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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-C₅₅H₈₄N₁₇O₂₁S₃) 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) [11]. 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 metalbleomycin complexes and catalyzes the formation of reactive oxygen species (Verma *et al.*, 2013) [13]. 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 (Pirinccioglu *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 Mehdi 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 0th day) soon after arrival and subsequently on 7th, 14th, 21st and 28th day of experiment to study the b.wt gains.

Haematology

Six (6) rats from each group were sacrificed on 14th and 28th 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 vacutainers {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/ μ L), Total Leukocyte Count (TLC-thousands/ μ L), Hemoglobin (Hb-g%) concentration, Packed Cell Volume/Haematocrit (PCV/Hct-%), Mean Corpuscular Hemoglobin (MCH-pg), Mean Corpuscular Volume (MCV-fL) 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 ($P < 0.05$) higher mean values of weekly b.wts were recorded in control group and significantly lower mean values were observed in group 2 rats on 7th, 14th, 21st and 28th 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 ($P < 0.05$) 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 14th and 28th 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 14th and 28th 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_2O_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 ($P < 0.05$) 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 (1st 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 ± 0.89 ^a	224.75 ± 0.79 ^a	237.33 ± 0.88 ^a	248.50 ± 0.76 ^a
Group 2	187.58 ± 0.99 ^d	183.50 ± 0.79 ^d	192.50 ± 0.76 ^d	202.17 ± 0.60 ^d
Group 3	203.42 ± 0.43 ^c	207.42 ± 0.51 ^c	214.50 ± 0.76 ^c	225.33 ± 0.80 ^c
Group 4	212.25 ± 0.61 ^b	216.67 ± 0.67 ^b	228.00 ± 0.58 ^b	236.17 ± 0.79 ^b
P Value	*	*	*	*

Values are Mean ± SE (n=12) on day 7th and 14th; One-way ANOVA

Values are Mean ± SE (n=6) on day 21st and 29th; 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	Group 1		Group 2		Group 3		Group 4	
	14 th	28 th	14 th	28 th	14 th	28 th	14 th	28 th
Hb (%)	16.10±0.36 ^b	16.55±0.12 ^b	11.98±0.21 ^d	10.95±0.17 ^d	14.46±0.95 ^c	14.55±0.10 ^c	17.45±0.17 ^a	17.55±0.15 ^a
TEC (mil/μL)	8.01±0.20 ^b	8.28±0.15 ^b	5.58±0.14 ^d	5.13±0.17 ^d	6.76±0.58 ^c	7.36±0.17 ^c	9.10±0.15 ^a	9.41±0.15 ^a
PCV/Hct (%)	47.68±0.31 ^b	48.53±0.12 ^b	40.13±0.49 ^d	39.56±0.44 ^d	43.75±0.19 ^c	44.73±0.39 ^c	49.85±0.20 ^a	50.93±0.32 ^a
TLC (thou/μL)	8.41±0.12 ^c	8.26±0.15 ^c	17.11±0.35 ^a	16.68±0.22 ^a	12.63±0.19 ^b	12.81±0.17 ^b	7.83±0.25 ^c	8.23±0.13 ^c
MCV (fL)	52.93±0.21 ^b	53.51±0.10 ^b	42.65±0.14 ^d	41.38±0.39 ^d	48.53±0.27 ^c	50.63±0.25 ^c	57.81±0.17 ^a	58.10±0.24 ^a
MCH (pg)	16.53±0.18 ^b	16.81±0.17 ^b	13.71±0.17 ^d	13.28±0.28 ^d	15.10±0.15 ^c	15.56±0.13 ^c	17.71±0.15 ^a	18.51±0.87 ^a
MCHC (g/dL)	33.01±0.14 ^b	34.30±0.13 ^b	31.48±0.13 ^d	30.45±0.11 ^d	32.20±0.11 ^c	32.53±0.12 ^c	35.11±0.20 ^a	35.16±0.27 ^a
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 anti-inflammatory and anti-oxidant activity capable of attenuating the toxic effects of BLM.

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References

- Bennett JM, Reich SD. Drugs five years later: bleomycin. *Annals of Internal Medicine* 1979;90(6):945-948.
- Dorr RT. Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. In *Seminars in Oncology* 1992;19(2):3-8.
- Kandhare AD, Bodhankar SL, Mohan V, Thakurdesai PA. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. *Chemico-biological Interactions* 2015;237:151-165.
- Liang X, Tian Q, Wei Z, Liu FE, Chen J, Zhao Y *et al.* Effect of Feining on bleomycin-induced pulmonary injuries in rats. *Journal of Ethnopharmacology* 2011;134(3):971-976.
- Pirinccioglu M, Kizil M, Kanay Z, Ketani A. The protective role of pomegranate juice against carbon tetrachloride-induced oxidative stress in rats. *Toxicology and Industrial Health* 2014;30(10):910-918.
- Sadowska AM, Specenier P, Germonpre P, Peeters M. Antineoplastic therapy-induced pulmonary toxicity. *Expert Review of Anticancer Therapy* 2013;13(8):997-1006.
- Sharma DK. Pathology of bleomycin induced lung injury in mice with special reference to its amelioration with baicalein [Doctoral dissertation, University of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana] 2018.
- Snedecor GW, Cochran WG. *Statistical Methods*. 8th Edn. IOWA State University Press. Ames, Iowa, USA, 1994.
- Sriram N, Kalayarasan S, Sudhandiran G. Epigallocatechin-3-gallate augments antioxidant activities and inhibits inflammation during bleomycin-induced experimental pulmonary fibrosis through Nrf2-Keap1 signaling. *Pulmonary Pharmacology and Therapeutics* 2009;22(3):221-236.
- Sudha V. Paraquat (PQ) induced toxicity and its amelioration with Pomegranate Seed Extract (PSE) in male albino Wistar rats (Doctoral dissertation, PV Narsimha Rao Telangana Veterinary University) 2018.
- Teucher, Olivares, Cori. Enhancers of iron absorption: ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research* 2004;74(6):403-419.
- Umezawa H, Maeda K, Takeuchi T, Okami Y. New antibiotics, bleomycin A and B. *The Journal of Antibiotics* 1966;19(5):200-209.
- Verma R, Kushwah L, Gohel D, Patel M, Marvania T, Balakrishnan S. Evaluating the ameliorative potential of quercetin against the bleomycin-induced pulmonary fibrosis in Wistar rats. *Pulmonary Medicine* 2013; doi: <http://dx.doi.org/10.1155/2013/921724>.