an antioxidant factor on Bleomycin-induced pulmonary fibrosis in rats.

2. MATERIAL AND METHODS

To the study ends, twenty rats were divided to five comparable groups, as (1) saline group (negative control group), (2) BLM group (positive control group), (3) Chamomile group, (4) Anthocyanoside group, (5) combination of Anthocyanoside and Chamomile group; henceforth, groups #1, 2, 3, 4 and 5, respectively. For seven days before injecting with a single intratracheal Bleomycin, the individual rats in groups #3, 4 and 5 were daily injected 100mg of Anthocyanoside (0.5 CC) and 50mg/kg of Chamomile (0.5 CC); however, the rats in groups # 1 and 2were saline injected intraperitoneally (IP)(0.5 CC). On the seventh day, all rats in groups #2, 3, 4 and 5 were saline injected including Bleomycine (0.3 CC) and the ones in group# 1 (control group) were saline injected as (IP)(0.3 CC). Then, for other 14 days, the rats in the experimental groups (3, 4 and 5) were injected the same as the pre-treatment injections; i.e. 100mg of Anthocyanoside (0.5 CC) and 50mg/kg of chamomile (0.5 CC), and the ones in the control and BLM group were saline injected as (IP)(0.5CC). To analyze the differences existed between the different groups in the study, ANOVA and Turkey post-hoc test were used in SPSS (0.05). Also, Excel software was used to draw the graphs.

3. FINDINGS

Duncan test shows rats have significant different amount of MDA in the groups with different letter (p-value<0.05).

Groups	Average	Standard deviation (SD)	Minimum	Maximum
Negative control	8.8[a]	0.163	2.34	9.23
Positive control	16.69[b]	0.243	8.68	20.30
Anthocyanoside	9.30[c]	0.173	2.01	10.15
Chamomile	10.38[d]	0.214	3.11	11.21
Combination of Anthocyanoside and Chamomile	9[c]	0.217	1.67	10.06

Table 1. Comparing the MDA (µ/g per tissue)for rats' lungs in the groups under study

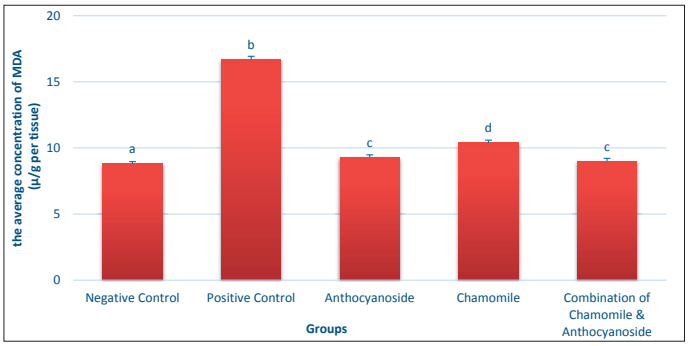

Figure 1. Comparing the MDA concentration (µ/g per tissue) for rats' lungs in the experimental groups, Duncan test shows rats have significant different amount of MDA in the groups with different letter(p-value<0.05)

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

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).

Acknowledgments

We would like to thank from Isfahan University of Medical Sciences and all coworkers of Professor Torabinejad Research Center.

CONFLICT OF INTEREST: NONE DECLARED.

REFERENCES

1. Burger RM, Peisach J, Horwitz SB. Activated bleomycin. A transient complex of drug, iron, and oxygen that degrades DNA," The Journal of Biological Chemistry. 1981; 256(22): 11636-11644.
2. Chenand J.Stubbe. Bleomycins: towards better therapeutics, Nature Reviews Cancer. 2005; 5(2): 102-112.
3. Sleijfer S. Bleomycin-induced pneumonitis. Chest. 2001; 120(2): 617-624.
4. Herman EH, Hasinoff BB, Zhang J. et al. Morphologic and morphometric evaluation of the effect of ICRF-187 on bleomycin-induced pulmonary toxicity. Toxicology. 1995; 98(1–3). 163-175.
5. Chandler DB. Possible mechanisms of bleomycin-induced fibrosis. Clinics in Chest Medicine. 1990; 11(1): 21-30.
6. McKay DL, Blumberg JB, A review of bioactivity and potential health benefit of Chamomile tea (MatricariarecutitaL.). Phytother Res. 2006; 20: 519-530.
7. Ganzera M, Schneider P, Stuppner H, Inhibitory effects of the essential oil of chamomile (Matricariarecutita L.) and its major constituents on human cytochrome 450 enzymes. J Life Sci. 2006; 78: 856- 861.
8. Janmejai K, Srivastava A, Mitali P, Sanjay G, Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. J Life Sciences. 2009; 85: 663-669.
9. Abate A, Yang G, Wong R, Schroder H, David K, Stevenson Phyllis A. Dennery A. Apigenin decreases hemin-mediated heme oxygenase-1 induction. Free Radical Biol Med. 2005; 39: 711-718.
10. Middleton Jr, E, Kandaswami C. Effects of flavonoids on immune and inflammatory cell functions. Biochemical pharmacology. 1992; 43(6): 1167-1179.
11. Hemmati AA, Arzi A, Adinehvand A, Mostofi NE, Mozaffari AR, Jalali A. Yarrow (Achilleamillefolium L.) extract impairs the fibrogenic effect of bleomycin in rat lung. Journal of Medicinal Plantes Research 2011; 5(10): 1843-1849.
12. Liantao T, Tao J, Xiaoli H, Dongling C. Effect of tranilast on bleomycin induced pulmonary fibrosis in a rat model. African Journal of Pharmacy and Pharmacology. 2011; 10: 1315-1320.
13. Evans WC. Trease and Evans' pharmacognosy.Elsevier Health Sciences, 2009..
14. Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi SW, Kawakishi S, Osawa T. Antioxidative activity of the anthocyanin pigments cyanidin 3-O-. beta.-D-glucoside and cyanidin. Journal of Agricultural and Food Chemistry. 1994; 42(11): 2407-2410.
15. Mavelli I, Rossi L, Autuori F, Braquet P, Rotilio G. Anthocyanosides inhibit cellular reactions of drugs producing oxy radicals. In Oxy radicals and their scavenger systems: proceedings of the Third International Conference on Superoxide and Superoxide Dismutase, Ellenville, New York, October 3-8, 1982/ editors, G. Cohen, RA Greenwald, 1983.
16. Yetkin A, Gul H, Gunay E, Akyurek N, Memis L, Gunay S, Sinan J, et al. The efficiency of proanthocyanidin in an experimental pulmonary fibrosis model: comparison with taurine. Inflammation. 2012; 35(4): 1402-1410.
17. Javadi A, Nasr R. The inhibitory effect of Diclofenac on pulmonary fibrosis-induced by bleomycin as an anti-cancer drug. Ahvaz Journal of Medical Sciences. 1997; 20: 51-62.
18. Javadi A, Taghavian M. The comparative study of inhibitory effect of Fenugreek, Sage, Romarin, Chamomile, Aminophilline, Betamethasone on bleomycin-induced pulmonary fibrosis. PhD Thesis in Pharmacy, Pharmacy Department, University of Medical Sciences, 1995.
19. Shoghmand A. The inhibitory effect of inhibitors on pulmonary fibrosis-induced by bleomycin as an anti-cancer drug using an animal model. PhD. Thesis in Pharmacy, Pharmacy Department, Isfahan University of Medical Sciences, 1997.
20. Amirghofran Z, Azadbakht M, Karimi MH. Evaluation of the immunomodulatory effects of five herbal plants. Journal of Ethnopharmacology. 2000; 72: 167-172.
21. Janmejai K, Srivastava A, Mitali P, Sanjay G,. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. J Life Sciences. 2009; 85: 663-669.