Journal of Medicinal Plants Research Vol. 5(10), pp. 1843-1849, 18 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Yarrow (*Achillea millefolium* L.) extract impairs the fibrogenic effect of bleomycin in rat lung

A. A. Hemmati[1,3], A. Arzi[1,3], A. Adinehvand[1], N. E. Mostofi[2], A. R. Mozaffari[2] and A. Jalali[1,4]*

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Jundishapur University of Medical Sciences, Ahwaz, Iran.
[2]Department of Histopathology, School of Medicine, Jundishapur University of Medical Sciences, Ahwaz, Iran.
[3]Physiology Research Center, Jundishapur University of Medical Sciences, Ahwaz, Iran.
[4]Toxicology Research Center, Jundishapur University of Medical Sciences, Ahwaz, Iran.

Accepted 23 February, 2011

The aim of the present study was to investigate the effect of yarrow (Achillea millefulium) extract on bleomycin-induced lung fibrosis in rat. Hydroalcoholic extract of yarrow was prepared using maceration method. Sprague Dawley rats weighing 180 to 220 g were given single intratracheal instillation of bleomycin (7.5 IU/kg) or vehicle (saline). They were treated with different doses of oral yarrow extract (400, 800, 1600 mg/kg/day) for two weeks. Histopathological examination of bleomycin-treated animals showed marked alveolar thickening associated with fibroblasts and myofibroblasts proliferation and collagen production in interstitial tissue leading to pulmonary fibrosis. Administration of yarrow extract impaired such damages in lung tissue with a dose-dependent manner. Contractility of lung parenchymal strips were also studied. The force generated by lung strips in response to potassium ions and sodium tungstate was recorded using an isometric transducer on a polygraph. The results indicated that lungs strips isolated from bleomycin-treated fibrotic lungs could generate significantly more contractions when compared to the animals that received yarrow extract after bleomycin. It can be concluded that yarrow extract may be able to impair the rate of fibroblast/myiofibroblast proliferation and collagen deposition in lung tissue due to bleomycin. The effect of yarrow may be attributed to active ingredients of the plant with anti-inflammatory and anti-oxidant properties.

Key words: Yarrow extract, bleomycin, pulmonary fibrosis, collagen, fibrobalst, myofibroblat, rat.

INTRODUCTION

Bleomycin is an effective antineoplastic agent, particularly when used in conjunction with other cytostatic drugs such as cisplatin and vinblastine. It binds to and damages DNA of tumor cells and has fewer side effects than most other antitumor drugs. Nonetheless, repeated systemic administration of bleomycin may result in lung inflammation that can progress to fibrosis. This side effect is due mostly to augmented concentration of reactive oxygen species such as superoxide and hydroxyl radicals (Tunon et al., 1995), decrease in nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP), and overproduction of mature collagen fibrils (Breuer et al., 1995; Foth, 1995). A variety of cells of the lung are affected by intratracheal or intravenous injection of bleomycin. Bleomycin stimulated alveolar macrophages undergo a sequence of changes that starts with cellular activation of the phagocytes that is associated with secretion of inflammatory cytokines and enzymes (Denholm and Rollins, 1993; Hamilton, 1993). Secreted enzymes, such as gelatinase, may then facilitate the migration of the phagocytes in the tissue and, thus, allow the activated macrophage to initiate widespread inflammatory reactions in the lung (Mori, 1995; Silva, 2010). Later on, macrophages may enter apoptosis due to lack of the protective mechanism coming from intracellular synthesis of heat shock proteins, a process

*Corresponding author. E-mail: amjalali@hotmail.com. Tel: 0098-611-3738380. Fax: 0098-611-3738381.

that is inhibited by bleomycin (Hamilton, 1993; Tanaka et al., 2010). Actin-containing non-muscle cells such as fibroblasts and myofibroblasts are the cells responsible for the synthesis and secretion of interstitial extracellular matrix proteins such as collagen. Such products colaborate in the alveolar walls are at the core of the fibrotic transformation of the lung and subsequently compromises respiratory function (Lan et al., 1997). Increased numbers of fibroblasts and myofibroblasts in interstitial areas, characteristic of pulmonary fibrosis, could results in an increased contractile activity of lung tissue. *In vivo* experiments showed that lung strips from fibrotic lungs exhibit contraction in response to pharmacological agents e.g. acetyl choline, sodium tungstate, pirylamine maleate or potassium chloride (Hemmati and Hicks, 1999). To the best of our knowledge there was no previous investigation to find an effective and safe natural product for treatment of bleomycin-induced lung fibrosis. Several drugs such as steroids, immunosupressive agents and antioxidants have been tested to prevent the pulmonary toxicity of bleomycin, but they have not led to a useful clinical treatment because of their adverse effects on other tissues or organs (Daniels and Ryu, 2006; Piguet et al., 1993).

Yarrow (*Achillea millefolium* L) is a plant of compositae family with numerous subspecies which are found in various regions of the world. It grow in eastern, southern, central Europe and Asia (Evans, 1996). This plant widely grown in different parts of Iran mainly Loresatn, Esfahan, Eelam and Fars provinves (Herbal Pharmacopea of Iran, 2003). Yarrow is widely used in Iranian folk medicine to treat diverse diseases including inflammation, pain and gastrointestinal disturbances.

In modern medicine, several studies have been done to verify the effects of yarrow. They have shown that this plant have anti-inflammatory (Tunon et al., 1995; Burk et al., 2010), antitumor (Tozyo et al.,1994), antioxidant, antimicrobial (Candan et al., 2003), liver protective activities (Lin et al., 2002), anti-secretory and gastro-protective activity (Baggio et al., 2002; Cavalcanti et al., 2006). Some report showed liver protection against carbon tetrachloride and acetaminophen-induced liver injury by yarrow (Gagdoli and Mishra,1995) and antioxidant and anti inflammatory agents have been showed to prevent the cell damages by free radicals (Kilinc et al., 1993; Bozin et al., 2008)

Information concering the *in vivo* anti-fibrogenic effect of a plant against bleomycin-induced pulmonary fibrosis has not been found in the literature. However, screening of protective potential against acute and chronic ulcers showed positive correlation with the folk medicinal use. This study was designed to find a natural agent to prevent or treat the fibrogenic effect of bleomycin. So the efficacy of hydroalcoholic extract of yarrow has been examined to control the fibrogenic effect of bleomycin in animal model of rat.

MATERIALS AND METHODS

Plant material

Yarrow flowers were collected from Isfahan region in central Iran. The plant was identified as A. millefulium L (domestic name: Boomadaran) in the department of pharmacognosy, school of pharmacy, Ahwaz Jundishapur University of Medical Sciences.

Preparation of extract

Maceration method was used to prepare hydroalcoholic extract of yarrow. The plant materials were dried under shade and then was ground into a fine powder using electric blender. 100 g of dried powder was placed in a becker, alcohol 70% + distilled water 30% was added, mixed properly and left in room temperature. After 72 h solvent was separated and filterated with Wathman filter paper. The extract was then concentrated using rotary evaporator. Density of product was measured by a picnometer.

Animals

Sprague Dawley rats of either sexes weighing 180 to 220 g were used throught the study. Animals were purchased from Razi Instituite (Karaj, Iran). They were housed in polycarbonate cages in groups of six and had free access to standard laboratory pellet diet (Shushtar Khorakdam Co, Iran) and tap water. The animals were maintained in holding room illuminated with 12 h light/dark cycle. Room temprature was set at 23 ± 2°C with a relative humidity of 45 to 55%.

All experiments were conducted in accordance with principles and guidelines of the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes and the guiding principles in the use of animals in toxicology" which were adopted by the Society of Toxicology.

All test groups of animals (n= 5-6) were given single intratracheal instillation of 7.5 IU/Kg of bleomycin sulfate in a volume adjusted not to exceed 0.2 ml. Control group received 0.2 ml of saline vehicle. After 24 h, all test groups were treated with either oral saline or 400, 800 and 1600 mg/kg of yarrow extract (oral) once a day for 2 weeks. The doses of the extract was selected based on its protective activity against chronic gastric lesions induced in rats which were accompanied by no signs of relevant toxicity even at very long chronic exposure (Cavalcanti et al., 2006). After such treatment, animals were killed and lungs removed for histological and pharmacological studies.

Histological studies

At the end of treatment schdules, animals were killed by ketamine overdose. Lungs was dissected out and washed in normal saline. After taking a tissue sample from left lung for pharmacological studies, the remaining lung tissue was fixed in 10% neutral formaline solution for at least 48 h. Tissue was then embeded in paraffin and sectioned at approximately 5 micron thickness. Tissue affixed to a glass slide deparffinised, rehydrated and stained with hematoxylin and eosin. The slides were then examined by light microscopy to evaluate changes due to bleomycin and also the effect of yarrow extract on lung.

Pharmacological studies

Two weeks after treatment, lung strip (4 x 4 x 20 mm) from left lobe of was prepared and suspended in an organ bath containing Krebs'

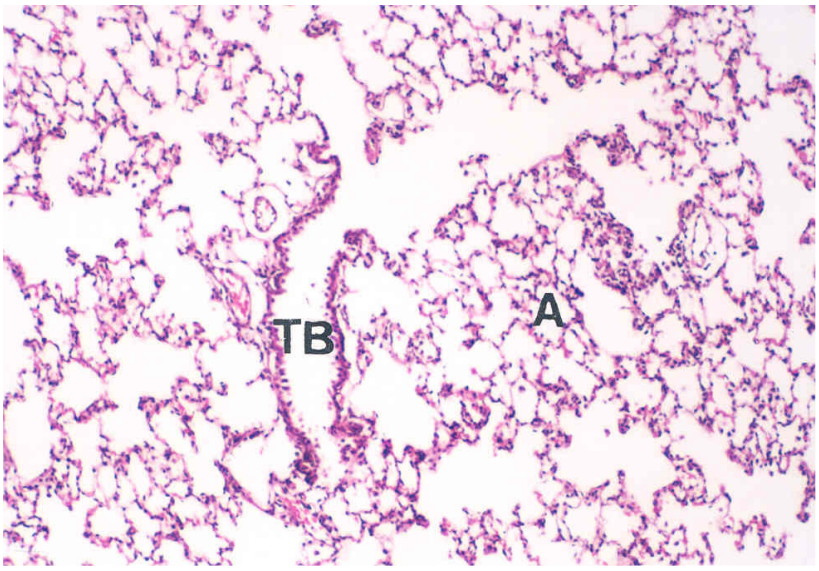

Figure 1. Photomicrograph of normal lung section. No septal thickening or inflammatory cells in alveolar spaces (A) or terminal Bronchile (TB) are seen (H and E x100).

solution, aerated with 95% O_2 and 5% CO_2 at 37°C. Contractile activity of lung strip to sodium tungstate and KCl was measured isometrically (using Harvard Oscillograph and UF1 isometric transducer). Contractile response of different groups were recorded and compared. Pharmacological study was performed in control, fibrotic (bleomycin) and treatment groups.

Statistical analysis

Statistical comparison was made by one-way ANOVA. Significant F values were tested with Tukey's test. Data are presented as mean±SEM. In all cases $p<0.05$ was considered significant.

RESULTS

Histology results

Lung tissue of rats in control group showed normal structure and no pathologic lesion was seen. Thin alveolar walls without inflammatory cells inside the alveoli was evident (Figure 1). In lungs from bleomycin-treated rats, there was a great change in tissue structure associated with infiltration of mononuclear cells, hyperplasia of type II pneumocytes and increased connective tissue in intralveolar septa (Figure 2). Numerous fibroblasts and myofibroblasts, together with collagenous fibre were found in alveolar structure indicating fibrosis (Figure 3). The lesions were diffuse in nature involving most lobes of the lung. Peribronchial or perivascular fibrosis was seen in most tissue sections. Yarrow extract treatment could reduce the structural damage of lung tissue due to bleomycin. In such tissues there were less infiltration of inflammatory cells in alveolar spaces. Thickness of alveolar walls has been reduced and less collagen deposition was observed. Numbers of fibroblasts and myofibroblasts had a pronounced reduction in lungs that are treated with yarrow extract following bleomycin instillation. Such reduction led to lower synthesis of collagen an therefore less fibrosis (Figure 4).

Pharmacolgy results

Tissue contraction of fibrotic lungs, in response to sodium tungstaste (ST) and KCl were significantly greater in lung strips of fibrotic group copmared to control group. Groups that were treated with yarrow extrcat showed less contractions than the bleomycin fibrotic group. The least contraction of lung strips was obtained in group that received 1600 mg/kg yarrow extrcat, indicating the less contractile cells in interstitium and less fibrosis (Figures 5 and 6). This protective activities of yarrow extract showed a dose-dependent manner.

DISCUSSION

The use of bleomycin as a therapeutic agent in the treatment of cancer is limited by adverse effects that can result in pulmonary fibrosis. The hallmark of the disease is characterized by the increase of extracellular matrix proteins, notably collagens, associated with the growth of interstitial cells within the alveolar septa (Quinones and Crouch, 1986; Moseley et al., 1986). Conventional therapy of human pulmonary fibrosis with corticosteroids

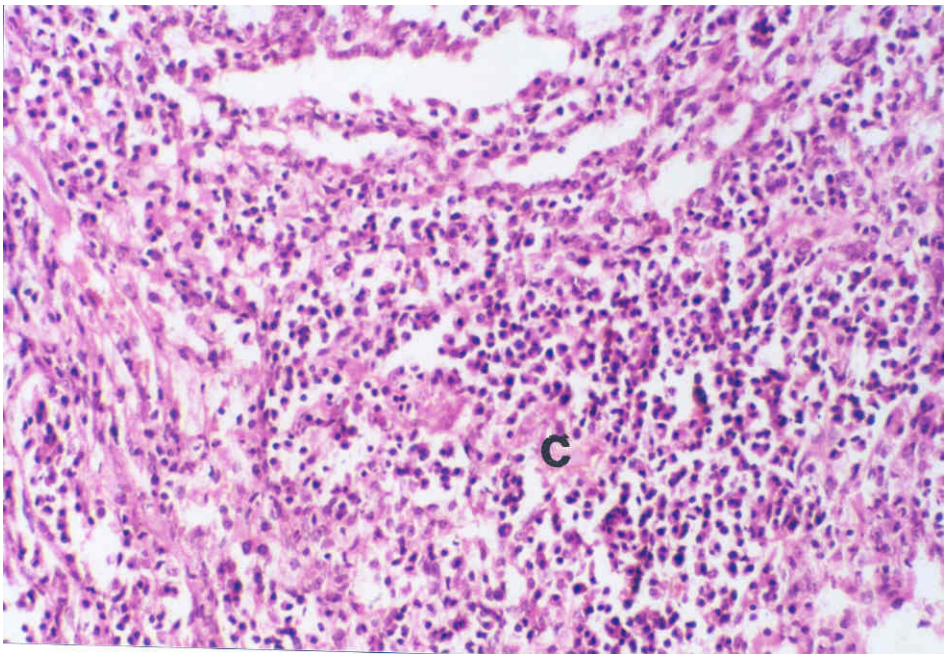

Figure 2. Photomicrograph of bleomycin treated lung section. Extensive infiltration of inflammatory cells in alveolar spaces associated with diffused fibrosis and collagene (C) production are seen (H and E x200).

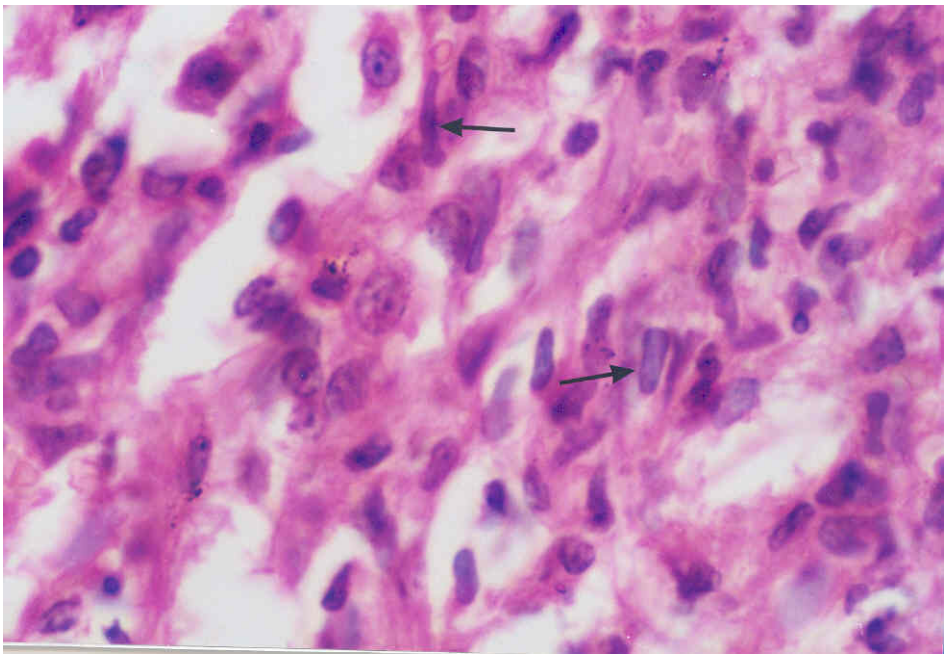

Figure 3. Photomicrograph of bleomycin treated lung section. Alveolar thickening is associated with proliferation of fibroblasts and myofibroblasts (arrow heads) (H and E x800).

or immunosuppressive agents generally does not lead to an ideal result. Various attempts in animal models designed to inhibit the initiation and progression of bleomycin-induced pulmonary fibrosis (Daniels and Ryu, 2006). However, such attempts have not led to a useful clinical trial because of the low efficacy and adverse

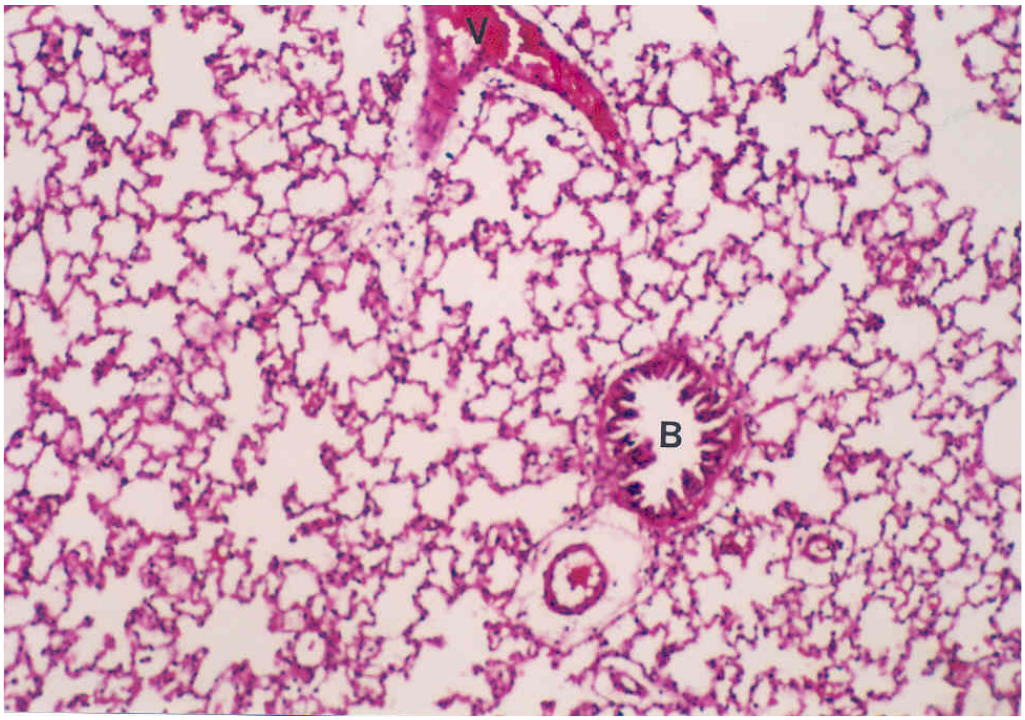

Figure 4. Photomicrograph of lung section after treatment with Yarrow extract (1600 mg/kg). Less alveolar thickening is associated with reduced numbers of inflammatory cell. No sign of peribronchial (B) or perivasculad (V) fibrosis is seen (H and E x100).

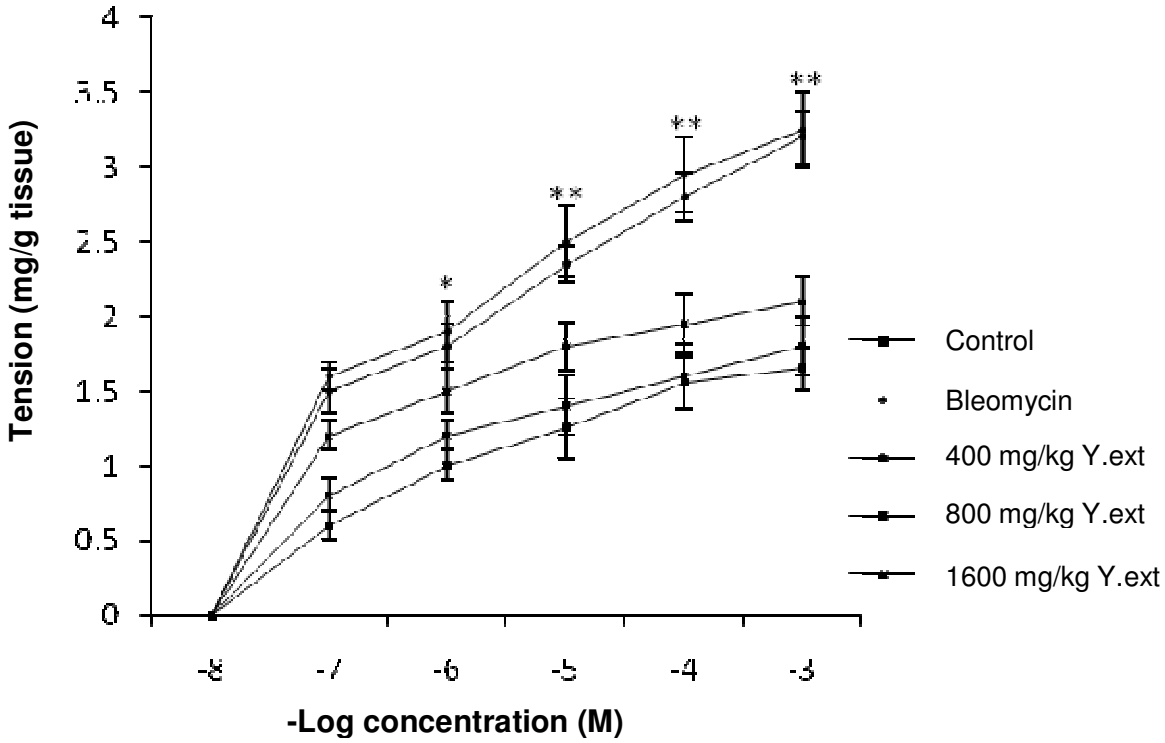

Figure 5. Dose response curvre of control, fibrotic and yarrow extract treated lung strips to sodium tungstate. Each point represents mean±sem (n=5). Values signifinatly different from control (non fibriotic) are indicated * ($p<0.05$) or **($p<0.01$).

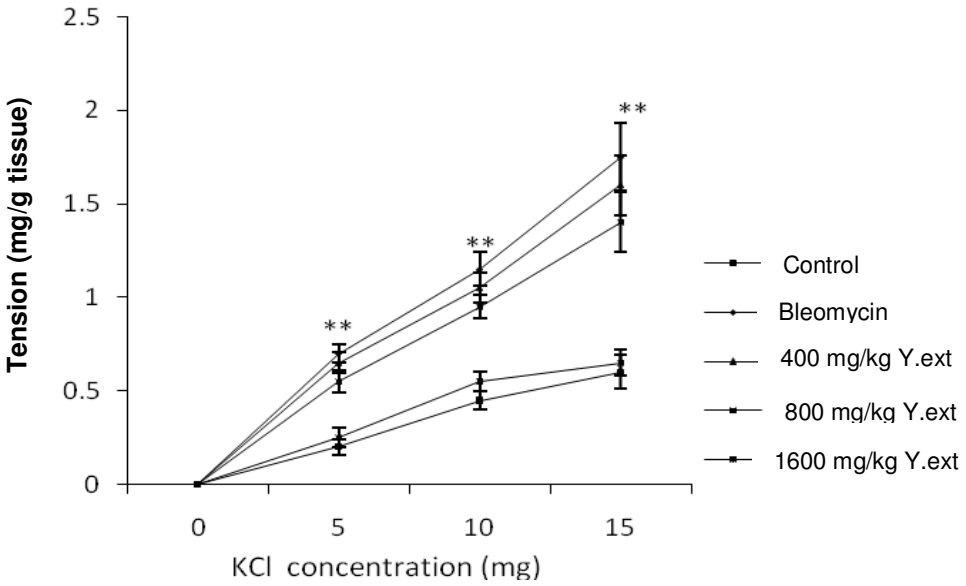

Figure 6. Dose response curvre of control, fibrotic and yarrow extract (Y.ext) treated lung strips to KCl. Each point represents mean±sem (n=5). Values signifinatly different from control (non fibrotic) are indicated * **($p<0.01$).

effects on lungs and other tissues (Piguet et al., 1993). The employment of medicinal plants e.g. yarrow in this disease may open a new era for management of pulmonary fibrosis.

The results presented in this research may help to find a new approach to prevent the development of bleomycin-induced pulmonary fibrosis by the use of herbal medicines. In the present study we evaluated the effect of yarrow extract on bleomycin-induced pulmonary fibrosis. The advantage of this work is the application of pharmacological procedures to study the mechanical function of fibrotic lung. Contractiltiy of fibrotic lung strips to sodium tungstate, mepyramine has been reported earlier which seems to be due to increase in population of myofibroblast in lung interstitum (Hemmati and Hicks, 1999). Pharmacological results of present study confirmed that yarrow extract is able to decrease the contractility of fibrotic lungs. Such phenomenon may atribute to the reduced numbers of collagen producing cells: fibroblasts and myofibroblasts. Similar result have not been reported elswhere.

Yarrow contains different chemical substances e.g. volatile oil (chamazulene, beta-pinene), sesquiterpene lactones (achillin, alphaperoxyachiolide), polyynes (potica epoxide), flavonoids (apigenine, rutin) and bteaine(L-stachydrine, Bbetonocine) (Gomez et al., 1999; Bozin et al., 2008). In addition to flavone derivatives, two caffeic acid derivatives: dicaffeoylquinicacid and chlorogenic acid have been identified in yarrow species (Innocenti et al., 2007). The main pharmacologically active principles were shown to be the essential oil (antimicrobial), proazulenes and other sesquiterpene lactones (antiphlogistic), dicaffeoylquinic acids (choleretic) and flavonoids (antispasmodic) (Kovats et al., 2010). Chronic administration of Yarrow reduced the glycemia rate which was correlated with the presence of sesquiterpene lactones in *A. millefolium* (Peris et al., 1995). Achilleine, an alkaloid isolated from *A. millefolium*, reduced the clotting time of rabbits (Cavalcanti et al., 2006). The flavonoid content exert spsamolyitic effect while the proazulene fraction has anti-edema and anti-inflammatory effect (Muller-Jakic et al., 1994). Our results suggest that anti-fibrogenic effect of *A. millefolium* could be due to more than one compounds of this plant. Therefore total extract of yarrow will have benefit from wide range of active ingredint in prevention of the lung fibrosis. The most active component of yarrow in pulmonary fibrosis needs to be verified by further investigations.

From this work we can not find the precise mechanism of yarrow extract. However; the antioxidant and anti inflammatory effect of this plant has been studied and reported before (Gomez et al., 1999; Bozin et al., 2008; Burk et al., 2010). Topical anti-inflammatory activity of the sesquiterpenes was already shown being caused by inhibition of the arachidonic acid metabolism (Kastner et al., 1993; Li et al., 2011). The anti-fibrogenic of yarrow may partly mediated by inhibition of human neutophil elastase and matrix metalloproteinase (Benedek et al., 2007). There are positive results on the wound healing activity of yarrow extract (Hemmati et al., 2002; Temmamogullari et al., 2009). The diversity and complexity of the effective compounds of yarrow species explains the broad spectrum of their activity. Fractions of

the plant which exert the antioxidant (Candan et al., 2003) and anti inflammatory action may be responsible for its effects on pulmonary fibrosis.

On the basis of the present findings, we conclude that after oral treatment with yarrow extract, rats exhibited no treatment-related toxicological or histopathological abnormalities. The doses of yarrow tested in rats were higher that anticipated for human consumption. Thus, it is likely that no long-term toxicological risk would occur with the doses of yarrow commonly consumed by humans. However, this extrapolation should be made with caution, since the real human risk cannot be assessed on the basis of the present study.

ACKNOWLEDGEMENTS

This article is a part of results from the Pharm D thesis performed in the school of Pharmacy and financially supported by Ahwaz Jundishapur University of Medical Sciences.

REFRENCES

Baggio CH, Freitas CS, Nhaducue PF, Rieck L, Marques MCA (2002). Action of crude aqueous extract of leaves of *Achillea millefolium* L.(Compositae)on gastro-intestinal tract. Rev. Bras. Pharmacog., 12: 31-33.

Benedek B, Kopp B, Melzig MF (2007). *Achillea millefolium* L.s.l.–Is the anti-inflammatory activity mediated by protease inhibition. J. Ethnopharm., 113: 312-317

Bozin B, Mimica DN, Bogavac M, Suvajdzic L, Simin N, Samojlik I, Couladis M (2008). Chemical Composition, Antioxidant and Antibacterial Properties of Achillea collina Becker ex Heimerl s.l. and A.pannonica Scheele Essential oils. Molecules, 13: 2058-2068.

Breuer R, Lossos IS, Krymsky M, Dagan A, Yedgar S (1995). Abatement of bleomycin-induced pulmonary injury by cell-impermeable inhibitor of phospholipase A_2. Life Sci., 57: 237-240.

Burk DR, Cichacz ZA, Daskalova SM (2010). Aqueous extract of *Achillea millefolium* L. (Asteraceae) inflorescences suppresses lipopolysaccharide-induced inflammatory responses in RAW 264.7 murine macrophages. J.M.P.R., 4(3): 225-234.

Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sokmen A, Akpulat HA (2003). Antioxidant and anti-microbial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan.(Asteraceae). J. Ethnopharma., 87: 215–220.

Cavalcanti AM, Baggio CH, Freitas CS, Rieck L, de SRS, DaSilva SJE, Mesia VS, Marques MCA (2006). Safety and antiulcer efficacy studies of *Achillea millefolium* L. after chronic treatment in Wistar rats. J. Ethnopharm., 107: 277-284.

Daniels CE, Ryu JH (2006). Treatment of idiopathic pulmonary fibrosis. Seminars in Respiratory and Critical Care Med., 27(6): 668-676.

Denholm EM, Rollins SM (1993). Alveolar macrophage secretion of a 92-kDa gelatinase in response to bleomycin. Am. J. Physiol., 265: 581-585.

Evans WC (1996). Trease and Evans pharmacognosy. W.B. Saunders Co. London.

Foth H (1995). Role of the lung in accumulation and metabolism of xenobiotic compounds - implications for chemically induced toxicity. Crit. Rev. Tox., 25: 165-205.

Gagdoli C, Mishra SH (1995). Preliminary screening of *Achillea millefolium*, Cichorium intybus and Capparis spinosa for antihepatotoxic activity. Fitoterapia, 66: 319–323.

Gomez MA, SJenz MT, Garcيa MD, FernJndez MA (1999). Study of the topical anti-inflammatory activity of Achillea ageratum on chronic and acute inflammation models. Z Naturforsch C, 54(11): 937-941.

Hamilton RF, Li L, Felder TB, Holian A (1993). Bleomycin induces apoptosis in human alveolar macrophages. Am. J. Physiol., 269: 318-325.

Hemmati AA, Arzi A, Amin M (2002). Effect of *achillea millefolium* extract in wound healing of rabbit. J. Nat. Remedies, 2: 164-167

Hemmati AA, Hicks R (1999). Increased myofibroblast contractile sensitivity in paraquat pretreated rat lung tissue. Life Sci., 65: 2325-2331.

Herbal Pharmacopea of Iran (2003). Ministry of Health, Tehran, Iran.

Innocenti G, Vegeto E, Dall'Acqua S, Ciana P, Giorgetti M, Agradi E, Sozzi A, Fico G, Tome F (2007). *In vitro* estrogenic activity of *Achillea millefolium* L. Phytomed., 14: 147-152.

Kastner U, Sosa S, Tubaro A, Breuer J, Rucker G, DellaLoggia R, Jurenitsch J (1993). Anti-edematous activity of sesquiterpene lactones from different taxa of the *Achillea millefolium* group. Planta Medica, 59: A669.

Kilinc C, Ozcan O, Karaoz E, Sunguroglu K, Kutluary T, Karaca L (1993). Vitamin E reduces bleomycin-induced lung fibrosis in Mice: Biochemical and Morphological studies. J. Basic Clin. Physio. Pharm., 43: 249-269.

Kovats N, Goloncser F, Acs A, Refaey M (2010). Quantification of the antibacterial properties of Artemisia absinthium, A.vulgaris, Chrysanthemum leucanthemum and *Achillea Millefolium* using the vibriofischeri bacterial bioassay. Acta Botanica Hungarica, 52(1-2): 137-144.

Lan TP, Weinbach J, Opolon P, Linares CG, Reynes JP, Grégoire A, Kremer E, Lemay AM, Hanston CK (2005). Bleomycin-induced pulmonary fibrosis susceptibility genes in AcB/BcA recombinant congenic mice. Physiol. Genomics, 23: 54-61.

Li H, Kim JY, Hyeon J, Lee HJ, Ryu JH (2011). *In vitro* Antiinflammatory Activity of a New Sesquiterpene Lactone Isolated from Siegesbeckia glabrescens. Phytother. Res., In press.

Lin LT, Liu LT, Chiang LC, Lin CC (2002). In vitro anti-hepatoma activity of 15 natural medicines from Canada. Phytother. Res., 16: 440-444.

Moseley PL, Hemken C, Hunninghake GW (1986). Augmentation of fibroblast proliferation by bleomycin. J. Clin. Invest., 78: 1150-1154.

Mori H, Tanaka H, Kawada K, Nagai H, Koda A (1995). Suppressive effects of tranilast on pulmonary fibrosis and activation of alveolar macrophages in mice treated with bleomycin: role of alveolar macrophages in the fibrosis. Jap. J. Pharm., 67: 279 289.

Muller JB, Breu W, Probstle A, Redl K, Greger H, Bauer R (1994). In vitro inhibition of cyclooxygenase and 5-lipoxygenase by alkamides from Echinacea and Achillea species. Planta Medica, 60: 37-40.

Peris JB, Stubing G, Vanaclocha B (1995). Fitoterapia aplicada. Colegio Oficial de Farmaceuticos,Valencia, pp. 374-375.

Piguet PF, Rosen H, Vesin C, Grau GE (1993). Effective treatment of the pulmonary fibrosis elicited in mice by Bleomycinor silica with anti-CD-11 antibodies. Am. Rev. Res. Dis., 147: 435-441.

Quinones F, Crouch E (1986). Biosynthesis of interstitial and basement membrane collagens in pulmonary fibrosis, Am. Rev. Res. Dis., 134: 1163-1171

Silva MT (2010). When two is better than one: macrophages and neutrophils work in concert in innate immunity as complementary and cooperative partners of a myeloid phagocyte system. J. Leuk. Bio., 8 (1): 93-106.

Tanaka KI, Tanaka Y, Namba T, Azuma A, Mizushima T (2010). Heat shock protein 70 protects against bleomycin-induced pulmonary fibrosis in mice. Biochem. Pharm., 80(6): 920-931.

Temmamogullari F, Hayat A, Baba F (2009). Effects of Yarrow on wound healing in rabbits. J. Anim. Vet. Adv., 8; 1204-1206.

Tozyo T, Yoshimura Y, Sakurai K, Uchida N, Takeda Y, Nakai H, Ishii H (1994). Novel anti tumor sesquiterpenoidsin *Achillea millefolium*. Chem. Pharmac. Bull., 42: 1096-1100.

Tunon H, Olavsdotter C, Bohlin L (1995). Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin bio-synthesis and PAF-induced exocytosis. J. Ethnopharma., 48: 61-76.