An Article Submitted to

Journal of Complementary and Integrative Medicine

Manuscript 1222

Biphasic Modulation of Neutrophil Migration by Aqueous Extracts of Uncaria tomentosa in Murine Lung.

Noelia Roque[*] David Cremonezzi[†] Celeste Maria Bigliani[‡]

Ezequiel Grondona[**] Paula Zunino[††] Andres A. Ponce[‡‡]

[*]Physiology Institute, School of Medicine, University of Cordoba, noeroque84@yahoo.com.ar

[†]Cátedra de Histología, School of Medicine, University of Cordoba, dccj35@yahoo.com.ar

[‡]Physiology Institute. School of Medicine, University of Cordoba, biglianiceleste@gmail.com

[**]Physiology Institute, School of Medicine, University of Cordoba, ezetag@hotmail.com

[††]Catedra de Quimica Organica, Fac Cs Ex Fisic y Nat. University of Cordoba, pauzun@efn.uncor.edu

[‡‡]University of Cordoba, andresaponce@daad-alumni.de

Biphasic Modulation of Neutrophil Migration by Aqueous Extracts of Uncaria tomentosa in Murine Lung.

Noelia Roque, David Cremonezzi, Celeste Maria Bigliani, Ezequiel Grondona, Paula Zunino, and Andres A. Ponce Prof

Abstract

In the history of medicine, the treatment of disease has been influenced by an intimate contact with nature. A mice model of lipopolysaccharide (LPS)-induced acute lung injury was used to evaluate the protective effects of long-term water-soluble administration ad libitum of Uncaria tomentosa extracts (20 gr/L; UTE) in lung inflammation. Swiss mice had LPS (1,67μg/ml) instilled intranasally 3hs before sacrificed, and were then pre-treated with UTE for 7, 15, 30 or 90 days or with a single dose of dexamethasone (2,5 mg/kg, DX). Inflammatory cell concentration was measured in the bronchoalveolar fluid (BALF) and histology was performed. No acute or chronic toxicity signs were observed in the clinical status. In addition, body weight, food consumption, organ weight, kidney, liver, and lung pathology were not found to be affected by the UTE treatments. UTE or DX significantly reduced the lung edema, exudation and lung injury histology for 7 and 90 day treatments. In addition, pre-treatment with UTE revealed a biphasic attenuated recruitment in BALF from neutrophils at 7 and 90 days induced by endotoxin exposure compared to the control ($p<0.05$). These data suggest that UTE initially induces a nonspecific response that is transient protection from PMNs migration into the lung mice.

KEYWORDS: cystic fibrosis, Uncaria tomentosa, lung, mice

INTRODUCTION

America is one of the richest ecosystems of fauna and flora in the world, with the Amazon environment being a source that every day provides new natural agents with antioxidant and anti-inflammatory activity (Ribnicky, et al. 2008).

Uncaria tomentosa from Amazonia is a widely used species in folk medicine for the treatment of several diseases, and appears to be a possible source of several active substances with useful properties. It possesses anti-inflammatory antispasmodic, antioxidant, DNA repairing, immunomodulatory, anti-tumor and anti-viral properties (Aguilar et al. 2002; Akesson, et al. 2003; Allen-Hall, et al. 2007; Aquino, et al. Bednarek, et al. 2004).

Acute respiratory processes are inflammatory disorders characterized by an excessive infiltration of neutrophils (polymorphonuclear leukocytes, PMNs). These inflammation is now accepted as a main aspect in the pathogenesis of lung disease in cystic fibrosis (CF). Its most distinctive feature is a manifest and constant invasion into the airways of PMNs, which damage the lung architecture by releasing harmful mediators, such as reactive oxygen species and proteolytic enzymes (Terheggen-Lagro, et al. 2005).

CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CFTR is also expressed in neutrophils and several neutrophil functions like cytokine production, migration, phagocytosis and apoptosis seem altered in CF and in some hospital from EE.UU, reported use of complementary and alternative therapies in CF patients (Reddy, et al. 2005; Conese, et al. 2003).

A mechanism of action for *Uncaria tomentosa* extract (UTE) produce the paradoxical effect of sometimes increasing and sometimes decreasing the PMN migration have not describe before. It has been established that preparations with the bark of UTE interfere with the production of various cytokines, and can also act on the proliferation of various components of the immune system as well as on phagocytosis and the complement system, thus producing an immunomodulatory activity Akesson, et al. 2003; Allen-Hall, et al. 2007; Spelman, et al. 2006).

In the present study, we demonstrate that UTE has a patent biphasic effect in murine neutrophil migration induced by LPS-injury in mice lung and it was established that the pre-treatment administration on different days of the UTE did not modify either the body weight or clinical status.

MATERIALS AND METHODS

Animals

Swiss albino mice were used in all experiments (weighing 25-30 g) under 12h light/dark cycle (with lights on 20:00-8:00) and a constant temperature (20 ± 2 °C).

Metabolism

The water and aqueous extract of UTE were accurately calculated before being made accessible to the mice. At 7, 15, 30 and 90 days after treatment, the water and UTE were collected and measured, with water and UTE intake being determined by the difference between the total amount of UTE or water that was given to the mice and the total remaining UTE or water in each group (n=8) at each time point. Weight was also measured at 7, 15, 30 and 90 days after treatment.

Preparation of extracts and treatment

The UTE was freshly prepared by decoction of the bark at a 20 g/L concentration. This involved the extract being purified for 45 minutes in boiling water, before being filtered and then left for 12 hours before use. The material was protected from direct light and stored at 4°C (Aguilar et al. 2002; Lemaire et al. 1999).

Mice had access to continuous commercial chow and were administered *ad libitum* water, or with the protective effects of UTE (equivalent doses at 0.75 g/kg) being studied for 7, 15, 30 or 90 days.

The general health of the mice was evaluated for approximately 15 minutes at two different daily time points.

The animal protocol was approved by the Animal Care Committee of the University of Córdoba, Argentina and the bark was authenticated by Paula Zunino (Department of Pharmacognosy, UNC; Córdoba)

Instillation of LPS and Bronchoalveolar Fluid (BALF)

Mice were anaesthetized with a short lasting inhalation and the acute lung inflammation was induced intranasally (i.n.) with *P. aeruginosa* LPS (serotype 10) (Sigma, St. Louis) in PBS solutions (60 µl) Wieland et al. 2002). During the experimental anaesthesia procedure, the mice were placed under a warming light and temperature was maintained close to 37ºC. Dissolved sterile saline or saline alone (as a vehicle control) was used at a dose of 100 µg LPS/kg mouse body

mass. After intratracheal instillation, all mice were put into the vertical position to ensure that the fluid was evenly distributed in both lungs. Control animals were treated in the same way with PBS (Roderick, et al. 1997; Song, et al. 1998).

A differential count of cells obtained by BAL was performed with cold PBS (4°C) on the sacrificed mice after a 3 hour LPS exposure. The mice were anesthetized by inhalation of ether and then fixed on a board at an angle of 50° in a supine position. After sterilization, a mid-line incision was performed on the neck to isolate the trachea. The trachea was cannulated with a sterile, 23-gauge needle, and 1 ml ice-cold of PBS was infused intratracheally with the fluid being collected by aspiration. This procedure was repeated three times by flushing, resulting in a total volume of 5-7 ml. The BAL fluid was stored on ice until being centrifuged at 300 *g* for 10 min. The supernatant was then discarded, leaving a 0.1-ml cell pellet. This pellet was resuspended in 1 ml of buffer (1% BSA and 0.1% sodium azide in PBS), and a 10-µl aliquot was used for cell count with a Trypan blue exclusion (0.4%) being used to determine cell viability. The cells were then collected from the BAL fluid for measurement of the differential count of cells obtained by May-Grünewald Giemsa staining before being, air-dried, and coverslipped. More than two hundred cells obtained by BALF, using morphologic criteria, were counted under the microscope to obtain the percentages of macrophages (MA) or polymorphonuclear neutrophils (PMNs). The absolute numbers of PMNs or MA were calculated as follows: number of BALF cells/ml × percent PMNs= PMN in BALF cells/ml (Çok *et al.* 2007, Freedman, et al. 2002). No treatment of PBS (pyrogen-free) occasionally causes a weak but significant increase in BALF cells (Hirano, 1997).

In the dexamethasone group, the mice were i.p. administered at a single dose of 2.5 mg/kg at 9:00 a.m. Food consumption; water intake and weight were carefully monitored in all treatments (Sadikot, et al. 2001).

Histopathology

In order to harvest the lungs, we cannulated the trachea and fixed the lungs by inflation with 4% paraformaldehyde. After overnight fixation, the tissue was embedded in paraffin, sectioned, and stained. Hematoxylin and eosin stains were made to determine the morphology and inflammatory infiltrate. The lung pathology was assigned microscopically one of four grades according to the severity of the inflammation, as follows: 1, normal histology; 2, mild focal inflammation; 3, moderate to severe focal inflammation with areas of normal lung tissue; and 4, severe inflammation to necrosis or severe inflammation throughout the lung. The cellular alterations were classified by a scoring system based on the proportions of cell populations (Freedman, et al. 2002).

Data analysis

Data were expressed as means ± standard errors of the means (SEM). Pearson´s correlations and the statistical significance of differences between the treatment and control groups was determined by factorial analysis of variance (ANOVA) and the Kruskal Wallis test followed Dunn´s multiple comparison for three or more variables. Differences were considered statistically significant for p values <0.05. Statistical analyses were performed using Info-Stat software (Córdoba, Argentina, 2008).

RESULTS

Survival analysis

We first examined whether the UTE model is associated with tolerance in *ad-libitum* administration for 90 days in Swiss Albino mice. When these values were compared with ingested water and the evolution of the body weight determined in untreated animals, no significant statistical differences were found (dates not show). All of the animals survived for the duration of the experiments.

Histopathology parameters

The relative weights after 90 days of treatment (water and treatment with the UTE respectively) were: kidneys (13.29 ± 0.10, n = 6 and 13.16 ± 0.10, n= 5); lung (12.00 ± 0.62, n= 6 and 10.99 ± 0.50, n= 5) and liver (56.07 ± 2.60, n= 6 and 60.98 ± 2.70, n= 5). Also, no changes were detected in the structure of organs evaluated histologically.

Bronchioalveolar fluid evaluation

Next, we wished to test the alveolar PMNs and MA obtained in BALF. In this study, we showed that for pre-treatments with DX on 7 or 90 days of treatment with UTE in mice, the number of PMNs in BALF were similar to basal and PBS, which was capable of recognizing up regulation and down regulation of the PMN. However LPS induced significantly more cells ($p < 0.05$) (Figure 1).The number of MA (MA thousand in BALF/ml) decreased from 7 to 90 days treatment for both UTE and DX (Figure 2), while an increase in the numbers of PMNs and MA was observed following i.n. administration of LPS, thus validating the efficacy of LPS (Fig 1 and 2).

In animals without LPS instillation and treated with UTE for 7, 15, 30 or 90 days, the numbers obtained in BALF with PMNs and MA showed no change either population cell, see Table 1 and 2.

Three hours after instillation, there was a 100% diffuse inflammation, with edema and congestion occurring in animals treated only with LPS. In the treatments with DX-LPS and UTE of 7, 15, 30 and 90 days and 3hs before LPS were reduced by (%) 64, 63, 88, 86, 60 respectively, with microscopic examination showing no ultrastructural lesions of the lungs or the airways. Lung histology of mice receiving PBS instillation showed physiologic lung architecture (Table 3).

Correlation in BALF

We then evaluated the hypothesis that protection from PMN and MA could be associated with leukocyte recruitment to the lung, because there was a strong correlation between the number of PMNs in BALF and MA in the lung content (r^2 =0.44, p<0.0001).

DISCUSSION

Medicinal plants are characterized by their multiple active ingredients, which, by acting together (synergy), can enhance exert a beneficial effect on the functioning of various organs (Song, et al., 1996; Williamson, 2005). While the purpose was not to identify the compounds of this plant, have two main classes of secondary metabolites, alkaloids and quinovic acid glycosides (Valerio and Gonzales 2005), we used *Uncaria tomentosa* as an water soluble extracts because its reported in complementary and alternative therapies in CF patients (i.e: http://www.umm.edu/altmed/articles/cystic-fibrosis-000045.htm) and the significant protective effect of UTE preparation in the murine lung experimental LPS inflammation has not been investigated thoroughly

We performed a test to elucidate the tolerance to UTE in a mouse model for 90 days. The fluid intake showed that the administration of UTE did not change the amount of liquid ingested or the evolution of body weight when compared with water.

No histological changes were found in the structure of tissues evaluated in animals treated for various time periods with the UTE. Finally, no detected variations in ambulation or in the general state of the animals (stains on the skin, hair loss, etc.).

On the other hand, in other assays conducted on healthy volunteers, UTE administered for 6 weeks at a dose of 5 grams of bark/kg did not cause any symptoms of toxicity, but also induced a significant increase in leukocytes

(Valerio and Gonzales, 2005). Moreover, several authors demonstrated an anti-inflammatory activity of UTE (Aguilar, et al. 2002; Aquino, et al. 1991; Akesson, et al. 2003; Lemaire, et al. 1999; Cisneros, et al. 2005).

Only instillation with LPS induced a significant increase in the concentration of PMNs in the BAL fluid, which was reduced in animals treated with DX or UTE for 7 or 90 days, but not in the experimental group with UTE-15 and UTE-for 30 days.

The exact mechanism of the biphasic regulation by UTE administration remains to be clearly understood. The biphasic mechanism in the modulation of PMN migration may be through the cytokines, like tumors cells (Spelman *et al.* 2006). A class of herbal medicines, known as immunomodulators, alters the activity of immune function through the dynamic regulation of informational molecules such as cytokines (Allen-Hall et al. Bednarek et al. 2004; Lemaire et al. 1999).

In accorded with our results, Bižanov and Tamošiūasn (2005) found IgG titres in oral immunization of mice treated with Sendai virus in combination with UTE significantly different reduced by day 14 after the last immunization with compared with 7 days immunization. In another hand, Dhuley et al. (1997) found *Withania somnifera* increased TNF- expression, while Davis and Khutan (1999) showed it decreased TNF- . Moreover, several mechanisms, such as synergistic interactions or biphasic response is also recognized in other plants (Williamson, 2005; Gaffney, et al. 2001; Sattayasai, et al. 2008). These differential effects could be due to the fact that some of the many active substances contained in the extract may act on different cellular systems when applied for different periods, and this suggested the presence of compounds with different actions on UTE tolerance in a different pathways within an aqueous herbal extract.

Furthermore, this biphasic effect could be by NO in lung mice. NO will have a pronounced effect on cellular recruitment/infiltration, immune cell function, tissue repair, and antioxidant protection and therefore promotes NO to the forefront of the regulation of host defense (Connelly, et al. 2001; Huang, et al. 1998).

In an analysis of the percentage of instillation with LPS and PBS prompted a significant increase in this parameter over the baseline in animals without any treatment with UTE (Hirano, 1997; Çok, et al. 2007). Concerning the effects of DX, it has been previously confirmed that this drug have anti-inflammatory effects and is widely used to relieve inflammation (swelling, heat, pain and redness) thereby producing a decrease in the number and effectiveness of the response of inflammatory cells, as postulated by Sadikot, et al. (2001).

When the results were analyzed to assess the concentration of macrophages in the BAL fluid, although no statistical significance was found, instillation with LPS produced a stight increase in this parameter, which was then attenuated by

DX and UTE in 30 and 90 day periods. Similar results regarding the migration of cellular macrophages were obtained by other authors (Cisneros, et al. 2005; Lemaire, et al. 1999). This increase is due to LPS being the main component of the outer membrane of Gram-negative, and is also a potent activator of response defense, macrophages and neutrophils among others. It is important that the method used to administer the three virulence factors, i.e., intranasal inoculation, induces only a local inflammatory response in the lungs, with no apparent systemic effects. Therefore, compared to a systemic administration of proinflammatory agents, the model used here more closely reflects the pulmonary inflammation observed in CF patients, in which systemic involvement is not usually observed (Conese, et al. 2003; Wieland, et al. 2002).

The fact that there was a significant correlation coefficient (r^2= 0.44) between the concentration of macrophages and PMNs with LPS instillation, indicates that chemotactic stimuli produced by different pathways, e.g. the vascular endothelium, or by macrophages, act as inducers of the migration of neutrophils into the alveoli.

With respect the exploratory analysis of samples obtained from histopathological mouse lung with LPS instillation, we found that 100% had infiltrated diffuse, edema and congestion, while DX and UTE also had a similar response, thereby reducing the parameters described previously.

CONCLUSIONS

The findings of this study show that the administration of UTE for periods of 7, 15, 30 or 90 days, did not induce alterations in the general state of animals, or in the histopathological characteristics of the organs evaluated (lungs, liver and kidneys). Furthermore, we found that UTE significantly reduced transient pulmonary edema and PMN infiltration in the mice model, for LPS instillation with a pre-treatment of DX or UTE-7 and UTE-90. The principal finding of the present study is the demonstration that the biphasic protection from PMNs migration into the lung was reduced by a 7 or 90 day administration of UTE. This work confirms the potential anti-inflammatory effects of UTE in lung mice. However further studies are necessary in order to identify the possible mechanisms of action of the components of the extract.

Table 1. PMNs obtained from BALF in Swiss lung Albino mice.

Treatments	PMNs in BALF x 10^5/ml
Water	14.02 ± 2.24
UTE-7 (8)	9.27 ± 4.21
UTE-15 (8)	5.13 ± 1.02
UTE-30 (7)	11.63 ± 3.23
UTE-90 (5)	11.34 ± 4.60

Polymorphonuclear neutrophils (PMNs) were obtained from the bronchoalveolar fluid (BALF) in Swiss Albino mice. The treatments instilled a solution with buffer phosphate (PBS) 3 hours prior to sacrifice. Water: only water drank, and *Uncaria tomentosa* extract (UTE) drank *ad libitum* for 7, 15, 30 and 90 days. Data are mean ± SEM. In parentheses: number of animals.

Table 2. Macrophages obtained from BALF in lung Swiss Albino mice.

Treatments	Macrophages x 10^5 / ml
Water	3.38 ± 1.39
UTE-7 (8)	2.95 ± 0.77
UTE-15 (8)	2.97 ± 0.95
UTE-30 (7)	2.29 ± 0.42
UTE-90 (5)	2.04 ± 0.28

Macrophages were obtained from the bronchoalveolar fluid (BALF) in Swiss Albino mice. The treatments were instilled i.n. with PBS 3 hours prior to sacrifice. Water: only water drank, and *Uncaria tomentosa* extract (UTE) drank *ad libitum* for 7, 15, 30 and 90 days. Data are mean ± SEM. In parentheses: number of animals.

Table 3. Lung histopathology from Swiss Albino mice.

.

	Infiltrate															
	Focal				Diffuse				Congestion				Edema			
Treatments	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Basal (8)	8	0	0	0	8	0	0	0	7	1	0	0	8	0	0	0
	100		**0**		**100**		**0**		**88**		**12**		**100**		**0**	
PBS (6)	4	1	1	0	6	0	0	0	5	0	1	0	3	2	1	0
	67		**33**		**100**		**0**		**83**		**17**		**50**		**50**	
LPS (8)	8	0	0	0	0	4	4	0	0	4	4	0	0	4	4	0
	100		**0**		**0**		**100**		**0**		**100**		**0**		**100**	
DX + LPS (11)	4	6	1	0	4	7	0	0	2	5	4	0	5	4	2	0
	36		**64**		**36**		**64**		**18**		**82**		**45**		**55**	
UTE 7 + LPS (8)	2	6	0	0	3	5	0	0	7	1	0	0	2	4	2	0
	25		**75**		**37**		**63**		**12**		**88**		**25**		**75**	
UTE 15 + LPS (8)	3	3	2	0	1	7	0	0	2	6	0	0	2	4	2	0
	37		**63**		**12**		**88**		**25**		**75**		**25**		**75**	
UTE 30 + LPS (7)	4	3	0	0	1	5	1	0	2	3	2	0	0	4	3	0
	57		**43**		**14**		**86**		**29**		**71**		**0**		**100**	
UTE 90 + LPS (5)	2	3	0	0	2	3	0	0	1	4	0	0	2	1	2	0
	40		**60**		**40**		**60**		**20**		**80**		**40**		**60**	

The microscopic analysis was performed on lung tissue obtained from Swiss Albino mice, subjected to the following treatments: Basal: untreated; PBS: only water drank and instilled with fosfosalino phosphate buffer (PBS) 3 hours prior to being sacrificed; LPS: instilled with lipopolysaccharide *Pseudomonas aeruginosa* (LPS) 3hs before being sacrificed; DX + LPS: treated only with dexamethasone (DX, ip; 2.5mg/kg) 5h before being sacrificed and LPS identical to previous condition. UTE + LPS: The *Uncaria tomentosa* extract (UTE) was administered *ad libitum* to animals for 7, 15, 30 and 90 days, and 3 hs before being sacrificed were instilled with LPS. The table shows the absolute frequencies of the attributes measured (focal infiltrate, diffuse, congestion and edema). The modalities of these attributes indicate the degree of intensity of each variable as 0: absence; 1: mild, 2: moderate and 3: intense. In bold are shown the percentages corresponding to the frequencies obtained. Parentheses indicate the number of animals.

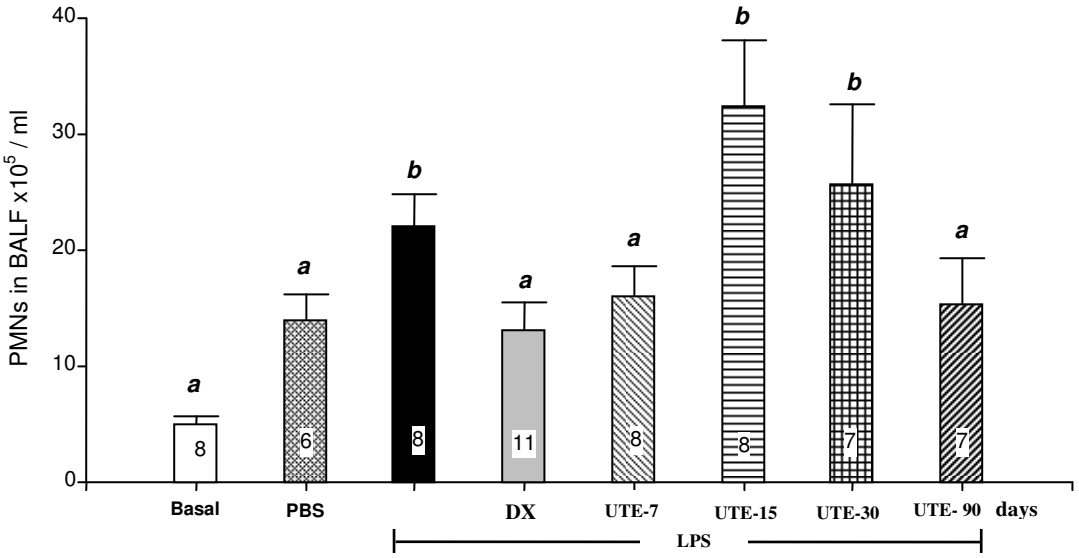

Figure 1. Polymorphonuclear neutrophils (PMNs) were obtained from bronchoalveolar fluid (BALF) in Swiss Albino mice: Basal untreated; PBS: instilled with buffer phosphate 3 hours previous to sacrificed. Animals subjected to the following treatments were instilled i.n.(1.67 mg/Kg) with Pseudomonas *aeruginosa* lipopolysaccharide (LPS) 3hs previous to being sacrificed; LPS i.n. only; DX (dexamethasone,i.p. only; 2.5mg/kg) 5 hs prior to sacrificed; and *Uncaria tomentosa* extract (UTE): administered *ad libitum* the for 7, 15, 30 and 90 days. Data are mean ± SEM. Different letters indicate significant differences (p <0.05). Within each bar is shown the number of animals used.

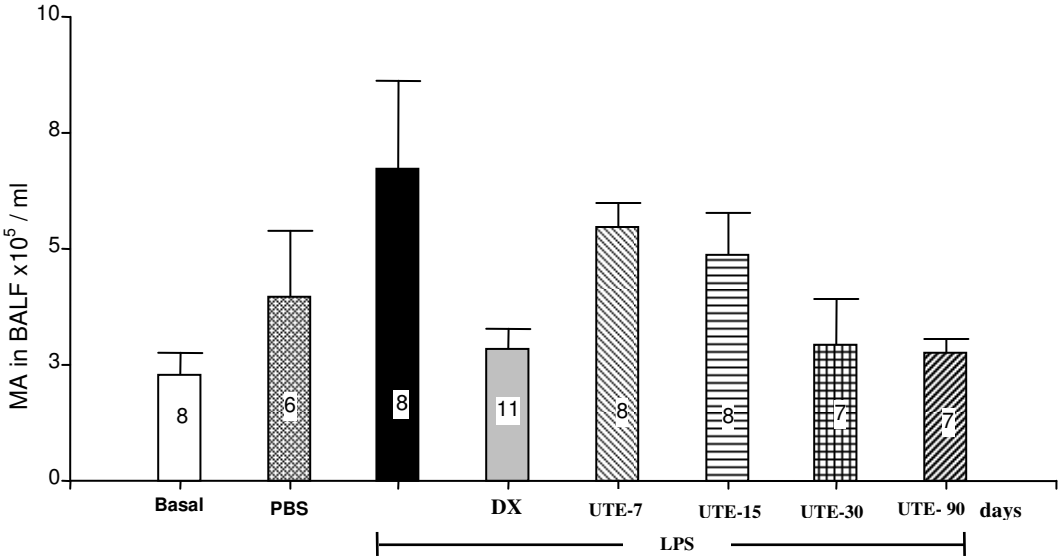

Figure 2. Macrophages (MA)were obtained from bronchoalveolar fluid (BALF) in Swiss Albino mice: Basal untreated; PBS: instilled with buffer phosphate 3 hours previous to sacrificed; and the animals subject to the following treatments were instilled i.n.(1.67 mg/Kg) with *Pseudomonas aeruginosa* lipopolysaccharide (LPS) 3hs previous to sacrificed; LPS i.n. only; DX (dexamethasone,i.p. only; 2.5mg/kg) 5 hs prior to being sacrificed; and the *Uncaria tomentosa* extract (UTE): administered *ad libitum* for 7, 15, 30 and 90 days. Data are mean ± SEM. Different letters indicate significant differences (p <0.05). Within each bar is shown the number of animals used.

REFERENCES

Aguilar JL, Rojas P, Marcelo A, Plaza A, Bauer R, Reininger E, Klaas CA, Merfort I (2002) Anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiaceae). J Ethnopharmacol 81: 271-276.

Akesson C, Lindgreen H, Pero RW, Leanderson T (2003) An extract of *Uncaria tomentosa* inhibiting cell division and NF-kB activity without inducing cell death. Int Immunopharmacol;3:1889-1900.

Allen-Hall L, Cano P, Arnason JT, Rojas R, (2007) Treatment of THP-1 cells with *Uncaria tomentosa* extracts differentially regulates the expression if IL-1beta and TNF-α. J Ethnopharmacol;109:312-317.

Aquino R, De Feo V, De Simone F, Pizza C, Cirino G (1991) Plant metabolites. New compounds and anti-inflammatory activity of *Uncaria tomentosa*. J Nat Products 54: 453-459.

Bednarek D, Łukasiak J, Kondracki M, Żurowska K, Falkiewicz B, Niemczuk K (2004) Analysis of phenotype and functions of peripheral blood leukocytes in cellular immunity of calves treated with *Uncaria tomentosa*. Bull Vet Inst Pulawy 48: 289-296.

Bižanov G, Tamošiūasn V (2005) Immune responses induced in mice after intragastral administration with Sendai virus in combination with extract of *Uncaria tomentosa*. Scand J Lab Anim Sci 32: 201-207.

Cisneros FJ, Jayo M, Niedziela L (2005) An *Uncaria tomentosa* (Cat's claw). Extract protects mice against ozone-induced lung inflammation. J Ethnopharmacol 96: 355-364.

Çok G, Uysal EF, Çakan A, Nart D, Özkök S, Veral A, Özhan HM (2007) The effect of erdosteine on irradiation-induced lung inflammation in rats. Turkish Res J 8: 52-55.

Conese M, Copreni E, Di Gioia S, De Rinaldis P, Fumarulo R (2003) Neutrophil recruitment and airway epithelial cell involvement in chronic cystic fibrosis lung disease. J Cyst Fibros 2: 129-135.

Connelly L, Palacios-Callender M, Ameixa C, Moncada S, Hobbs AJ (2001) Biphasic Regulation of NF-κB activity underlies the pro- and anti-inflammatory actions of nitric oxide. J Immunol 166: 3873-3881.

Davis L, Kuttan G (1999) Effect of *Withania somnifera* on cytokine production in normal and cyclophosphamide treated mice. Immunopharmacol Immunotoxicol 21: 695-703.

Dhuley JN (1997) Effect of some Indian herbs on macrophage functions in ochratoxin a treated mice. J Ethnopharmacol 58: 15-20.

Freedman SD, Weinstein D, Blanco PG, Martinez-Clark P, Urman S, Zaman M, Morrow JD, Alvarez JG (2002) Characterization of LPS-induced lung inflammation in cftr-/- mice and the effect of docosahexaenoic acid. J App Physiol 92: 2169-2176.

Gaffney BT, Hügel HM, Rich PA (2001) *Panax ginseng* and *Eleutherococcus senticosus* may exaggerate an already existing biphasic response to stress via inhibition of enzymes which limit the binding of stress hormones to their receptors. Med Hypotheses 56: 567-572.

Hirano S (1997) Quantitative time-course profiles of bronchoalveolar lavage cells following intratracheal instillation of lipopolysaccharide in mice. Ind Health 35: 353-358.

Huang, FP, Niedbala W, Wei XQ, Xu D (1998) Nitric oxide regulates Th1 cell development through the inhibition of IL-12 synthesis by macrophages. Eur J Immunol;28:4062-4070.

Lemaire I, Assinewe V, Cano P (1999) Stimulation of interleukin-1 and –6 production in alveolar macrophages by the neotropical liana, *Uncaria tomentosa* (Uña de Gato). J Ethnopharmacol 64: 109-115.

Reddy MM, Ponce AA, Quinton PM. (2005) Outside neurons/inside epithelia: novel activation of CFTR Cl^- and HCO_3 conductance. Adv Exp Med Biol 558: 1-52.

Roderick J, Szarka N, Wang L, Gordon PN, Smith RH (1997) A murine model of pulmonary damage induced by lipopolysaccharide via intranasal instillation. J Immunol Methods 202: 49-57.

Sadikot RT, Jansen ED, Blackwell TR, Zoia O, Yull F, Christman JW, Blackwell TS. (2001) High-dose dexamethasone accentuates nuclear factor-B activation in endotoxin-treated mice. Am J Respir Crit Care Med 164: 873-878.

Sattayasai J, Tiamkao S, Puapairoj P (2008) Biphasic effects of *Morus alba* leaves green tea extract on mice in chronic forced swimming model. Phytother Res 22: 487-492.

Song Z, Johansen HK, Moser C, Høiby N (1996) Effects of Chinese medicinal herbs on a rat model of chronic *Pseudomonas aeruginosa* lung infection. APMIS 104: 350-354.

Song Z, Kharazmi A, Wu H, Faber V, Moser C, Johansen HK, Rygaard J, Høiby N (1998) Effects of *Ginseng* treatment on neutrophil chemiluminescence and immunoglobulin G subclasses in a rat model of chronic *Pseudomonas aeruginosa* pneumonia. Clin Diagn Lab Immunol 5: 882–887.

Spelman K, Burns JJ, Douglas N, Winters N, Ottersberg S, Tenborg M (2006) Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. Altern Med Rev 11: 128-150.

Terheggen-Lagro G, Rijkers C, van der Ent CK (2005) The role of airway epithelium and blood neutrophils in the inflammatory response in cystic fibrosis. J Cyst Fibros 4(supp 2): 15-23.

Valerio LG, Gonzales GF (2005) Toxicological aspects of the South American herbs Cat's claw (*Uncaria tomentosa*) and Maca (*Lepidium meyenii*). Toxicol Rev 24: 11-35.

Wieland CW, Siegmund B, Senaldi G, Vasil ML, Dinarello CA, Fantuzzi G (2002) Pulmonary inflammation induced by *Pseudomonas aeruginosa* lipopolysaccharide, phospholipase C, and exotoxin A: role of interferon regulatory factor 1. Infec Immunity 70: 1352-1358.

Williamson EM (2005) Interactions between herbal and conventional medicines. Expert Opin Drug Saf 4: 355-378.