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Anti-inflammatory Potential of Whole Pomegranate Fruit Juice (POM) against Bleomycin Induced Lung Injury in Rats

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ABSTRACT

The ameliorative potential of whole Pomegranate fruit juice was studied against Bleomycin (BLM) induced lung injury. A total of 48 male *Wistar* albino rats were procured and divided into 4 groups consisting of 12 rats in each. The group 1 (control), group 2 BLM toxic control {@ 5 mg/kg body weight (b.wt) *via* single intra- tracheal (IT) instillation}, group 3 administered with BLM (@ 5 mg/kg b.wt *via* single IT instillation) and Pomegranate juice (@ 1 mL/rat once daily orally) and group 4 rats administered with Pomegranate juice only (@ 1 mL/rat once daily orally). Grossly, in group 2 rats, lung showed congestion, haemorrhage and edema. Histopathological studies revealed interstitial pneumonia, pulmonary edema, neutrophilic infiltration in perivascular and peribronchiolar areas on 14th day of experiment. In addition to these lesions complete loss of architecture of lung alveoli, severe hyperplasia of bronchiolar epithelial cells, peribronchiolar infiltration and mild fibrosis in perivascular and peribronchiolar areas was noticed on 28th day of experiment. Group 3 rats showed moderate improvement in both gross and histopathological lesions. The cytokine activity in group 2 showed a significant ($P<0.05$) increase in tumor necrosis factor- *Alpha* (TNF-α), transforming growth factor-*Beta* 1 (TGF-β1) and interleukin (IL)-10 while treatment with POM in group 3 exhibited significant decreased in the levels of proinflammatory cytokines. The current study concluded the anti-inflammatory effect of POM against bleomycin.

HIGHLIGHTS

- Inflammatory cytokines play important role in the pathogenesis of pulmonary toxicity.
- Pomegranate fruit juice showed significant anti-inflammatory activity.

Keywords: Chemotherapeutic agent, Gross pathology, Histopathology, Cytokines

The cancer is the second leading cause of death worldwide, accounting for 10 million deaths in 2020 (*World Health Organization,* 2021). Bleomycin (BLM) is a chemotherapeutic agent which belongs to glycopeptidic antineoplastic antibiotic that is used in the treatment of various neoplastic diseases including head and neck squamous cell carcinomas, ovarian and testicular carcinoma, lymphomas, malignant pleural effusions (Latta *et al*., 2015). Like other anticancer drugs bleomycin also produce adverse side effects particularly on lung tissue. The mechanism of bleomycin induced lung injury involves oxidative damage, relative deficiency of the deactivating enzyme bleomycin hydrolase, genetic susceptibility and release of inflammatory cytokines (Reinert *et al*., 2013). Pomegranate (*Punica granatum)* has been tremendously used as a source of traditional remedies for thousands of years in Indian subcontinent's ancient Ayurveda system of medicine due to its long traditional medicinal value (Mayilsamy and Shwetha, 2015). Pomegranate being rich in tannins and flavonoids possesses potent anti-oxidant, anti-inflammatory and remarkable anti-cancer promoting activity (Mansouri *et al.,* 2014).

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The present study was done to evaluate the efficacy of whole pomegranate fruit juice (POM) against bleomycin induced lung injury.

MATERIALS AND METHODS

A total of forty eight (48) male albino *Wistar* rats weighing between 180-220 g were procured from M/S Vyas labs. The rats were randomly divided into four (4) groups consisting of twelve (12) animals in each. Group 1 served as Control (Saline @ 0.9 mL/ rat/single dose/Oral). Group 2 rats were administered BLM Sulphate (BLM @ 5 mg/kg b.wt/single dose/IT instillation). Group 3 were administered BLM Sulphate (BLM @ 5 mg/kg b.wt/single dose/IT instillation) + whole Pomegranate fruit Juice (@ 1 mL/rat once daily orally for 28 days) and Group 4 with whole Pomegranate fruit juice (@ 1 mL/rat once daily orally for 28 days).

Detailed necropsy was conducted on 14th and 28th day of the experiment and gross changes in lungs were noticed. Pieces of lung were collected in 10 % neutral buffered formalin for histopathological study. Samples were processed, sectioned (4-5 microns) and stained with Hematoxylin and Eosin as per standard protocol (Luna, 1968). Small pieces of lung tissue were kept frozen for estimation of tissue cytokines. Levels of TNF-alpha, interleukin 10 and transforming growth factor- Beta 1 were measured in lung homogenate using a commercially available ELISA kit (Krishgen Biosystem, Mumbai) according to the manufacturer's instructions.

Data obtained were subjected to statistical analysis by applying one way Analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 15.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P<0.05$ (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

On necropsy, normal appearance of lungs was observed in groups 1 and 4 on 14th and 28th day of experiment. In group 2, rats showed macroscopic changes like congestion, haemorrhages and mild emphysematous areas of lungs on day 14 (Fig. 1) and the gross changes *viz*., swollen, emphysematous, edematous, haemorrhagic, and collapsed on day 28 of experiment (Fig. 2).

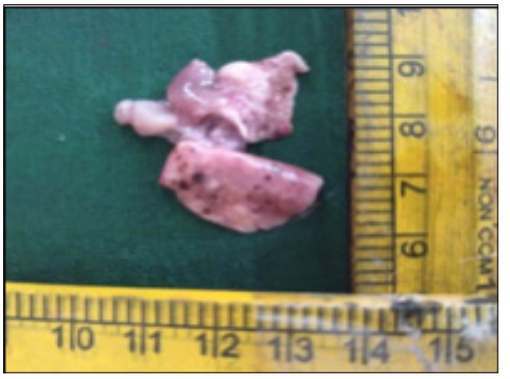

Fig. 1: BLM treated rat on 14th day showing collapsed and haemorrhagic lungs with rough surfaces

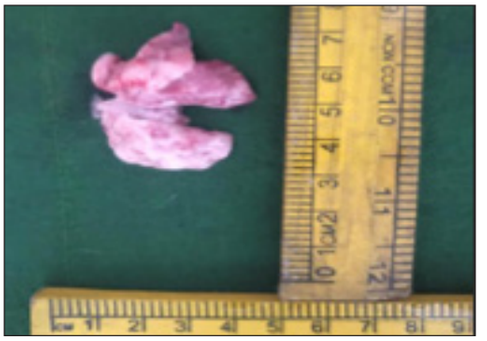

Fig. 2: BLM treated rat on 28th day showing depressed areas, swollen, emphysematous, edematous, haemorrhagic and collapsed lungs

However, the severity of lesions significantly reduced in group 3, including mild emphysema with focal petechial haemorrhages on 14th day (Fig. 3) along with mild edema on 28th day of experiment.

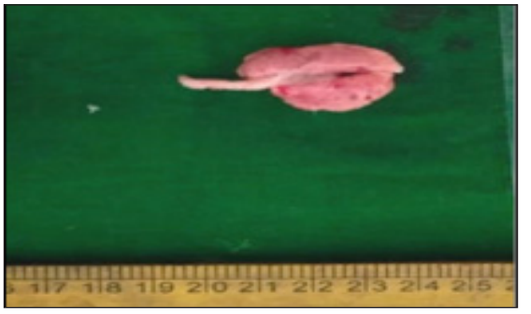

Fig. 3: BLM + Pomegranate juice treated rat on 14th day showing mild emphysema with focal petechial haemorrhages on lungs

Microscopically, lung sections of group 2 on 14th day showed thickened alveolar septa with inflammatory cell infiltration (pneumonitis) predominantly neutrophils, reduced alveolar lumen, red hepatisation with marked dilation and congestion of pulmonary artery, extravasation of erythrocytes into the alveolar septa and alveolar lumen (Fig. 4), perivascular and peribronchiolar neutrophillic infiltration (Fig. 5).

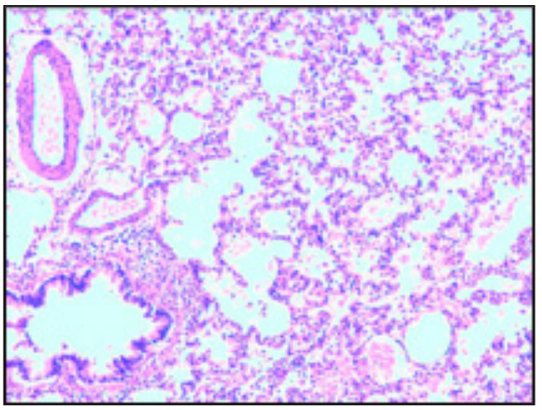

Fig. 4: Photomicrograph of lung showing red hepatisation with marked dilation and congestion of pulmonary vessel (arrow), extravasation of RBCs into the alveolar septa (Group 2, day 14): H&E × 100

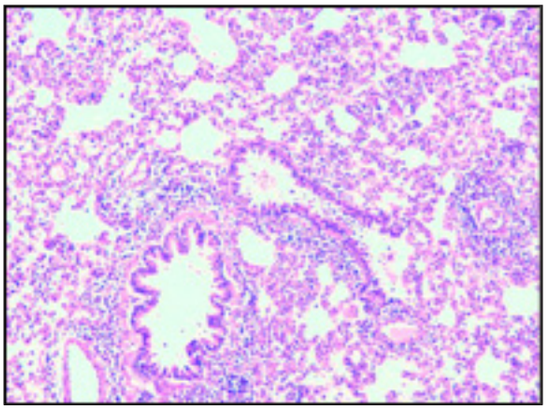

Fig. 5: Photomicrograph of lung showing leucocytic infiltration in perivascular and peribronchiolar area (Group 2, day 14): H&E × 100

These changes are principally due to selective accumulation of BLM in alveolar epithelial cells of lungs due to lack or lowest levels of the enzyme BLM hydrolase. On 28th day similar types of lesions with increased severity was noticed (Fig. 6 & 7). In addition, thickening of alveolar septa due to severe MNCs infiltration and hyperplasia of bronchiolar epithelium was noticed.

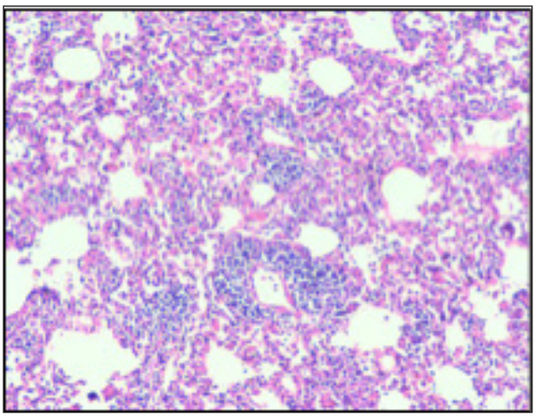

Fig. 6: Photomicrograph of lung showing distorted architecture of lung alveoli, haemorrhages, increased thickening of alveolar septa and severe infiltration (Group 2, day 28): H & E × 100

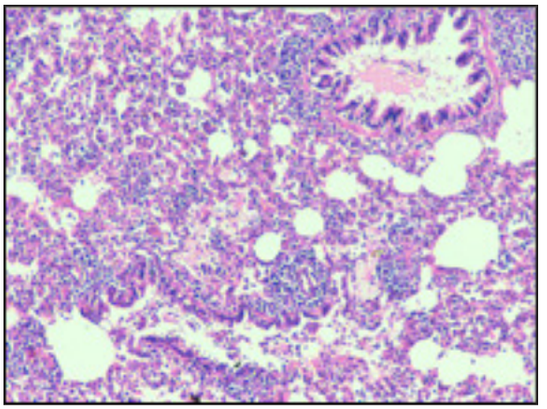

Fig. 7: Photomicrograph of lung showing complete obliteration of most of the lung alveoli due to severe infiltration of MNCs, interstitial pneumonia, severe bronchitis and MNCs infiltration in peribronchiolar areas (Group 2, day 28): H& E×100

Similar observations were reported by Zaghloul and Salem (2017) and Xu *et al*. (2019) in their experimental studies. ROS may be responsible for most of the BLM induced cellular damage that occurs in acute pulmonary injury. The lung sections of group 3 on day 14 appeared to be similar to that of group 2 sections but with mild degree of changes like focal areas of thickening of the alveolar

walls due to leucocytic infiltration and mild congestion of pulmonary vessels (Fig. 8), mild to moderate neutrophilic infiltration in the perivascular area and mild hyperplasia of the bronchiolar epithelium.

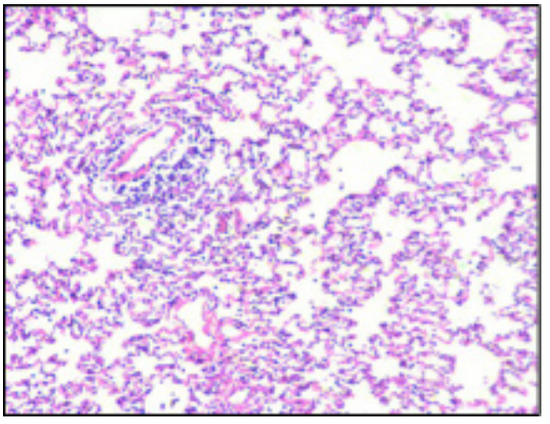

Fig. 8: Photomicrograph of lung showing mild perivascular leucocytic infiltration, mild thickening of inter alveolar septa and extravasation of erythrocytes into alvelolar spaces (Group 3, day 14): H&E × 100

The lung sections of group 3 on day 28 showed reconstructive appearance of alveolar epithelium. Additionally, mild alveolar wall thickening, mild emphysema and mild infiltration of MNCs within the lumen of alveoli and alveolar septa (Fig. 9) were observed.

The decrease in the severity of lesions might be due to presence of high concentration of anti oxidants and flavonols in POM juice. Earlier studies have shown that polyphenolic phytochemicals in the pomegranate play important role in the modulation of inflammatory cell signaling in colon cancer cells (Adams *et al*., 2006).

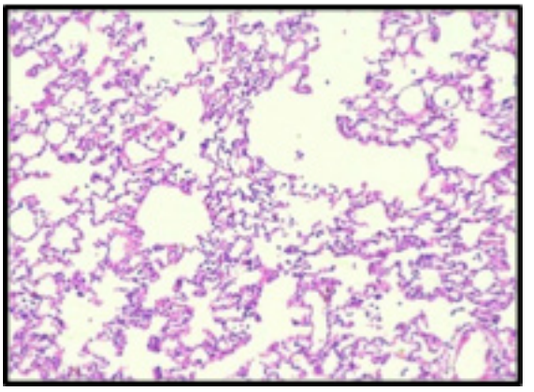

Fig. 9: Photomicrograph of lung showing mild emphysema and mild MNCs infiltration within the lumen of alveoli, alveolar septa and perivascular area (Group 3, day 28): H&E × 100

Lung injury induced by bleomycin showed significant increase of inflammatory cytokines TNF alpha and TGF beta and IL 10 in group 2 compared to other groups (Table 1).

Previous studies have reported significantly increased IL-10 levels during pulmonary inflammation and fibrosis and accounted increased IL-10 level to be a compensatory anti inflammatory response syndrome hypothesized to be increased and commensurate with the pro-inflammatory response (Doughty *et al.,* 1998). In the present study also to compensate the pro-inflammatory response, due to production and stimulation of TNF alpha and TGF-β_1 in the pulmonary tissue concurrent upregulation of IL-10 was noticed in the group 2. The findings in the present study are in agreement with earlier studies of Hou *et al.* (2018), Saghir *et al.* (2019). Significant elevation in the concentration of inflammatory cytokines might be

Table 1: Cytokines levels in lungs of different groups

	Parameters					
	TNF-*Alpha* (pg/mL tissue)		**TGF-β1 (pg/mL tissue)**		**IL-10 (pg/mL tissue)**	
	Day 14	**Day 28**	**Day 14**	**Day 28**	**Day 14**	**Day 28**
Group 1	227.35 ± 2.12^{c}	227.92 ± 1.59^{c}	246.34 ± 1.69^{c}	254.37 ± 2.05^{c}	20.87 ± 0.25^{c}	23.16 ± 0.32^{c}
Group 2	458.75 ± 5.36^{a}	637.27 ± 3.90^{a}	528.97 ± 2.02^{a}	620.01 ± 2.98^{a}	35.35 ± 0.40^{a}	43.17 ± 0.23^{a}
Group 3	349.73 ± 3.71^{b}	344.65 ± 3.70^{b}	328.37 ± 3.32^{b}	350.58 ± 2.60^{b}	25.85 ± 0.24^{b}	27.61 ± 0.60^{b}
Group 4	231.10 ± 4.04^{c}	232.46 ± 1.75^{c}	245.87 ± 2.08^{c}	252.76 ± 3.09^{c}	21.34 ± 0.25^{c}	23.09 ± 0.23^{c}

Values are Mean ± SE (n=6); One-way ANOVA Means with different superscripts in a column differ significantly at P<0.05 (*).

due to pulmonary inflammation and oxidative damage induced by BLM. Treatment with POM juice reduced the inflammation of lung tissue by significantly reducing the levels of inflammatory cytokines. These findings are in accordance with previous studies reporting anti-inflammatory effect of POM in different experimental models (Shara Francesca *et al*., 2021; Ritu *et al*., 2016).

CONCLUSION

It was concluded that POM juice inhibits bleomycin induced lung injury by suppressing inflammatory cytokines production.

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