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Investigating the protective effects of bromelain against inflammatory marker alterations induced by cadmium pulmonary intoxication in rat

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Abstact

A total of 66 albino Wistar rats were subjected to the following treatments in 11 groups: Group 1 (negative control); Group 2 and 3: received Cadmium Chloride ($CdCl_2$) 400 µg/rat intratracheally (IT) and sampled after 5 and 10 days, respectively; Group 4 and 5: received bromelain 20 mg/kg orally (PO) from 14 days before until 5 and 10 days after $CdCl_2$ instillation, respectively; Group 6 and 7: received bromelain 40 mg/kg from 14 days before until 5 and 10 days after $CdCl_2$ instillation, respectively; Group 8: received bromelain 40 mg/kg for 24 days; Group 9 and 10: received Celecoxib 25 mg/kg PO from one day before until 5 and 10 days after $CdCl_2$ instillation, respectively; Group 11: received Celecoxib for 11 days. Serum protein analysis revealed that intratracheal Cadmium administration resulted in an insignificant rise in all globulin fractions on day 5 and 10 post-injection. Low dose bromelain treatment for 24 days in $CdCl_2$ exposed rats showed a significant decrease in serum total protein and all globulin fractions. However, $CdCl_2$ plus high dose bromelain treatment for 24 days, significantly increased all the mentioned analytes. Bronchoalveolar lavage fluid γ-globulin concentration was decreased in all cadmium and/or bromelain treated groups. However, these changes were not significant compared to the control group. Serum LDH activity was significantly increased 5 days after cadmium intoxication while bromelain or celecoxib coadministration resulted in an insignificant decrease in enzyme activity level. In the histopathologic examination, severe interstitial pneumonia and fibrinous bronchopneumonia were observed in cadmium exposed rats and low dose bromelain administration for 24 days resulted in the reduction of these complications in lung tissue. In brief, bromelain administration can be considered as a supportive or alternative treatment to alleviate $CdCl_2$ induced systemic and bronchoalveolar inflammatory changes, especially when administered in the lower dose.

Keywords: Cadmium, Bromelain, Pulmonary intoxication, Bronchoalveolar lavage fluid, Protein electrophoresis

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Introduction

Cadmium is a toxic heavy metal of occupational and environmental hazard with an extremely long biological half-life that makes it a cumulative toxin (Waalkes 2000). Cadmium has serious adverse effects in various organs and tissues including nephrotoxicity, hepatotoxicity and pneumotoxicity as documented by epidemiological studies and reports (Nogue´ et al. 2004, Nordberg et al. 2007). Occupational exposure to airborne cadmium may take place by welding, smelting, automobile emissions, manufacturing of electric equipment, alloys and pigments (Bell et al. 1997). Cigarette smoking is also considered to be one of the most significant sources of human cadmium exposure (Bernhoft 2013). Inhalation of high concentration of Cd fumes can cause acute pulmonary damage, pneumonitis, pulmonary emphysema and altered surfactant production resulting in prolonged impairment of pulmonary function (Bell et al. 1997).

Cadmium poisoning has also been reported in ruminants, horses and companion animals with various clinical symptoms which include a reduction in growth and weight gain, food intake, anemia, enlargement of joints, inflammation of liver parenchyma and renal changes, testicular degeneration and necrosis, and abortion. Moreover, poisoned animals by cadmium may present tumors, teratogenicity because this mineral causes changes in DNA and increase progesterone and 17-β estradiol plasma concentrations. These hormonal changes disturb follicular development and cause difficulty in maintaining a pregnancy (Reis et al. 2010, Serpe et al. 2012, Massanyi et al. 2014, Lane et al. 2015).

Although the mechanisms of Cd toxicity are not yet fully understood, several investigations reported evidence for interference with essential metals, induction of oxidative stress, inhibition of DNA repair, interference with apoptosis (Bertin and Averbeck 2006) and induction of pulmonary inflammatory response (Coccini 2012).

Many research findings suggest cadmium to have pro-inflammatory properties that cause up-regulation of the mediators and markers of inflammation (Olszowski et al. 2012).

Hence, administration of drugs/agents with antiinflammatory characteristics might be beneficial in preventing or reducing cadmium-induced organ damage.

Bromelain is a complex mixture of protease extracted from the fruit or stem of the pineapple plant which is widely administered for its well-recognized properties, such as its anti-inflammatory, immunomodulatory, antithrombotic, fibrinolytic, and anticancer effects, in addition to being a wound healing and circulatory improvement agent. Bromelain gained universal acceptability as a phytotherapeutic agent due to its history of safe use and lack of side effects (Kelly 1996, Pavan et al. 2012, Rathnavelu et al. 2016). However, to the best of our knowledge, bromelain was not evaluated as a protective agent in cadmium-induced pulmonary toxicity.

Hence, the present experimental study was performed to assess the possible protective effects of bromelain in comparison to celecoxib, as an anti-inflammarory drug, against cadmium acute intratracheal exposure and its local and systemic inflammatory consequences through hematology, serum and bronchoalveolar lavage fluid protein analysis, C reactive protein and lactate dehydrogenase enzyme activity level, as markers of inflammation and lung tissue histopathology.

Materials and Methods

Laboratory animals

A total of 66 Albino male rats (Wistar strain) weighing 250-300 g with the age of 4 to 5 months were housed in groups of six,

in plastic cages, in an air-conditioned room maintained at a temperature of 24±2 °C and relative humidity of 55±5%, with a 12-h light/12-h dark illumination cycle. They were fed a commercial laboratory pellet diet and tap water ad libitum. All procedures were done under ethical guidelines for care and use of laboratory animals, discarding of dead animals and protection of the researcher against animal bites and were approved by the Experimental Animals Committee of Shahid Chamran University of Ahvaz, Iran.

Experimental design

Animals were equally and randomly divided in 11 groups and subjected to the following experiment:

Group 1 (negative control): 400 µl saline (0.9%sodium chloride) intratracheally (IT) and sampled after 10 days.

Group 2: 400 µg/rat Cadmium Chloride ($CdCl_2$) (Sigma, USA) IT and sampled after 5 days (Damiano et al,1990).

Group 3: 400 µg/rat $CdCl_2$ IT and sampled after 10 days.

Group 4: 400 µg/rat $CdCl_2$ IT and 20 mg/kg bromelain (Acros Organics, Belgium) (Al-Otaibi et al, 2015) orally by gavage (PO) daily from 14 days before- until 5 days after cadmium instillation.

Group 5: 400 µg/rat $CdCl_2$ IT and 20 mg/kg bromelain PO daily from 14 days before- until 10 days after cadmium instillation.

Group 6: 400 µg/rat $CdCl_2$ IT and 40 mg/kg bromelain (Sudjarwo, 2005) PO daily from 14 days before- until 5 days after cadmium instillation.

Group 7: 400 µg/rat $CdCl_2$ IT and 40 mg/kg bromelain PO daily from 14 days before- until 10 days after cadmium instillation.

Group 8: 40 mg/kg bromelain PO daily for 24 days.

Group 9: 400 µg/rat $CdCl_2$ IT and 25 mg/kg Celecoxib (Exir Pharmaceutical co., Iran) (Roh et al., 2010) PO daily from one day before- until 5 days after cadmium instillation.

Group 10: 400 µg/rat $CdCl_2$ IT and 25 mg/kg Celecoxib PO daily from one day before- until 10 days after cadmium instillation.

Group 11: 25 mg/kg Celecoxib PO daily for 11 days.

Intratracheal injection method

The rats were anesthetized by administration of ketamine (60 mg/kg, IP) plus xylazine (5 mg/kg, IP). After anesthesia, they were placed on a slant wooden board with an angle of 60 degrees, so that their back was against the board and were suspended from their incisors on a wire. The tongue was gently pulled out and held aside from the oral cavity with a blunt forceps. The syringe containing the inoculum (400 µl saline/rat in control group or 400 µg $CdC1_2$/rat delivered in 400 µl saline in exposed groups) was attached to a curved gavage needle and the needle was inserted into the pharynx. The plunger was pushed evenly to deliver the inoculum and the needle was pulled out of the pharynx as soon as possible. The nostrils were blocked by fingers, and the tongue restraint was continued until at least 2 deep breaths were completed but for no longer than 15 secs. The rats were held upright for a few seconds to allow inoculum to be inhaled into the lung (Bell et al., 2000).

Blood collection

At the end of the experimental period, 24 hr after the last dose, all rats were anesthetized with chloroform (Merck, Germany). Blood sampling was performed via the cardiac puncture through the diaphragm to prevent any damage to the lungs. Blood samples were collected into sterile tubes with and without anticoagulant (EDTA).

Whole blood samples (with EDTA) were subjected to hematologic assessment while the remaining samples were used for serum

separation and stored at −20 °C until performing further biochemical analysis.

Bronchoalveolar lavage

The euthanized rats were placed on a dorsal position, and a midline incision was made from the mandible to the peritoneal cavity.

The ribs were then retracted and the right bronchus was ligated with nylon suture to prevent penetration of lavage fluid into the right lung and preserving it for subsequent histopathologic assessment.

A small incision was made in the trachea approximately1 inch above the bifurcation where a plastic catheter was inserted and the bronchoalveolar lavage was performed 2 successive times, each with 2.5 ml saline. The aspirated washes were combined and kept on ice.

The bronchoalveolar lavage fluid (BALF) samples were centrifuged at 300 × g for 10 min and the supernatant was separated from the pellet. The supernatant was stored at −20 °C for further biochemical analysis (Bergmann et al. 2000).

Hematologic assessment

Total erythrocyte count (RBC), hematocrit value (HCT), hemoglobin concentration (Hb), and total white blood cells (WBC) were determined by the BC-2800Vet hematology analyzer (Mindray, China).

Total protein, C reactive protein and Lactate dehydrogenase analysis

Serum C reactive protein (CRP) concentration was assessed using a commercial ELISA kit (Monobind Inc., Germany).

Lactate dehydrogenase (LDH) activity in serum and total protein concentration in serum were determined photometrically with Parsazmun kits (Iran) using a biochemistry autoanalyzer (BT-1500, Biotechnica, Italy).

Total protein concentration in BALF samples was measured photometrically by the Bradford method using a commercial kit (Nadford, Navand Salamat, Iran) (Hay and Westwood, 2002).

Protein electrophoresis

Serum and BALF protein electrophoresis was performed on cellulose acetate membranes using Tris Hippurate buffer solution and Ponceau S staining according to manufacturer's instructions (Cellogel electrophoresis kit, Italy) (Ambler and Rodgers, 1980).

Histopathologic assessment

The right lung and its accompanying bronchus were excised from each rat. The tissues were then stored in 10% formalin fixative and the fixative was replaced after 24 h for better fixation. The tissues were subsequently embedded with paraffin, cut into 4 μm, and stained with Hematoxylin and Eosin for light microscopic observation.

Statistical analysis

Statistical analysis was performed using the SPSS statistical program version16 (SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard error (SE) for different groups. Data were analyzed statistically using analysis of variance (ANOVA) and Tukey's post hoc tests.$P< 0.05$ was considered statistically significant.

Results

Hematology

Leukogram assessment revealed no significant difference in total counts between groups (Table 1). Although total leukocyte count was higher in groups 7 (cadmium and high dose bromelain, 24 d) and 9 (cadmium and celecoxib, 6d), this alteration was not statistically significant compared to other groups ($P>0.05$).

There was no significant difference in erythrocyte counts, hemoglobin concentration, and hematocrit ($P>0.05$) (Table 1).

Table 1. Means ± SE of hematologic analysis as mean ± SE in different groups

Group**	WBC ($\times 10^3$/µl)*	RBC ($\times 10^6$/µl)	HGB (g/dl)	HCT (%)
1	8.56±0.76	7.38±0.20	13.24±0.08	43.82±0.55
2	6.87±2.42	7.17±0.55	12.56±0.97	41.71±2.96
3	8.08±2.29	7.69±.59	12.56±1.06	44.70±3.76
4	8.82±1.95	7.35±0.28	12.88±0.44	42.11±1.41
5	7.82±0.53	7.23±0.48	11.34±0.94	39.50±2.84
6	8.98±1.40	7.31±0.49	12.25±0.88	40.00±2.85
7	12.20±1.78	8.62±0.24	14.48±0.40	47.98±1.14
8	8.45±1.26	8.37±0.08	14.27±0.16	46.22±0.31
9	11.10±1.78	7.35±0.12	12.56±0.23	40.80±0.80
10	6±0.47	7.68±0.31	12.42.±0.43	43.55±1.30
11	6.25±1.61	6.83±0.46	11.60±0.83	37.97±2.17

* There was no significant difference in any leukogram parameter between groups.
** Group treatments: 1: Cont; 2: CdCl2 (5d); 3: CdCl2 (10d); 4: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (5d); 5: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (10d); 6: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (5d); 7: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (10d); 8: Bromelain 40 (24d); 9: Celecoxib (1d) + CdCl2 + Celecoxib (5d); 10: Celecoxib (1d) + CdCl2 + Celecoxib (10d); 11: Celecoxib (11d).

Serum and BALF protein analysis

Serum protein

The maximum serum total protein and albumin levels were observed in group 7 (cadmium and high dose bromelain, 24 d) which were significantly different when compared to group 5 (cadmium and low dose bromelain, 24 d) ($p<0.05$)(Table 2). There was no significant difference in total protein and albumin level in other groups ($P>0.05$).

The highest serum α1 globulin concentration was in groups 3 (cadmium) and 7 (cadmium and high dose bromelain, 24 d) while the lowest amounts were in groups 5 (cadmium and low dose bromelain, 24 d) and 11 (celecoxib) so that the concentration in the later mentioned groups were significantly different from group 3 ($P<0.05$) (Table 2).

Serum α2 globulin concentration in cadmium receiving groups (group 2 and 3) and Serum β globulin in group 3 were significantly higher than the amounts in group 5 ($P<0.05$).

Group 5 had the lowest γ globulin level compared to other groups, however, this difference was not statistically significant ($P>0.05$).

BALF protein

BALF total protein and electrophoresis showed that γ globulin concentration was decreased in all cadmium and/or bromelain treated groups except the ones that received celecoxib when compared to the control group (Table 3). Accordingly, mean γ globulin level was significantly lower in groups 4 (cadmium and low dose bromelain, 19 d) and 8 (bromelain group) in comparison to group 11 (celecoxib) ($P<0.05$). Total protein and other protein fraction concentrations did not alter significantly ($p>0.05$) (Table 3).

LDH analysis

Serum LDH assessment revealed that the highest enzyme activity was in group 2 (five days after cadmium administration) (Table 4). Although bromelain or celecoxib treatment resulted in a reduction in enzyme level after five days (group 4, 6 and 9), these variations were not statistically significant ($P>0.05$). Conversely, LDH activity was significantly decreased in groups 3, 7 and 8 (10 days after cadmium administration) compared to group 2 ($P<0.05$).

Table 2. Means ± SE of serum Protein analysis as mean ± SE in different groups.

Group**	Total protein (g/dl)	Albumin (g/dl)	α1- globulin (g/dl)	α2-globulin (g/dl)	β-globulin (g/dl)	γ-globulin (g/dl)
1	7.40±0.79^{ab*}	4.12±0.27^{a}	1.17±0.23^{ab}	0.52±0.16^{ab}	1.54±0.13^{ab}	0.75±0.05
2	7.07±0.58^{ab}	3.87±0.29^{ab}	0.93±0.08^{ab}	1.03±0.09^{a}	1.35±0.09^{ab}	0.86±0.12
3	7.02±0.95^{ab}	3.55±0.31^{ab}	1.96±0.29^{a}	0.89±0.18^{a}	1.88±0.36^{a}	1.04±0.10
4	7.90±0.50^{ab}	4.10±0.22^{ab}	1.72±0.35^{ab}	0.87±0.19^{ab}	1.67±0.19^{ab}	0.94±0.07
5	5.40±0.40^{a}	2.90±0.20^{b}	0.42±0.09^{b}	0.30±0.03^{b}	0.83±0.07^{b}	0.55±0.06
6	7.82±0.41^{ab}	4.07±0.19^{ab}	0.79±0.25^{ab}	0.51±0.04^{ab}	1.00±0.12^{ab}	0.96±0.25
7	8.76±0.81^{b}	4.50±0.34^{a}	1.82±0.39^{ab}	0.61±0.09^{ab}	1.56±0.49^{ab}	1.07±0.17
8	7.80±0.70^{ab}	4.10±0.34^{ab}	1.40±0.44^{ab}	0.45±0.08^{ab}	1.08±0.32^{ab}	1.01±0.19
9	6.20±0.15^{ab}	3.43±0.14^{ab}	1.11±0.42^{ab}	0.39±0.03^{ab}	0.83±0.09^{ab}	0.88±0.18
10	6.10±0.58^{ab}	3.26±0.18^{ab}	0.80±0.43^{ab}	0.58±0.12^{ab}	0.99±0.25^{ab}	0.64±0.11
11	6.60±0.50^{ab}	3.65±0.18^{ab}	0.34±0.02^{b}	0.46±0.02^{ab}	1.20±0.10^{ab}	1.10±0.11

* Different letters in each column represent a significant difference between groups (P<0.05).
** Group treatments: 1: Cont; 2: CdCl2 (5d); 3: CdCl2 (10d); 4: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (5d); 5: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (10d); 6: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (5d); 7: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (10d); 8: Bromelain 40 (24d); 9: Celecoxib (1d) + CdCl2 + Celecoxib (5d); 10: Celecoxib (1d) + CdCl2 + Celecoxib (10d); 11: Celecoxib (11d).

Table 3. Means ± SE BALF protein analysis as mean ± SE in different groups

Group**	Total protein (mg/dl)	Albumin (mg/dl)	α1- globulin (mg/dl)	α2-globulin (mg/dl)	β-globulin (mg/dl)	γ-globulin (mg/dl)
1	10.00±0.00	6.36±0.81	0.74±0.06	1.48±1.31	0.48±0.30	2.19±0.39^{ab*}
2	17.50±7.50	10.21±5.38	3.25±1.30	1.45±0.12	1.58±0.47	1.00±0.22^{ab}
3	7.50±2.50	3.16±0.69	1.23±0.75	1.25±0.79	1.08±0.30	0.78±0.03^{ab}
4	7.50±2.50	3.75±1.57	0.79±0.31	1.48±0.47	1.00±0.25	0.47±0.11^{a}
5	7.50±2.50	4.09±1.36	0.84±0.28	1.17±0.39	0.71±0.23	0.66±0.22^{ab}
6	17.50±7.50	6.04±2.68	2.81±0.86	3.32±1.73	3.57±1.73	1.75±0.49^{ab}
7	10.00±0.00	4.06±0.00	1.50±0.00	1.39±0.00	2.44±0.00	0.61±0.00^{ab}
8	7.50±2.50	3.55±1.59	0.82±0.23	1.73±0.58	0.99±0.06	0.38±0.16^{a}
9	17.50±7.50	7.62±2.92	3.11±1.43	4.22±1.87	1.41±0.79	1.12±0.47^{ab}
10	10.00±0.00	3.74±0.78	1.04±0.08	0.94±0.09	1.92±0.02	2.35±0.63^{ab}
11	10.00±0.00	2.71± 0.27	1.45±0.02	0.96±0.28	1.98±0.87	2.91±0.85^{b}

* Different letters in each column represent a significant difference between groups (P<0.05).
** Group treatments: 1: Cont; 2: CdCl2 (5d); 3: CdCl2 (10d); 4: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (5d); 5: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (10d); 6: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (5d); 7: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (10d); 8: Bromelain 40 (24d); 9: Celecoxib (1d) + CdCl2 + Celecoxib (5d); 10: Celecoxib (1d) + CdCl2 + Celecoxib (10d); 11: Celecoxib (11d).

Table 4. Means ± SE of serum LDH activity and CRP concentration as mean ± SE in different groups

Group**	LDH (Iu/l)	CRP (µg/ml)
1	997.50±255.27[ab*]	0.01±0.01
2	1888.8±300.90[a]	0.00±0.00
3	789.80±226.30[b]	0.00±0.00
4	1078.5±270.41 [ab]	0.15±0.02
5	980.00±282.93 [ab]	0.12±0.04
6	1341.8±274.88 [ab]	0.07±0.01
7	464.00 ±86.27[b]	0.03±0.03
8	637.60±87.63[b]	0.00±0.00
9	1061.00±149.45 [ab]	0.02±0.02
10	645.67±123.07 [ab]	0.04±0.01
11	906.00±343.43 [ab]	0.05±0.04

* Different letters represent a significant difference between groups (P<0.05).

** Group treatments: 1: Cont; 2: CdCl2 (5d); 3: CdCl2 (10d); 4: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (5d); 5: Bromelain 20 (14d) + CdCl2 + Bromelain 20 (10d); 6: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (5d); 7: Bromelain 40 (14d) + CdCl2 + Bromelain 40 (10d); 8: Bromelain 40 (24d); 9: Celecoxib (1d) + CdCl2 + Celecoxib (5d); 10: Celecoxib (1d) + CdCl2 + Celecoxib (10d); 11: Celecoxib (11d).

There was no significant difference between cadmium treated groups after 10 days. However, the minimum enzyme activity was in group 7 (cadmium and high dose bromelain, 24 d).

LDH activity could not be measured in BALF samples due to low enzyme activity.

CRP analysis

Serum CRP analysis revealed no significant variation in different groups (p>0.05) (Table 4). Moreover, CRP concentration was in the normal range in all groups.

Histopathology results

In the microscopic examination of the lungs of all rats of group 2 and 3 (cadmium exposure), severe interstitial pneumonia (Fig. 1) along with fibrinous bronchopneumonia was observed.

Low dose bromelain treatment for 24 days in cadmium exposed rats (group 5) resulted in moderate interstitial pneumonia and cellular infiltration (Fig. 2). However, bromelain treatment in other groups (groups 4, 6 and 7) was not as effective in reducing cadmium-induced pulmonary histopathologic damage.

Celecoxib treatment for 6 and 11 days plus cadmium injection (group 9 and 10, respectively) produced a low grade of interstitial pneumonia.

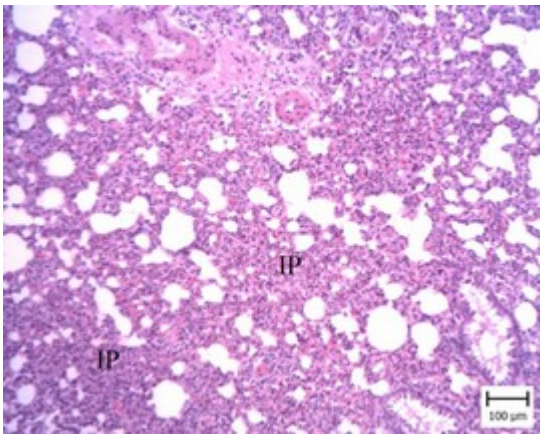

Fig. 1. Rat lung of group 2 (Cadmium exposure, sampled at day 5). Severe interstitial pneumonia (IP). Note the thickening of the alveolar walls (Hematoxylin and Eosin staining) (Bar: 100 µm).

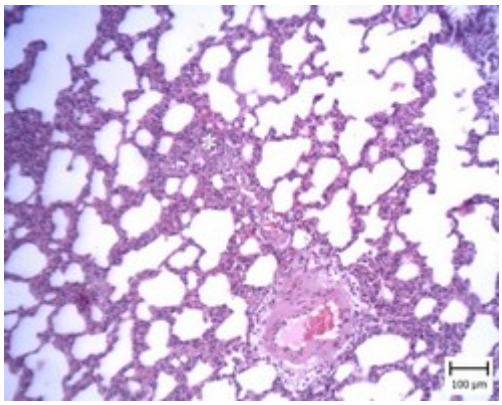

Fig. 2. Rat lung of group 5 (bromelain treatment for 24 days plus cadmium). Note the moderate interstitial pneumonia (asterisk) (Hematoxylin and Eosin staining) (Bar: 100 µm).

Discussion

Cadmium is a toxic heavy metal that can induce acute pneumotoxicity and inflammation when inhaled in high concentrations (Coccini et al., 2012; Blum et al., 2014). The effects of bromelain with anti-inflammatory properties were investigated to protect against cadmium pulmonary intoxication through this study.

Analysis of blood leukocyte count in this experiment revealed that Cadmium intratracheal injection did not generate any significant alteration in total leukocyte count. Although the rats which were treated with cadmium and high dose bromelain for 24 days or celecoxib for 6 days displayed a mild leukocytosis, thease alterations were not significant.

Long-term low-level exposure to cadmium was previously proved to induce leukocytosis so that increased blood cadmium level is associated with higher WBC counts (Parks et al. 2006). However, in an experiment conducted by Hounkpatin et al. (2013) neither low dose and high dose cadmium exposure made any significant change in total leukocyte count in rats that were followings the present study in which no significant alteration in the blood WBC count was detected following intratracheal Cd instillation. Simsek et al. (2009) reported a significant decrease in leukocyte counts in rats treated with cadmium.This variation in the results of different studies may be attributed to different routes and doses of cadmium administration.

Erythrocyte counts, hemoglobin concentrations, and hematoctrit, in the present study, remained unaffected. Accordingly, these treatments did not have major adverse effects on erythrocytes and can be considered safe to red blood cells.

Serum protein analysis in the present study revealed that intratracheal Cadmium administration resulted in an insignificant rise in all globulin fractions on the day 5 and 10 post-injection. This can presumably be attributed to an inflammatory response and elevated levels of acute-phase proteins which are mainly in α and β globulin fractions. Most research findings suggest that cadmium has proinflammatory effects and can up-regulate inflammatory mediators and markers including NF-κB, IL-6, TNF-α, IL-1β and IL-8 which can, in turn, alter the production and serum concentration of particular acute-phase proteins (Olszowski et al. 2012, Blum et al. 2014).

On the other hand, Cd exposed rats which were treated with low dose bromelain for 24 days in the current experiment showed a significant decrease in serum total protein and all protein components. However, high dose bromelain treatment for 24 days in the rats instilled with Cd, increased serum total protein level, albumin ($P<0.05$), α1 and γ globulin fractions ($P>0.05$). Total protein, albumin,α and β globulin fractions were also decreased in all celecoxib receiving rats (exclusively or in combination with Cd) for 6 and 11 days. It appears that bromelain administration in the lower dose (20 mg/kg) was more effective in reducing cadmium-induced protein changes which were similar to celecoxib effects as an anti-inflammatory drug.

BALF protein analysis in the current experiment represents a notable increase in total protein concentration in 5 days following Cd instillation, although this change was not statistically significant. Administration of 20 mg/kg bromelain was effective in reducing the protein level while 40 mg/kg bromelain or celecoxib could not recover this Cd effect after 5 days. Moreover, albumin, α1, α2 and β globulin fraction followed almost the same pattern as total protein in the mentioned groups though none of these alterations was statistically significant. Elevated levels of BALF protein can be correlated with pulmonary inflammation and increased vascular permeability. The reduction in BALF protein observed after 10 days (group 3) compared to that seen 5 days following $CdCl_2$ exposure (group 2), could indicate that vascular permeability decreased over time (Van Vyve et al., 1995).

Bell et al. (1997) also recorded a significant rise in BALF total protein concentration following Cd and/or Selenium intratracheal treatment. It may indicate lung tissue inflammation and increased alveolar/capillary permeability.

The increased protein level in BALF samples was also observed following intratracheal administration of Cadmium Oxide or Cadmium Chloride in rats (Dogra et al. 2002).

The concentration of BALF γ globulin in the current experiment was decreased in all cadmium and/or bromelain treated groups except the ones that received celecoxib which was near the amount in the control group. It is most likely due to decreased immunoglobulin concentration which is the main component of this globulin fraction. Cadmium, like other metals, was demonstrated to have immunomodulatory effects (Kaplan et al. 2013) and associated with some changes in the humoral immune response (Skoczyńska et al. 2002). Oral administration of cadmium to mice was showed to increase susceptibility to the herpes virus, suppress T- and B-cell proliferation, but enhanced macrophage phagocytosis (Thomas et al. 1985). Another study revealed that levels of hypersensitivity and IgG antibody titers were reduced in school-aged children exposed to cadmium (Ritz et al. 1998).

Moreover, intratracheal exposure to cadmium salts (CdCl2 and CdO) in rats was showed to reduce the number of primaries (IgM) antibody-forming cells in lung associated lymph nodes and spleen. It appears that both Cd salts have potential to suppress the local lung immune response, but the effect of CdO is more severe and prolonged (Dogra et al. 2002). The possible mechanisms of Cd-induced immunosuppression include stress response, a shift to a T-helper2 cell population, and induction of apoptosis (Kaplan et al. 2013). Bromelain treatment could not reverse $CdCl_2$ induced BALF γ globulin changes which might be due to inadequate impact of this medication on the immune system at the dose and period administrated in this study.

In the current study, LDH activity was measured in serum as a systemic injury marker. Cytoplasmic cellular enzymes, like LDH in the extracellular space, although of no further metabolic function in this space, are of benefit because they serve as indicators suggestive of disturbances of the cellular integrity induced by pathological conditions (Nillawar et al., 2012; Blum et al., 2014). It was significantly increased 5 days after intratracheal administration of cadmium in all heavy metal treated groups ($p<0.05$). The enzyme activity was subsequently reduced significantly on day 10 of the experiment following cadmium instillation ($p<0.05$). Administration of bromelain or Celecoxib, similarly resulted in a decrease in serum LDH activity level so that the minimum enzyme activity among all was observed in group 7 which received Cd and 40 mg/kg bromelain for 24 days, though these alterations were not statistically significant ($p>0.05$). Also, enzyme activity in BALF samples was too low to be measured. It might be due to the short half-life of the enzyme after it was released to the bronchoalveolar fluid as well as excessive fluid dilution (Radi et al., 2011).

These findings suggest that Cd intratracheal administration resulted in increased serum LDH activity as a systemic tissue injury marker. Also, BALF protein level followed a similar trend, although it was not statistically significant. The pattern of serum LDH activity and BALF protein in time revealed that the maximum tissue damage occurred 5 days post Cadmium instillation while it was recovered to the previous state after 10 days. These findings were consistent with the results of other studies. Yamadori et al. (2010) recorded a rise in BALF LDH activity following intermittent inhalation of cadmium nitrate aerosols though these changes were not significant.

In another research conducted by Bell et al. (1997) guinea-pigs that were treated intratracheally with 0.3 mg/kg Cadmium alone or in combination with Selenium, showed a significant increase in total protein, LDH activity, β-glucuronidase and

alkaline-phosphatase of bronchoalveolar-lavage-fluid at24-hour post-exposure. They suggested that both metals can cause pulmonary cell injury or death and elevated lysosomal activity.

Similar findings were achieved by Hirano et al. (1989) who reported increased protein content and LDH activity in BALF samples 2 days after instillation of Cadmium Oxide as well as Cadmium Chloride into rat lungs at doses of 0.5, 1, 2, 5 and 10 µg Cd/rat.

In an experiment on the effects of inhaled Cadmium oxide nanoparticles on pulmonary injury and systemic immunity in mice, increased levels of total protein, LDH activity, cytokine markers of inflammation and phagocytic activity of circulating phagocytes were observed one day after final Cd exposure (Blum et al. 2014).

Major histopathology findings in the present study included severe interstitial pneumonia and fibrinous bronchopneumonia following cadmium acute exposure which agreed with earlier findings reported by Bergman et al. (2000). They investigated the bioavailability and pulmonary toxicity of Zinc-Cadmium Sulfide (ZnCdS) in rats. In histopathologic assessment, pulmonary interstitial inflammation with thickening of alveolar walls was observed. Moreover, Driscoll et al. (1992) reported chronic interstitial inflammation with increased alveolar wall thickening, increased number of mononuclear cells and type II cell hyperplasia following cadmium exposure in rats which were consistent with the results of the present study. The possible structural changes in the lung tissue of rats after oral exposure to Cd and/or mercury (Hg), was also investigated through an experimental study conducted by Naidoo et al. (2019). Collapsed alveolar spaces, presence of inflammatory cells and thickening of the alveolar walls were the major changes to the alveoli.

Bromelain treatment in this study could efficiently recover Cd-induced systemic and bronchoalveolar inflammatory changes, especially when administered in the lower dose (20 mg/kg). Bromelain anti-inflammatory properties were demonstrated earlier. It was shown that bromelain can reduce the majority of inflammatory mediators including IL-1β, IL-6 and TNF-α when immune cells are already stimulated in the condition of inflammation-induced over-production of cytokines (Hale et al. 2005; Onken et al. 2008). Bromelain showed significant anti-inflammatory activity in a murine model of allergic airway disease which was indicated by reduced BALF total leukocytes, CD4+ and CD8+ T cells as well as serum IL-4, IL-12, IL-17 and IFN-γ in bromelain-treated animals (Secor et al. 2012). Also, bromelain was capable to modulate the expression of transforming growth factor (TGF)-β, one of the major inflammation regulators in patients with osteomyelofibrosis and rheumatoid arthritis (Leipner et al. 2001; Bierie and Moses 2006; Rathnavelu et al. 2016). Various trials indicate that administration of bromelain might be effective in reducing swelling, bruising, pain and the average number of days for the complete disappearance of pain and postoperative inflammation (Tassman et al. 1965, Howat and Lewis 1972).

In the current experiment, bromelain administration in the higher dose (40 mg/kg), was not effective in the reduction of inflammation following Cd exposure but it did not exert any significant toxic or adverse effects. Bromelain was shown to have very low toxicity with an LD50 higher than 10 g/kg in laboratory animals (Taussig et al. 1975). Increasing dosages of bromelain up to 750 mg/kg/day in toxicity assessments, revealed no toxic effects after six months in the tested dogs. Rats which were treated with 1500 mg/kg per day did not display any changes in food intake, histology of heart, growth, spleen, kidney, or hematological parameters. Also, bromelain had no carcinogenic or teratogenic effects in the mentioned rats

(Moss et al. 1963, Pavan et al. 2012). However, this plant-derived protease was reported to cause allergic reactions, including asthma, especially following occupational exposure. Earlier studies revealed that bromelain can induce IgE-mediated reactions of both the immediate type and the 'late-phase reaction of immediate type reaction' with predominantly respiratory symptoms (Gailhofer et al., 1988). Furthermore, there is evidence for immunological cross-reaction between the two plant proteases bromelain and papain in human subjects (Baur and Fruhmann 1979). Hence, the adverse changes in the bromelain treated group (high dose) in this study might be attributed to the probable allergic reactions following drug administration for a long period.

The present study revealed cadmium administration to induce no significant change in serum CRP level, nor do other treatments. CRP is considered as a reliable inflammatory marker that can be increased in response to inflammation up to several hundred folds (Das 2011). Many studies demonstrated cadmium to be a pro-inflammatory factor that can upregulate CRP production (Hsu et al. 2009; Lin et al. 2009). In a cross-sectional study conducted by Lin and coworkers on the US citizens, higher urinary cadmium levels were found to be significantly associated with elevated blood CRP (Lin et al. 2009). Also, the relationship between blood Cd level and inflammation in maintenance hemodialysis patients was examined in a cross-sectional study. The results revealed that blood cadmium level was positively and significantly correlated with inflammatory risk (hs-CRP> 3 mg/L) (Hsu et al. 2009).

However, Messner and Bernhard (2010) found that increased serum Cd concentrations did not increase in human CRP levels and suggested that there is no correlation between cadmium levels and systemic inflammatory markers.

In another research, the incubation of primary human arterial endothelial cells with cadmium for 6 and 24 hours resulted in down-regulation of several of pro-inflammatory genes including COX-2 and CXCL2 chemokine (Bernhard et al. 2006).

These contradictory findings in cadmium effects might be attributable to the experimental arrangement (e.g. different biological systems analyzed and different doses of cadmium applied) (Olszowski et al. 2012). Further research including detailed histological and gene expression analyses should solve this inconsistency.

In total, findings in the present study demonstrate that acute intratracheal exposure to $CdCl_2$ can cause persistent pulmonary as well as systemic cell injury and inflammatory response. Furthermore, it seems that while induction of some pulmonary or systemic damage biomarkers was reversible, others persisted for at least 10 days after Cadmium exposure suggesting continued injury and/or diminished ability to repair. Persistent adverse pulmonary effects such as those observed in this experiment can represent an important occupational health threat. Bromelain administration can be considered as a supportive or alternative treatment to alleviate $CdCl_2$ induced systemic and bronchoalveolar inflammatory changes, especially when administered in the lower dose. Further research is required on various aspects of bromelain administration before it can be recommended as a clinical medication in cadmium-induced pulmonary intoxication.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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