

# *Punica granatum* and *Citrillus colocynthis* Aqueous Extracts Protect Mice from LPS-induced Lung Inflammation and Inhibit Metalloproteinases-2 and -9

Fatma Zioud<sup>1,2</sup>, Viviana Marzaioli<sup>3</sup>, Jamel El-Benna<sup>3,4</sup>, Rafik Bachoual<sup>1,2,\*</sup>

<sup>1</sup>Faculty of Sciences of Gabes, University of Gabes, TUNISIA.

<sup>2</sup>Laboratory of Plant Improvement and Valorization of Agroresources, National School of Engineering of Sfax, Sfax, TUNISIA.

<sup>3</sup>INSERM U1149, CNRS ERL8252 Inflammation Research Center, Paris, FRANCE.

<sup>4</sup>University of Paris Diderot, Sorbonne Paris City, Inflammex Laboratories, Faculty of Medicine, Xavier Bichat, Paris, FRANCE.

## ABSTRACT

**Aim:** Plants used in traditional medicine produce diverse active metabolites exhibiting various medicinal properties. For several centuries *Citrillus colocynthis* and *Punica granatum* have been used to treat inflammation, cancer, edema, bacterial infections and diabetes. The objectives of this work were to study the effect of *C. colocynthis* and *P. granatum* peel aqueous extracts, on activity of metalloproteinases, enzymes implicated in chronic inflammatory diseases such Chronic Obstructive Pulmonary Disease (COPD) and emphysema. **Materials and Methods:** Lung inflammation was induced in the mice (Strain BALB/c) by intra-tracheal instillation of lipopolysaccharide (5 µg/mouse) and *P. granatum* peel extract (PGE) or *C. colocynthis* peel extract (CCPAE) (200 mg/Kg body weight) was injected intraperitoneally prior to LPS administration. Bronchoalveolar lavage and lung tissue were collected to assess inflammatory cells count and total protein content. Metalloproteinases activity and expression was detected by RT-PCT and zymography techniques, respectively. **Results:** Results showed that PGE and CCPE decreased markedly neutrophils count and proteins leakage into Bronchoalveolar Lavage Fluid (BALF). Gelatin zymography assay showed that PGE inhibited MMP-2 and MMP-9 activity in BALF and in lung homogenates. However, CCPE inhibited MMP-2 and MMP-9 in lung homogenates but only MMP-2 in BALF. Furthermore, both PGE and CCPAE inhibit MMP-2 and -9, expression in the lung. **Conclusion:** Our results showed that PGE and CCPAE exhibit anti-inflammatory and, antimetalloproteinases especially MMP-2 and -9, activities. According to these findings, these natural products could be used as a potential source of new compounds with anti-inflammatory and anti-metalloproteinases activity.

**Key words:** Lung inflammation, COPD, Emphysema, MMP-2, MMP-9, Mice.

Submission Date: 11-01-2019;

Revision Date: 15-03-2019;

Accepted Date: 07-05-2019

DOI: 10.5530/ijper.53.3.82

Correspondence:

Dr. Rafik Bachoual,

Faculty of Sciences of Gabes, Erriadh City- 6072, Gabes, TUNISIA.

Phone: +216 00 75392089

E-mail: rafik.bachoual@fsg.mu.tn

## INTRODUCTION

*Punica granatum*, a member of the *Punica-ceae* family, is a small tree originating from Asia. India, Iran and China are the most productive countries of these fruits.<sup>1</sup> The pomegranate tree is widely cultivated in the Mediterranean countries such as Tunisia, Morocco, Spain, Italy and Greece.<sup>2</sup> The ripe pomegranate fruit is composed of juicy seeds, surrounded by a leathery yellow peel. In Tunisian<sup>3</sup> and Indian<sup>4</sup> folk medicine, dried pomegranate peel is used to treat dis-

orders such as colitis, headache, aphthae, diarrhea, dysentery and ulcers.<sup>5</sup> Pomegranate extracts have been shown to have antioxidant activity.<sup>6</sup> In the West Indies, this peel was employed against irregular fevers. The peel of the trunk, twigs and roots are characterized by their deworming properties. It has recently been shown that the aqueous extract of the pomegranate peel attenuates the inflammation induced by Lipopolysaccharide (LPS) in mice and it is



www.ijper.org

explained by the inhibition of the recruitment of the inflammatory cells as well as the inhibition of the activity of the myeloperoxidase.<sup>7</sup>

*Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae), commonly known as “bitter apple” is a plant that grows abundantly in Tunisia and widely in other parts of the world. In the Tunisian traditional medicine, this plant has been used to treat various diseases including, hypertension and rheumatism,<sup>3</sup> while in other countries it is used to treat constipation, oedema bacterial infections, cancer and diabetes and it is used as an abortifacient.<sup>8,9</sup> The ethnobotanical uses of this plant include its use as cathartic, purgative and vermifuge and for the treatment of fever, cancer, amenorrhoea, jaundice, leukemia, rheumatism and tumour.<sup>8</sup>

Many secondary metabolites from *C. colocynthis*, including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids, have been previously reported<sup>10-12</sup> and could explain the biological activity of this plant.

Several inflammatory disorders including Chronic Obstructive Pulmonary Disease (COPD), emphysema, rheumatoid arthritis, atherosclerosis and cancer are considered as major health care problem. The pathophysiology of these diseases is due to inflammatory/anti-inflammatory, oxidant/antioxidant and proteinase/antiproteinase imbalance. Neutrophils play a key role in host defenses against invading micro-organisms,<sup>13</sup> but excessive activation of these cells is involved in tissue damage associated with inflammatory disorders.<sup>14,15</sup> In response to a variety of agents, Polymorphonuclear neutrophils migrate to inflammatory sites, where they release proteases, bactericidal peptides and large quantities of ROS in a process known as the respiratory burst.<sup>14</sup> Oxygen reduction by neutrophil NADPH oxidase, a multicomponent enzyme system, yields superoxide anion ( $O_2^{\cdot-}$ )<sup>16</sup> while myeloperoxidase (MPO) produces hypochloric acid from hydrogen peroxide.<sup>17</sup> Matrix Metalloproteinases (MMPs) are the main proteases involved in tissue damage especially MMP-2 and MMP-9 play an important role in these disorders notably in airway obstruction including COPD and emphysema.<sup>18</sup> These diseases exhibited glucocorticoid resistance still today; no specific medicine to control such disorders is available. Thus, searching for new therapies and specific targets notably inhibiting these metalloproteinases are the main goal of scientific research.<sup>19</sup> *P. granatum* and *C. colocynthis* are used in traditional Tunisian medicine to treat some inflammatory diseases such pulmonary and articular inflammation. In these pathologies, parameters like protein concentration, cellularity, neutrophil infiltration and metalloproteinases are increased in the site of inflammation. To protect and

to treat these diseases, as a target, is the inhibition of inflammation as well as metalloproteinases, especially MMP-2 and -9 which are known to be involved in pulmonary diseases such as COPD and Emphysema. To investigate the effect of our products one animal model of inflammation was used. Animals were pre-treated with either *P. granatum* or *C. colocynthis* aqueous extract and compared to the positive control (Animal treated with 5 µg of LPS and to the negative control animal treated with saline). The aim of this work was to examine the effect of aqueous peel extract of *Citrullus colocynthis* and *Punica granatum* on MMPs activity and expression in BALF and lung homogenates on LPS-induced lung inflammation in mice.

## MATERIALS AND METHODS

### Preparation of CCPAE and PGE

In this study, three batch of Tunisian *C. colocynthis* (Collected from the island of Jerba in South of Tunisia) and *P. granatum* peel (Hammouri variety), were dried at 37°C, blended and suspended in sterile 0.9% NaCl, then centrifuged at 2000 rpm for 3 min. From each batch, the supernatants of different preparations of CCPAE or PGE were used for the experiments. The results obtained with different preparations from different batches are reproducible and the same dose effect responses were found.

### Animals

BALB/c mice aged 7 weeks and weighing 22-26 g were purchased from the animal facility of Faculty of sciences of Gabes. Animals were housed in standard wire-topped cages and the temperature-controlled units. Food and water were supplied *ad libitum*. The experiments were approved by our Institutional Committee on Animal Care and use and the experimental protocol complied with Tunisian legal requirements for animal's studies.

### Experimental Design

Lipopolysaccharide (LPS) of *Escherichia coli* O55: B6 (Sigma) was used to induce lung inflammation. To investigate the effect of each of CCPAE and PGE, animals were divided randomly into four groups with 8-10 mice in each group.

**Group 1:** Control mice received saline (NaCl 0.9%).

**Group 2:** CCPAE or PGE mice received 200 mg/kg of *C. colocynthis* or *P. granatum* extract by intraperitoneal route (i.p) and saline by intratracheal route (IT).

**Group 3:** LPS mice received saline by IP route and LPS (5 µg/mouse) by IT route.

**Group 4:** CCPAE + LPS or PGE + LPS mice received 200 mg/kg of *C. colocynthis* or *P. granatum* extract by IP route and LPS (5 µg/mouse) by IT route.

The mice received an intraperitoneal injection of 200 mg/kg CCPAE or PGE on day 0 (D0), followed by 200 mg/kg on day 1 (D1). 3 h after the second injection, animals received a cocktail of anesthetics (75 mg/kg ketamine (Virbac Santé Animale) plus 1 mg/kg medetomidine (Pfizer)), before intratracheal LPS instillation (5 µg/mouse). The mice were aroused by an intraperitoneal injection of 1 mg/kg atipamezol (Pfizer), a medetomidine antagonist and were killed 24 h later.

### Biochemical Assays

#### Bronchoalveolar Lavage (BAL) and Lung Sampling

The mice were anesthetized by an intraperitoneal injection of 50 mg of penthotal (Sigma) and killed by exsanguination. The lungs were lavaged twice with 1 ml of physiological saline, removed from the chest cavity and immediately placed at -80°C until use. The lavage fluid (2 mL) was immediately placed on ice. Free alveolar cells were recovered from the lavage fluid by centrifugation at 400 g for 15 min at 4°C. The cell pellet was suspended in 150 µL of physiological saline and an aliquot was used to determine the total white cell count with a hemocytometer. For differential counts, the cell suspension was cytopun (Cytospin-2, Shandon Products Ltd.), fixed in methanol and stained with Diff Quick solution (Medion Diagnostics, Plaisir, France). Three hundred cells were counted with an oil immersion lens (1000).

The total protein concentration in the supernatant was measured with the Quick-Start Bradford assay (Bio-Rad, Marnes-la-Coquette, France).

#### Zymography

2 mL of grinding reagent were added to a lung sample (100 mg) which is centrifuged at 3000 rpm at 4°C. the supernatant is recovered and stored at -20°C for assays of protein and to test the activity of metalloproteinases. Samples of total proteins lung grinded were separated under non-reducing conditions on 12% polyacrylamide gels containing 1 mg/mL gelatin or casein, as described previously. Gels were loaded with 4-10µg of total proteins sample and run under Laemmli standard conditions. After electrophoresis, gels were washed twice in 100 mL of 2.5% Triton X-100 (30 min each) under constant mechanical agitation and incubated in activation buffer (50 mM Tris-HCl pH 7.5, 5 mM CaCl<sub>2</sub>, 1 µL ZnCl<sub>2</sub>, 0.1 mM NaN<sub>3</sub>) at 37°C for 72 h. Gels were stained with Coomassie blue. Visualization of MMP-2 and -9 activities was obtained by incubation of the gels

in acetic acid 45% and methanol 10%, H<sub>2</sub>O and were quantified with Image J software.

### Semi-quantitative RT-PCR

#### RNA Extraction and Real Time PCR (RT-qPCR)

RT-qPCR in lung homogenates was performed to examine the mRNA expression of proteases genes. Total RNA was extracted from lung tissues using TRIzol (PureZOL RNA Isolation Reagent). For each sample, total RNA content was determined by measuring the absorbance by NanoDrop (Spectrophotometer, INanoDrop ND-1000) at 260 nm and the purity was determined using A260/A280 ratio. The concentrations of extracted mRNA in lung samples were determined using RT-qPCR assay (iQ<sup>TM</sup> Universal SYBR Green One-Step Kit, Bio-RAD), On one step a total of 1µg mRNA sample from each group was reversely transcribed into cDNA and amplified for 35 cycles on a Light Cycler 1.5<sup>®</sup> thermocycler using the SYBR GREEN PCR mix. Genes analyzed were MMP-1 and MMP-2. The following sets of primers were used: MMP-2 sense 5-CACACCAGGTGAAGGATGTG-3 antisense 5-AGGGCTGCATGCAAATATC-3, MMP-9 sense 5-TTCTCTGGACGTCAAATGTGG-3 antisense 5-CAAAGAAGGAGCCCTAGTTCAAGG-3. Gene expression was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene.

#### Statistical Analysis

Data were reported as mean ± standard error. The Newman-Keuls multiple comparisons test was used and *P* values <0.05 were considered to denote significant differences.

## RESULTS

### Effect of CCPAE and PGE on BALF Protein Content and Cellularity in LPS Treated Mice

Intra-tracheal administration of 5 µg of LPS to mice induced a significant increase in BALF protein content after 24h, compared with animals treated with either the vehicle, CCPAE or PGE alone (Data not shown). The BALF protein content after intra-tracheal LPS challenge was significantly lower when animals were pretreated with 200 mg/kg of either of CCPAE or PGE (Data not shown). Intra-tracheal LPS administration also induced a significant increase in both the BALF total cell count (*p*<0.05 vs. vehicle, CCPAE or PGE) and the BALF neutrophil count after 24 h (*p*<0.05 vs. vehicle, CCPAE or PGE alone). Neither the vehicle, CCPAE nor PGE at 200 mg/kg modified the BALF cell count.

However, intra-peritoneal CCPAE or PGE injection at 200 mg/kg significantly reduced the BALF total cell count after intra-tracheal administration of LPS ( $p < 0.05$  vs. LPS alone). Simultaneous administration of LPS and CCPAE or PGE significantly reduced neutrophils recruitment compared to LPS alone ( $p < 0.05$  vs. LPS) (Data not shown).

#### CCPAE and PGE inhibit MMP-2 activity in BALF

To investigate the effect of PGE and CCPAE on matrix metalloproteinases (MMP-2) activity in BALF, the technique of zymography was used. Results showed that the activity of this enzyme increased following LPS intra-tracheal instillation compared to animals treated either with saline, PGE or CCPAE alone (Figure 1 A and B). However, this increase was suppressed when mice were pre-treated with 200 mg/kg of either PGE or CCPAE. A decrease of MMP-2 activities, 25% and 20%, respectively, were observed (Figure 1A and B). However, neither the saline or PGE or CCPAE at 200 mg/kg modified the MMPs activity in BALF. These results suggest that PGE and CCPAE at a dose of 200 mg/kg both inhibit the BALF activity of MMP-2.

#### CCPAE and PGE inhibit MMP-9 activity in BALF

To investigate the effect of PGE and CCPAE on matrix metalloproteinases (MMP-9) activity in BALF, the technique of zymography was used. Results showed that the activity of this enzyme increased following LPS intra-tracheal instillation compared to animals treated either with saline, PGE or CCPAE alone (Figure 2 A and B). However, this increase was suppressed when mice were pre-treated with 200 mg/kg of either PGE. A decrease of MMP-9 activity, 20%, was observed ( $p < 0.001$ ) (Fig-

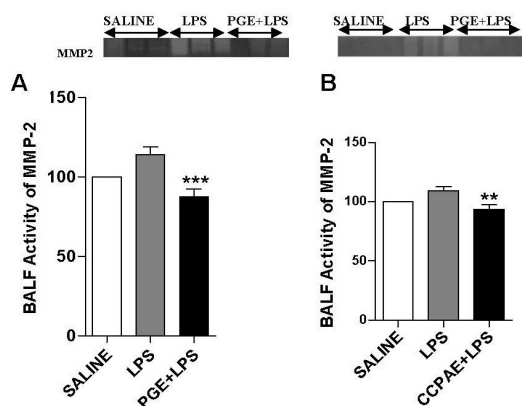
ure 2 A). However, no effect on the activity of MMP-9 was observed in animal pre-treated with 200 mg/kg of CCPAE and challenged with LPS (Figure 2 B). The activity of MMP-9 in animals treated with PGE or CCPAE at 200 mg/kg alone are comparable to the control mice treated with saline (Figure 2 A and B). These results suggest that PGE at a dose of 200 mg/kg inhibit the BALF activity of MMP-9. However, at this dose CCPAE had no effect on the activity of MMP-9

#### CCPAE inhibit MMP-2 and MMP-9 activity in Lung Tissues

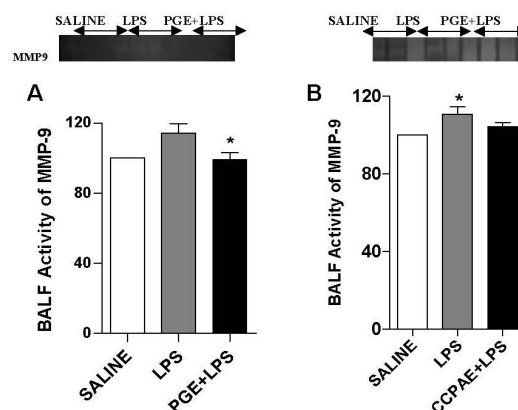
To investigate the effect of CCPAE on matrix metalloproteinases (MMP-2, MMP-9) activity, in lung homogenates, the technique of zymography was used. Results (Figure 3 A and B) showed that the activity of these enzymes increased following LPS intra-tracheal instillation compared to animals treated either with saline or CCPAE alone. However, this increase was suppressed when mice were pre-treated with 200 mg/kg of CCPAE. A decrease of both, MMP-2 (Figure 3 A) and MMP-9 (Figure 3 B) activities, 15% and 28%, respectively, were observed. However, neither the saline or CCPAE at 200 mg/kg modified the MMPs activity in lung homogenate.

#### CCPAE inhibits MMP-2 and MMP-9 Gene Expression

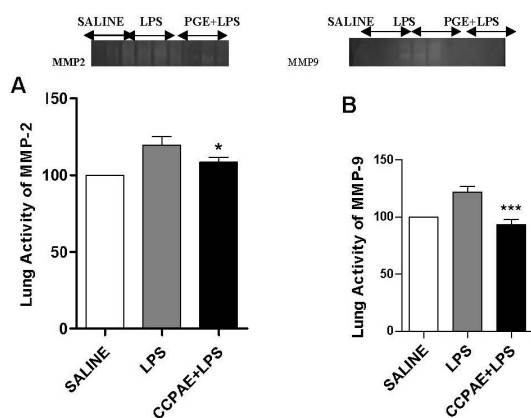
To investigate the effect of CCPAE on matrix metalloproteinases (MMP-2, MMP-9) gene expression, in lung tissues, the technique of RT-PCR was used. Results (Figure 4A and B) showed that the expression of MMP-2, MMP-9 genes increased following LPS intra-tracheal instillation compared to animals treated either with saline or CCPAE alone. However, this increase was suppressed when mice were pre-treated with 200 mg/kg of



**Figure 1:** Effect of *Citrullus colocynthis* or *Punica granatum* Peel on the BALF activity of MMP-2 in LPS-treated mice. Activity was measured in BALF 24 h after intratracheal instillation of Lipopolysaccharide (LPS 5  $\mu$ g/mouse) or in controls. LPS induced a massive increase in the MMP2 activity content, which was significantly attenuated by CCPAE (200 mg/kg) ( $n=8$ , mean  $\pm$  SEM,  $P < 0.05$ ).



**Figure 2:** Effect of *Citrullus colocynthis* or *Punica granatum* peel on the BALF activity of MMP-9 in LPS-treated mice. Activity was measured in BALF 24 h after intratracheal instillation of Lipopolysaccharide (LPS 5  $\mu$ g/mouse) or in controls. LPS induced a massive increase in the MMP9 activity content; CCPAE has no effect on the activity of MMP-9 in the BALF.



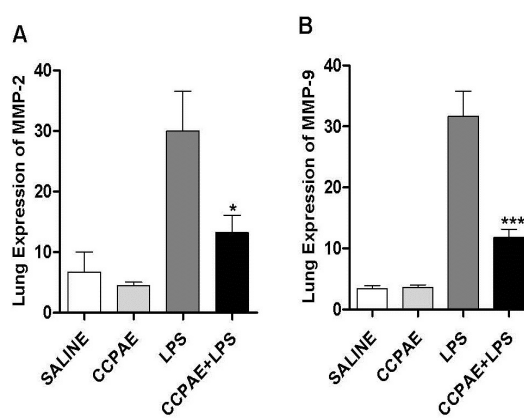
**Figure 3: Effect of *Citrullus colocynthis* peel on the lung homogenates activity of MMP-2, and MMP-9 in LPS-treated mice. Activity was measured in lung homogenates 24 h after intratracheal instillation of lipopolysaccharide (LPS 5 µg/mouse) or in controls. LPS induced a massive increase in the MMP-2, MMP-9 and MMP-1 activity content. CCPAE (200 mg/kg) significantly attenuated the increase of MMP-2 and MMP-9 activity ( $n = 8$ , mean  $\pm$  SEM,  $P < 0.05$ )**

CCPAE. A decrease of both MMP-2 ( $P < 0.01$ ) (Figure 4 A) and MMP-9 ( $P < 0.001$ ) (Figure 4 B) genes expression were observed. However, neither the saline or CCPAE at 200 mg/kg modified the MMPs expression in lung homogenate.

## DISCUSSION

In this study, we show that each of CCPAE and PGE attenuated LPS-induced lung inflammation in mice and reduces the activity of MMP-2 and -9. BALF from mice exposed to LPS contained a large amount of protein, reflecting high-permeability pulmonary edema. The BALF protein concentration was significantly reduced by CCPAE and PGE treatment, suggesting that each of CCPAE and PGE reduce lung vascular permeability and edema and might therefore protect the integrity of the alveolar capillary membrane. The reduction in neutrophils infiltration could explain these beneficial effects, as neutrophils are considered as primary cellular effector of alveolar capillary damage in some inflammatory diseases such COPD and asthma.<sup>20,21</sup>

Many scientific approaches were used to induce lung inflammation in animal models. The model of *Pseudomonas aeruginosa* inflammation has been established<sup>22</sup> since 1997. Intraperitoneal injection of 100 µg *Pseudomonas aeruginosa* LPS to C57BL6 mice induces over-recruitment of neutrophils in BALF, parenchyma and alveolar spaces, increases levels of inflammatory cytokine expression in BALF (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ) and also increases oxidative stress and alveolar damage. Other authors showed that a high dose of LPS induces the release of IL-1 $\beta$  and TNF with the recruitment of neu-



**Figure 4: Effect of *Citrullus colocynthis* peel on MMP-2 and MMP-9 expressions in the lungs of LPS-treated mice. Expression was measured in lung homogenates 24 h after intratracheal instillation of lipopolysaccharide (LPS 5 µg/mouse) or in controls. LPS induced a massive increase in the MMP expression content, which was significantly attenuated by CCPAE (200 mg/kg) ( $n = 8$ , mean  $\pm$  SEM,  $P < 0.05$ ).**

trophils into the alveoli which aggravates lung damage by increasing pulmonary permeability, edema formation and cell death.<sup>23</sup> Stimulation of human respiratory tract epithelial cells by LPS induces an inflammatory reaction via TGF release, overexpression of MUC5AC genes, EGFR receptor phosphorylation and overactivation of metalloproteases.<sup>24</sup>

Persistent asthma, Chronic Obstructive Pulmonary Disease (COPD) and emphysema are chronic inflammatory lung diseases.<sup>25,26</sup> These affections involve different types of inflammatory cells and soluble mediators.<sup>19</sup> COPD is characterized by alveolar neutrophils recruitment, the destruction of the alveolar epithelium and flooding of the alveolar spaces with proteinaceous exudates.<sup>27</sup> In this study, BALF from mice exposed to LPS showed an increase in protein amount, reflecting high-permeability pulmonary edema. However, the BALF protein concentration was significantly reduced by PGE or CCPAE treatment at a dose of 200 µg/mL suggesting that these products reduce lung vascular permeability and edema and might therefore protect the integrity of the alveolar capillary membrane. The reduction in neutrophil infiltration could explain these beneficial effects, as neutrophils are considered a primary cellular effector of alveolar capillary damage in COPD and asthma.<sup>20,21</sup> The anti-inflammatory, anti-oxidant and anti-oncological characteristics of pomegranate and colocynth were already investigated by other studies, it was shown that pomegranate and colocynth reduce overexpression of inflammatory mediators and overcome cellular degradation.<sup>6,7,28-30</sup> Pomegranate fruit is characterized by high level of polyphenols involved in antioxidant activity

and anti-inflammatory properties. Anti-inflammatory and anti-oxidant activities of Punicalagin (PUN), an ellagitannin isolated from pomegranate, induce an up-regulation of HO-1 in murine macrophages.<sup>31</sup> *Punica granatum* juice, at 5 and 8ml/kg, abrogate the effect of trinitrobenzene sulfonic acid in provoked colitis in rats.<sup>32</sup> Polyphenol of pomegranate fruit is known with its potent anti-oncological activity in lung, breast and cervical cells. It has anti-proliferative activity against prostate cancer cells by inducing apoptosis and inhibiting angiogenesis.<sup>30</sup> Moreover, pomegranate juices, its seed oil, inhibited cell growth, increased cell adhesion, decreased cell migration in breast cancer cells and inhibited cancerous lesion in murine mammary gland organ culture.<sup>33</sup> It was shown that colocynth is a main source of flavonoids; alkaloids; glycosides; saponins; phytosterols; steroids; proteins and triterpenoids. These compounds have potent antioxidant activity. The methanolic extract of colocynth fruit shows an ability to scavenge free radicals.<sup>34</sup>

The beneficial effects of pomegranate and colocynth are attributed to phenolic compounds, including punicalagin isomers, ellagic acid derivatives and anthocyanins (Delphinidin, cyanidin and pelargonidin 3-glucoside-sand 3,5-diglucosides). These compounds scavenge free radicals and inhibit lipid oxidation *in vitro*.<sup>35,36</sup> The protective effect of PGE was attributed to its ability to inhibit Myeloperoxidase,<sup>7</sup> however CCPAE inhibited NADPH oxidase activity *in vitro*, an effect possibly explaining the antiinflammatory action observed with these two compounds *in vivo* since MPO and NADPH oxidase stimulates murine macrophages to produce reactive oxygen species.<sup>37,38</sup> Secreted ROS enhance the secretion of TNF- $\alpha$ , IL-8 and other proinflammatory cytokines.<sup>39</sup> In particular, alveolar macrophage-derived TNF- $\alpha$  and IL-8 recruit PMN to sites of inflammation.<sup>40</sup> MPO and NADPH oxidase inhibition by PGE and CCPAE respectively, could attenuate these inflammatory reactions.

The activity and the expression of various MMPs have been reported in many pathological conditions, notably COPD, emphysema, allergic lung inflammation and arthritis, moreover these enzymes are also involved in cancer. Metalloproteinases regulate recruitment, influx and transmigration of inflammatory cells from vasculature to the site of inflammation in tissue. They are also implicated in the availability and activity of inflammatory mediators, such as cytokines and chemokines and they are involved in creating chemokine gradients in tissue to recruit inflammatory cells to the site of injury or inflammation and can also regulate survival of inflammatory cells.<sup>41</sup>

Implication of MMP-2 in the recruitment of inflammatory cells was clearly demonstrated, using allergic lung inflammation model. Corry *et al.* reported a reduction in the influx of inflammatory cells to the alveolar space in MMP-9-null mice. In the same model the role of MMP-2 in leukocyte migration to the inflammation site was also demonstrated.<sup>42,43</sup> Besides it was shown that MMP-9 exhibited more potent effect on inflammation than MMP-2 since various chemokines are affected by lack of MMP-9 resulting in disturbed influx of both neutrophils and eosinophils.<sup>43</sup> Hence, MMP-9 plays an important role in reepithelization in tissue repair in the lung. Activated neutrophils released MMP-9 which cleaves and inactivates the serine protease inhibitor  $\alpha$ 1-antitrypsin known with its potent inhibition of neutrophil elastase. Thus, MMP-9 can this way indirectly promote the activity of neutrophil elastase also implicated in lung injury.

In this study, we have demonstrated that LPS increased lung and BALF MMP-2 and MMP-9 activities and expressions. In fact, intraperitoneal injection of 200 mg/kg of PEG reduced BALF MMP-2 and MMP-9 activities. Intraperitoneal injection of CCPAE at 200 mg/kg reduced only BALF MMP-2 activity and no effect on MMP-9 activity was observed. However, these extracts reduced MMP-2 and MMP-9 activities and expression in lung homogenates. These finding suggest that, intraperitoneal injection of PEG or CCPAE at 200 mg/kg significantly reduced metalloproteinases activities and expression involved in tissues destruction which provide a hopeful therapeutic protocol against BPCO and emphysema. Further studies are needed to identify the precise compounds responsible for MMP-2 and -9 activities inhibition observed in this work. It is also interesting to test the effect of different mix of PGE and CCPAE using a model of animal pulmonary inflammation.

## CONCLUSION

In conclusion, *Punica granatum* and *Citrillus colocynthis* peel aqueous extracts attenuated inflammation induced by intratracheal endotoxin instillation in mice, leading to a decrease in the BALF protein concentration, total cellularity and neutrophils content but it also inhibits MMP-2 and -9, two metalloproteinases involved in many inflammatory and destructive tissues diseases.

## ACKNOWLEDGEMENT

This work was supported by the Faculty of Sciences of Gabes, University of Gabes and the Laboratory of

Plant Improvement and Valorization of Agroresources, National School of Engineering of Sfax, Sfax, Tunisia.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

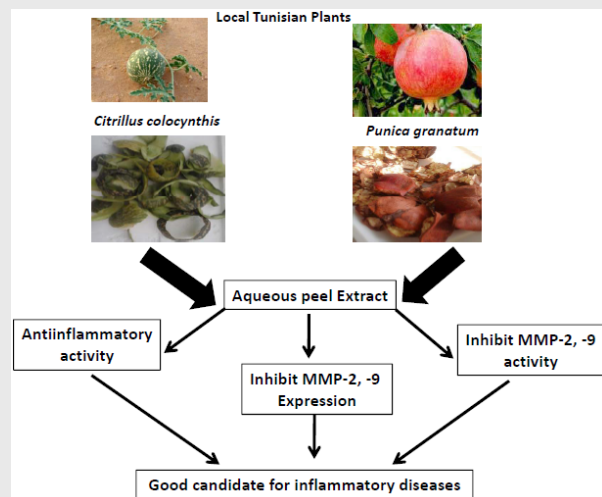
## ABBREVIATIONS

**PGE:** *Punica granatum* Extract; **CCPAE:** *Citrullus colocynthis* peel aqueous extract; **COPD:** Obstruction pulmonary disease; **LPS:** Lipopolysaccharide; **BALF:** Bronchoalveolar lavage fluid; **MMP:** Matrix metalloproteinase; **MPO:** Myeloperoxidase.

## REFERENCES

- Melgarejo P, Valero D. Series A: Mediterranean Seminars. Spain. 2012;103.
- Wald E. Le grenadier : *punica granatum*. 2009;NAN12052.
- Boukef K, Souissi HR, Balansard G. Contribution to the study of plants used in traditional medicine in Tunisia. *Plant Med.* 1982;16(4):260-79.
- Nagaraju N, Rao KN. A survey of plant crude drugs of Rayalaseema, Andhra Pradesh, India. *India J Ethnopharm.* 1990;29(2):137-58.
- Kirthikar KR, Basu BD. *Punica granatum*. In: *Indian Medicinal Plants*. Sri Satguru Publications. 2000;5:1508-13.
- Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress atherogenic modifications to LDL and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am J Clin Nutr.* 2000;71(5):1062-76.
- Bachoual R, Talmoudi W, Boussetta T, Braut F, ElBenna J. An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase *in vitro* and attenuates lung inflammation in mice. *Food and Chemical Toxicology.* 2011;49(6):1224-8.
- Delazar A, Gibbons S, Kosari AR, Nazemiyeh H, Modarresi M, Nahar L, et al. Flavone C-glycosides and cucurbitacin glycosides from *Citrullus colocynthis*. *DARU J Pharm Sci.* 2006;14(3):109-14.
- Gurudeeban S, Satyavani K, Ramanathan T. Bitter apple (*Citrullus colocynthis*): An Overview of Chemical Composition and Biomedical Potentials. *Asian Journal of Plant Sciences.* 2010;9(7):394-401.
- Yankov LK, Hussein SM. Fatty acid from the oil of the seeds of *Citrullus colocynthis*. *Dokl Bolg Akad Nouk.* 1975;28:209-12.
- Hatam NA, Whiting DA, Yousef NJ. Cucurbitacin glycosides from *Citrullus colocynthis*. *Phytochemistry.* 1989;28(4):1268-71.
- Maatooq GT, El-Sharkawy SH, Affif MS, Rosazza PN. CP Hydroxybenzoyl-glycoflavanones from *Citrullus colocynthis*. *Phytochemistry.* 1997;44(1):187-90.
- Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase and bacterial killing. *Blood.* 1998;92(9):3007-17.
- Babior BM. The respiratory burst of phagocytes. *J Clin Invest.* 1984;73(3):599-601.
- Babior BM. Phagocytes and oxidative stress. *Am J Med.* 2000;109(1):33-44.
- El-Benna J, Dang PM, Gougerot-Pocidallo MA, Elbim C. Phagocyte NADPH oxidase: a multicomponent enzyme essential for host defenses. *Arch Immunol Ther Exp.* 2005;53:199-206.
- Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol.* 2005;77(5):598-625.
- Cataldo DD, Gueders MM, Rocks N, Sounni NE, Evrard B, Bartsch P, et al. Pathogenic role of matrix metalloproteinases and their inhibitors in asthma and chronic obstructive pulmonary disease and therapeutic relevance of matrix metalloproteinase inhibitors. *Cell Mol Biol.* 2003;49(6):875-84.
- Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. *Eur Respir J.* 2003;22(4):672-88.
- Pesci A, Balbi B, Majori M, Cacciani G, Bertacco S, Alciato P, et al. Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease. *Eur Respir J.* 1998;12(2):380-6.
- Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2003;167(3):418-24.
- Rezaiguia S, Garat C, Delclaux C, Meignan M, Fleury J, Legr P, et al. Acute bacterial pneumonia in rats increases alveolar epithelial fluid clearance by a tumor necrosis factor-alpha-dependent mechanism. *J Clin Invest.* 1997;99(2):325-35.
- Kolomaznik M, Nova Z, Calkovska A. Pulmonary Surfactant and Bacterial Lipopolysaccharide: The Interaction and its Functional Consequences. *Physiol Res.* 2017;66:S147-57.
- Matt XG, Shao JA N. Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. *Proc Natl Acad Sci USA.* 2005;102(3):767-72.
- JrLemanske RF, Busse WW. Asthma. *JAMA.* 1997;278:1855-73.
- Ward PA. Phagocytes and the lung. *Ann N Y Acad Sci.* 1997;832(1):304-10.
- DiStefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I, et al. Increased expression of nuclear factor-kB in bronchial biopsies from smokers and patients with COPD. *European Respiratory Journal.* 2002;20(3):556-63.
- Mehta R, Lansky EP. Breast cancer chemopreventive properties of pomegranate (*Punica granatum*) fruit extracts in a mouse mammary organ culture. *Eur J Canc Prevent.* 2004;13(4):345-8.
- Zioud F, Mahmoud AB, Boussetta T, El-Benna J, Bachoual R. An aqueous *Citrullus colocynthis* peel extract inhibits neutrophil reactive oxygen species production and attenuates lung inflammation in mice. *Journal of Medicinal Plants Research.* 2015;9(31):829-37.
- Adaramoye O, Erguen B, Nitzsche B, Hopfner M, Jung K, Rabien A. Punicalagin, a polyphenol from pomegranate fruit, induces growth inhibition and apoptosis in human PC-3 and LNCaP cells. *Chem Biol Interact.* 2017;25:100-6.
- Xu X, Guo Y, Zhao J, He S, Wang Y, Lin Y, et al. Punicalagin, a PTP1B inhibitor, induces M2c phenotype polarization via up-regulation of HO-1 in murine macrophages. *Free Radic Biol Med.* 2017;110:408-20.
- Riaz A, Khan RA, Afroz S, Mallick N. Prophylactic and therapeutic effect of *Punica granatum* in trinitrobenzene sulfonic acid induced inflammation in rats. *Pak J Pharm Sci.* 2017;30(1):155-62.
- Ya L, Sha L, Xiao M, Ren-You G, Jiao-Jiao Z, Hua-Bin Li. Dietary Natural Products for Prevention and Treatment of Breast Cancer. *Nutrients.* 2017;9(7):728.
- Kumar S, Kumar D, Manjusha SK, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharmaceutica.* 2008;58(2):215-20.
- Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem.* 2000;48(10):4581-9.
- Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin and pelargonidin. *J Agri Food Chem.* 2002;50(1):166-71.
- Lincoln JA, Lefkowitz DL, Cain T, Castro A, Mills KC, Lefkowitz SS, et al. Exogenous myeloperoxidase enhances bacterial phagocytosis and intracellular killing by macrophages. *Infect Immun.* 1995;63(8):3042-7.
- Gelderman MP, Lefkowitz DL, Lefkowitz SS, Bollen A, Moguilvsky N. Exposure of macrophages to an enzymatically inactive macrophage mannose receptor ligand augments killing of *Candida albicans*. *Proc Soc Exp Biol Med.* 1998;217(1):81-8.
- Nelson S, Summer WR. Innate immunity, cytokines and pulmonary host defense. *Infect Dis Clin North Am.* 1998;12(3):555-67.
- Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest.* 2001;119(5):1329-36.
- Butler GS, Overall CM. Matrix metalloproteinase processing of signaling molecules to regulate inflammation. *Periodontol.* 2000. 2013;63(1):123-48.
- Corry DB, Kiss A, Song LZ, Song L, Xu J, Lee SH, et al. Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. *FASEB J.* 2004;18(9):995-7.
- Corry DB, Rishi K, Kanellis J, Kiss A, Song LZ, Xu J, et al. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nat Immunol.* 2002;3(4):347-53.

## PICTORIAL ABSTRACT



## SUMMARY

- Punica granatum* and *Citrillus colocynthis* are medicinal plants employed in Tunisian traditional medicine in treatment of inflammatory diseases. The present study investigates the anti-inflammatory and anti-Matrix metalloproteinases of peel aqueous extract of these plants. Lung inflammation was induced in mice by intra-tracheal instillation of lipopolysaccharide and *P. granatum* or *C. colocynthis* peel extract (200 mg/Kg bw) was injected prior to LPS administration. Inflammatory parameters and metalloproteinases activity and expression were studied. Results showed that PGE and CCPAE exhibit anti-inflammatory and antimetalloproteinases MMP-2 and -9, activity. These natural products could be used as a potential source of new compounds with anti-inflammatory and antimetalloproteinases activity and could be an alternative to treat chronic inflammatory diseases.

## ABOUT AUTHORS



**Dr. Rafik Bachoual** has completed his Ph.D from Faculty of Pharmacy of Chatenay Malabry, Paris XI University, France. He worked on the Contribution of different genetic mechanisms in fluoroquinolones resistance in *Escherichia coli*, *Campylobacter coli*, *campylobacter jejuni* and *Bacteroides fragilis*. He was a Postdoctoral fellow at Washington University School of Medicine of Saint Louis at USA. After, he joined Laboratory of Physiopathology and Epidemiology of respiratory failure, INSERM U700, Faculty of Medicine Xavier Bichat. He investigated the Role of cigarette smoke-diesel particles interaction in the physiology of bronchial and alveolar remodeling in Chronic Obstruction Pulmonary disease (COPD). Dr. Bachoual is now working as Professor of Microbiology and immunology at the Department of Sciences of life at the Faculty of Sciences of Gabes Tunisia. His main fields of research are antimicrobial and anti-inflammatory agents; their mechanism of action and resistance. He is working also on natural products and drug discovery.

**Cite this article:** Zioud F, Marzaioli V, El-Benna J, Bachoual R. *Punica granatum* and *Citrillus colocynthis* Aqueous Extracts Protect Mice from LPS-induced Lung Inflammation and Inhibit Metalloproteinases-2 and -9. Indian J of Pharmaceutical Education and Research. 2019;53(3):503-10.