Effect of bleomycin (BLM) induced toxicity on body weights and hematological parameters and protective role of whole pomegranate juice in rats

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Abstract
The ameliorative potential of whole Pomegranate fruit juice was studied against Bleomycin (BLM) induced toxicity on body weights (b.wts) and hematological parameters in rats. A total of 48 male albino Wistar rats were procured and divided into 4 groups consisting of 12 rats in each. The group 1 - control, group 2 - BLM [@ 5 mg/kg b.wt via single intra-tracheal (IT) instillation], group 3 - BLM [@ 5 mg/kg b.wt via single IT instillation] + Pomegranate juice (@ 1 mL/rat once daily orally) and group 4 - Pomegranate juice (@ 1 mL/rat once daily orally). The experiment was carried out for a period of 28 days and six rats from each group were sacrificed on 14th and 28th day of the experiment. Significantly (P<0.05) reduction in b.wts were recorded in group 2 rats. All the hematological parameters were significantly reduced except total leukocyte count in BLM treated rats.

Keywords: bleomycin, pomegranate juice, intra-tracheal instillation, body weight, hematology, wistar rats

Introduction
Pulmonary complications due to anti-cancerous therapy are common and are an important cause of respiratory failure (Sadowska et al., 2013) [6]. Bleomycin (BLM-C₅H₆N₇O₂S₅) is an antibiotic with antineoplastic (cytotoxic glycopeptide), antiviral and antibacterial water-soluble peptide extracted from fungus Streptomyces verticillus by Umezawa et al. (1966) [12]. BLM is commonly used (either alone or in combination with other chemotherapeutic agents) in the treatment of Kaposi’s sarcoma, cervical cancer, germ-cell tumors, squamous cell carcinomas of the head and neck and malignant pleural effusions (Sriram et al., 2009) [9]. However the major limitation of BLM in a dose-dependent manner is interstitial pneumonitis and pulmonary fibrosis (Bennett and Reich, 1979) [1]. The primary mechanism of action of BLM involves the drug's ability to oxidatively damage DNA by binding to metal ions, including iron, undergoes redox cycling by forming metalllobleomycin complexes and catalyzes the formation of reactive oxygen species (Verma et al., 2013) [3]. Free radicals, once generated, target biomacromolecules such as DNA, lipids and proteins with the ultimate progression of lipid peroxidation (LPO), resulting in damage to the lung (Liang et al., 2011) [4] and low concentration of bleomycin hydrolase, a member of the cysteine proteinase family in the skin and lung tissue has contributed to the hypothesis for the unique BLM sensitivity found in these sites (Dorr, 1992) [2].

Pomegranate have anti-oxidant, anti-cancerous, anti-atherosclerotic properties and is effective scavenger for free radicals due to its high levels of phenolic acids, flavonoids and polyphenolic compounds like punicalagins, gallic acid and ellagic acid derivatives (Pirincicoglu et al., 2014) [5]. Hence, the present experiment was designed to study the toxic effects of BLM and its amelioration with Pomegranate juice in male albino Wistar rats.

Materials and Methods

Experimental animals
A total of forty eight (48) male albino Wistar rats weighing between 180–220 g were procured from M/S Vyas labs (CPCSEA registered No. 17/22/C.V.Sc, Hyd/IAEC1), Hyderabad. The
experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC approval no. 19/22/C.V.Sc, Hyd/IAEC-Rats/29.02.2020).

Chemical source
BLM was procured from Mehdhi Nawaz Jung (MNJ) cancer hospital in Hyderabad under the trade name Bleocel 15 manufactured by CELON LABS (Celon laboratories Pvt. Ltd., Hyderabad) India.

Pomegranate juice preparation
Fresh Pomegranate fruits (*Punica granatum* Linnaeus) were obtained from local market, Hyderabad. They were washed, drained and cut into halves. The Pomegranate peel, all white pulpy part and seeds were together squeezed with electric blender. On daily basis, Pomegranate juice was prepared and administered through oral gavage to the experimental animals.

Experimental design
A total of 48 male albino *Wistar* rats were randomly divided into four (4) groups consisting of twelve (12) animals in each. Group 1 - Control (Saline @ 0.9 mL/rat/single dose/Oral) Group 2 - BLM Sulphate (BLM @ 5 mg/kg b.wt/single dose/IT instillation) Group 3 - 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) Group 4 – Whole Pomegranate fruit juice (@ 1 mL/rat once daily orally for 28 days).

Body weight (B.wt) gain (s)
Individual b.wts of all the rats were recorded by using electronic balance on day one (initial b.wt on 0\(^{th}\) day) soon after arrival and subsequently on 7\(^{th}\), 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) day of experiment to study the b.wt gains.

Haematology
Six (6) rats from each group were sacrificed on 14\(^{th}\) and 28\(^{th}\) day of experiment. On the day of sacrifice, 2-3 mL of blood was collected from retro-orbital plexus with the help of capillary tube in an anticoagulant coated vaccutainers (K3-EDTA tube, 13 mm x 75 mm, 4 mL. (Rapid Diagnostics Pvt. Ltd., Delhi)) to carry out all hematological parameters. Prior to blood collection, the selected experimental rats were put to fast for 12 hours. All the blood samples were used for estimation of Total Erythrocyte Count (TEC-millions/μL), Total Leukocyte Count (TLC-thousands/μL), Hemoglobin (Hb-g%) concentration, Packed Cell Volume/Haematocrit (PCV/Hct-%), Mean Corpuscular Hemoglobin (MCH-pg), Mean Corpuscular Volume (MCV-μL) and Mean Corpuscular Hemoglobin Concentration (MCHC-g/dL) by using automatic whole blood analyzer (Huma count, med source ozone biomedical Pvt. Ltd., Faridabad, Haryana) and results were tabulated for statistical analysis.

Statistical analysis
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) \(^{[8]}\).

**Results and Discussion**

**Effect of BLM on b.wts**
Significantly \((\text{\(P<0.05\)})\) higher mean values of weekly b.wts were recorded in control group and significantly lower mean values were observed in group 2 rats on 7\(^{th}\), 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) day respectively. This might be due to primary pulmonary inflammatory events, anorexia and oxidative stress at sub cellular level in different vital organs induced by BLM. This observation is in accordance with the earlier studies of Kandhare et al. (2015) \(^{[3]}\). Group 3 rats showed a significant increase in the mean values of b.wt gain during respective days of experiment when compared to that of group 2 rats, which could be due to the protective effect of Pomegranate juice (anti-inflammatory and anti-oxidative property) against BLM induced toxicity (Table 1).

**Effect of BLM on haematological parameters**
A significant \((\text{\(P<0.05\)})\) reduction in the mean values of Hb concentration, TEC, PCV, MCV, MCH and MCHC were recorded in group 2 rats when compared over all other groups (1, 3 and 4) on 14\(^{th}\) and 28\(^{th}\) day of the experiment. The mean values were significantly varied between group 1 and group 4 and mean values of group 3 showed a significant increase when compared to group 2 on 14\(^{th}\) and 28\(^{th}\) day of the experiment. It was opined that the BLM binds with Fe\(^{2+}\), thereby causing iron deficiency and anaemia directly, indirectly ROS induces stress in red blood corpuscles. According to Sharma (2018) \(^{[7]}\), organic peroxides and H\(_2\)O\(_2\) can be produced during BLM reaction leading to release of iron from the haeme protein results in decreased Hb concentration in the BLM group. The above hypothesis was positively correlated in the present study with the significantly decreased erythrocyte indices in group 2 when compared to group 1 rats.

A significant \((\text{\(P<0.05\)})\) increase in the mean values of TLC was observed in group 2 when compared with other groups of rats (1, 3 and 4). These observations were similar to the findings of Kandhare et al. (2015) \(^{[3]}\). BLM induced pulmonary toxicity and generation of ROS is associated with intense inflammatory process that leads to influx of neutrophils (1\(^{st}\) line of defence) into the alveoli (pneumonitis) within 3-7 days after that, chemotaxis of macrophages and lymphocytes within 6-30 days in to the alveoli that result in increased TLC in group 2. There was a significant improvement in the mean values of group 3 when compared with group 2. The significant improvement in haemograms could be due to Pomegranate juice being a rich source of iron, Vitamin A, C and E. The Vitamin C present in the Pomegranate juice enhances the absorption of iron and regulates the blood count (Teucher et al., 2004) \(^{[11]}\). In addition, anti-oxidant defense action of Pomegranate juice against free radical induced oxidative stress in different tissues including blood cells and protects cells against oxidative damage (Sudha, 2017) (Table 2).
Table 1: Weekly body weight gain (g) in different group (s)

| Group | On Day 7 | On Day 14 | On Day 21 | On Day 28 |
|-------|----------|-----------|-----------|-----------|
| Group 1 | 214.58 ± 0.89a | 224.75 ± 0.79a | 237.33 ± 0.88a | 248.50 ± 0.76a |
| Group 2 | 187.58 ± 0.99a | 183.50 ± 0.79a | 192.50 ± 0.76a | 202.17 ± 0.60a |
| Group 3 | 203.42 ± 0.43a | 207.42 ± 0.51a | 214.50 ± 0.76a | 225.33 ± 0.80a |
| Group 4 | 212.23 ± 0.61a | 216.67 ± 0.61b | 228.00 ± 0.58b | 236.17 ± 0.79b |

P Value  
* * * * *

Values are Mean ± SE (n=6) on day 7th and 14th, One-way ANOVA
Values are Mean ± SE (n=10) on day 21st and 28th, One-way ANOVA
Means with different superscripts in a column differ significantly at P<0.05(*)

Table 2: Haematological parameters in different group (s)

| Group | Days | 14th | 28th | 14th | 28th | 14th | 28th | 14th | 28th |
|-------|------|------|------|------|------|------|------|------|------|
| Group 1 | Hb (%) | 16.10±0.30 | 16.55±0.12c | 11.98±0.21d | 10.95±0.17d | 14.46±0.95c | 14.55±0.10c | 17.45±0.17c | 17.55±0.15c |
| Group 2 | TEC (mil/μL) | 8.01±0.20 | 8.28±0.15 | 5.58±0.14 | 5.13±0.17 | 6.76±0.58 | 7.36±0.17 | 9.10±0.15 | 9.41±0.15 |
| Group 3 | PCV/Hct (%) | 47.68±0.31 | 48.53±0.12 | 40.13±0.49 | 39.56±0.44 | 43.75±0.19 | 44.73±0.39 | 49.85±0.20 | 50.93±0.32 |
| Group 4 | TLC (thou/μL) | 8.41±0.12 | 8.26±0.15 | 17.11±0.35 | 16.68±0.22 | 12.63±0.19 | 12.81±0.17 | 7.83±0.25 | 8.23±0.13 |
| Group 1 | MCV (fl) | 52.93±0.21 | 53.51±0.10 | 42.65±0.14 | 41.38±0.39 | 48.53±0.27 | 50.63±0.25 | 57.81±0.17 | 58.10±0.24 |
| Group 2 | MCH (pg) | 16.53±0.18 | 16.81±0.17 | 13.71±0.17 | 13.28±0.24 | 15.10±0.15 | 15.56±0.13 | 17.11±0.15 | 18.51±0.87 |
| Group 3 | MCHC (g/dL) | 33.01±0.14 | 34.30±0.13 | 31.48±0.13 | 30.45±0.11 | 32.20±0.11 | 32.53±0.12 | 35.11±0.20 | 35.16±0.27 |

P Value  
* * * * *

Values are Mean ± SE (n=6) on day 14th and 28th; One-way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*)

Conclusion
The BLM (5 mg/kg b.wt) causes significant reduction in b.wts and marked decrease in haematological parameters by free radical induced damage and Pomegranate juice is a potent antioxidant and anti-inflammatory capable of attenuating the toxic effects of BLM.

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