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Haemato-protective effect of pomegranate juice against coexposure of Isoniazid, Rifampicin and Pyrazinamide in rats

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Abstract
The protective effect of pomegranate juice (fruit juice extract) were studied against anti-TB drugs (INH+RIF+PZA) induced haematological alterations. A total of twenty four male wistar albino rats of 3 months age were procured for the study. The rats were randomly divided into four groups, consisting of six in each group. Isoniazid @27mg/kg BW, Rifampicin @54mg/kg BW and Pyrazinamide @135mg/kg BW were administered daily orally to groups 2, 3, &4 from day 1 to 28. Group 1 was maintained as normal control. Group 2 was kept as toxic control (administered anti-TB drugs, p/o). Groups 3 and 4 were administered (p/o) with Enalapril @5mg/kg BW and Punica granatum (fresh juice extract) @1ml/rat, respectively from day 1 to 28. Body weights were measured, whole blood collection done on day 14th and 28th for estimation of haematology. The results of haematological parameters in the present study revealed that, the means of TEC, TLC, Hb and PCV were significantly ($p<0.05$) reduced in group 2 and increased significantly ($p<0.05$) in groups 3 & 4 at different time intervals. In conclusion, Punica granatum was found to possess haemoprotective action and it was comparable to enalapril. The beneficial effects of pomegranate juice extract could be attributed to antioxidant actions of the phytoconstituents.

Keywords: Pomegranate juice, Isoniazid, Rifampicin and Pyrazinamide TEC, WBC, Hb and PCV

Introduction
Tuberculosis (TB) is a disease of zoonotic importance in human beings and all species of animals. Advanced studies were carried out previously in treating and curing the disease and suggested that the first line of antituberculous drugs like Isoniazid, Rifampin, Pyrazinamide and Ethambutol are the main treatment strategies of active tuberculosis. TB treatment may cause significant hematological disorder and also have serious side effects apart from other toxic effect [1]. Prolong usage of combination of these drugs may cause abnormalities of red cells, white cells, platelets, and clotting factors. The aim of this study was to determine hematoprotective effect of Pomegranate juice against coexposure of TB drugs in rats.

Material and Methods
The experimental study was conducted in male Wistar albino rats of uniform age (about 3 months) were procured from Jeeva life science Pvt. Limited, Hyderabad.

Drugs
a) Isoniazid Tablets (Macleods Pharmaceuticals Pvt. Ltd., Kachigam, Daman)
b) Rifampicin Capsules (LUPIN Ltd., Chikalthana, Aurangabad)
c) Pyrazinamide Tablets (LUPIN Ltd., Kartholi, Bar Brahmana, Jammu)
d) Enalapril Tablets (Dr. Reddy's Laboratories Ltd., Khol, Baddi, H.P)
e) Pomegranate Fruit (Local Market)

A total of 24 male *Wistar albino* rats of 3 months old were procured and acclimatized at least 1 week for the study. The rats were randomly divided into 4 groups consisting of 6 rats in each and kept in polypropylene cages and maintained with 12 hour dark/light cycle at college animal house. All the rats were provided with feed and water *ad libitum* throughout the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (I/2018-31/IAEC/CVSc., Hyd, Dated 16/07/2018). All the groups were maintained as per the following treatment schedule for 28 days.

Experimental design

The rats were randomly divided into four groups, consisting of six in each group. Isoniazid @27mg/kg BW, Rifampicin @54mg/kg BW and Pyrazinamide @135mg/kg BW were control. Group 2 was kept as toxic control (administered anti-TB drugs, p/o). Groups 3 and 4 were administered daily orally to groups 2,3,&4 from day 1 to 28. Group 1 was maintained as normal administered (p/o) with Enalapril @5mg/kg BW and Punica granatum (fresh juice extract) @1ml/rat, respectively from day 1 to 28. Blood collection was carried out on day 14th (24 h after the last dose of anti- TB Drugs) and 28th (24h after the last treatment dose) in heparinized vials from the rats in each group for hematological parameters and haematology was done by RMD Cell Count-1600 Automatic Hematology Analyzer.

Results

Total erythrocyte count (TEC)

The TEC (10^6/µl) in the group 2 (5.51±0.26 and 4.04±0.28, respectively) was significantly ($p<0.05$) lower when compared to group 1 (9.36±0.12 and 8.72±0.34, respectively) during 14th and 28th day. Treatment groups 3 (6.40±0.28 and 7.53±0.15, respectively) and 4 (6.67±0.23 and 7.99±0.22, respectively) revealed a significant ($p<0.05$) improvement in the total erythrocyte count at different time intervals in comparison to group 2. There was time-related reduction in the value of toxic control group 2 as there was significant ($p<0.05$) difference on day 28 when compared to day 14. The values of groups 3 and 4 were comparable without any significant difference.

White blood cell (WBC) count

The WBC (103/µl) in group 2 (10.52±0.40 and 9.12±0.17, respectively) was significantly ($p<0.05$) higher in comparison to group 1 (15.78±0.23 and 15.66±0.35, respectively) during 14th and 28th day, while groups 3 (12.58±0.48 and 13.06±0.47, respectively) and 4 (12.98±0.39 and 13.85±0.45, respectively) showed significantly ($p<0.05$) lower values in comparison to group 2. There was time-related reduction in the value of toxic control group 2 as there was significant ($p<0.05$) difference on day 28 when compared to day 14. The values of groups 3 and 4 were comparable without any significant difference.

Haemoglobin (Hb) concentration

The mean Hb (g/dl) concentration in group 2 (12.41±0.34 and 11.50±0.65, respectively) did not differ significantly as compared to groups 1 (14.01±0.17 and 14.49±0.22, respectively), 3 (13.04±0.15 and 13.62±0.35, respectively) and 4 (13.06±0.37 and 13.85±0.36, respectively) during 14th and 28th day. The results of the one way ANOVA of group 2 showed significant ($p<0.05$) decrease in Hb concentration in comparison to group 1, whereas treatment groups 3 and 4 showed significant ($p<0.05$) improvement on 14th and 28th day. The values of groups 3 and 4 were comparable without any significant difference.

Packed cell volume (PCV)

The mean PCV (%) in group 2 (39.59±0.71 and 38.48±0.72, respectively) was significantly ($p<0.05$) lower as compared to group 1(43.02±0.63 and 42.84±0.45, respectively) at 14th and 28th day. The treatment groups 3 (42.68±0.39 and 42.50±0.53, respectively) and 4 (43.25±0.24 and 43.16±0.44, respectively) showed significant ($p<0.05$) improvement and the values were comparable to group I at respective time intervals. The values of groups 3 and 4 were comparable without any significant difference.

Discussion

The effect of antituberculous drugs on blood haematology was assessed, and TEC, TLC, Hb and PCV in toxic control group (2) were significantly lowered, as compared to other groups at respective time intervals (14th and 28th day). Decrease in Hb in toxic group 2 may be due to destruction of RBCs or hemolysis resulted due to toxic metabolites of these drugs and induced oxidative stress leading to peroxidation of lipids causing disruption of the membrane lipid functions. These findings are in agreement with the report of Sharma *et al* [2] suggesting that the change in the shape of erythrocytes, increase in neutrophil count and decrease in lymphocytes could be due to peroxidation of lipids and response of body against foreign toxic substances. In our investigation, the TLC was decreased in toxic control group and these findings are in correlation with Thatoi and Khadanga [3, 4] who reported decreased haematological indices in the patient, who received therapy against tuberculosis and suggested that the tumor necrosis factor–α (TNF-α), and other cytokines released by activated monocytes suppress the erythropoietin production leading to anaemia. Shareef [5] also reported anaemia in several tuberculosis patients.

However, these findings are in contrast with the studies of Arundhathi *et al*. [6], who reported a significant elevation in the leucocytic count suggesting the improved phagocytic activity of the blood cells leading to improved immune status and further they reported that the significant rise in WBC count could possibly due to the stimulation of immune system against the invading antigens and also to an IL-1β mediated rise in the respective colony stimulating factors.

The treatment groups 3 and 4 revealed significant improvement in the haematological parameters and the present results suggest that improvement in the haematological parameters might be due to restoration of antioxidant activity. It was demonstrated that the flavonoids present in the PJ served an important role in preventing oxidation of hemoglobin by various factors, such as production of hypochlorous acid. Flavonoids bind to haemoglobin and inhibit oxidation of the haemoglobin molecule by oxidizing agents. In addition to flavonoids, PJ exhibits antioxidant activity that is much higher than that of red wine, green tea and other natural juices [7]. Antioxidant capacity in erythrocytes has been identified to be increased following two weeks of PJ supplementation in healthy individuals [8]. Therefore, the increased haemoglobin levels observed in the current study may be due to the phytochemicals contained in PJ juice have protected haemoglobin from the oxidizing species. PCV also exhibited a significant increase in PJ treated group. The high concentration of antioxidants in PJ is likely to have protected RBC and resulted in their subsequent reduced destruction. Similarly, Biu *et al*. [9] reported the protective effect of neem extract against haemotoxicity in hepatotoxic rats. In conclusion, the results of the present investigation enunciated that Pomegranate juice extract revealed haemoprotective might be due to active phytoconstituent of juice extract.

Table 1: Haematological alteration in different groups of rats

Group	TEC (106/µl)		WBC (103/µl)		Hb(g%)		PCV (%)	
	14th Day	28th Day	14th Day	28th Day	14th Day	28th Day	14th Day	28th Day
1. Control	9.36±0.12 c	8.72±0.34 c	15.78±0.23 ab	15.66±0.35 ab	14.01±0.17 cb	14.49±0.22 cb	43.02±0.63 c	42.84±0.45 c
2. INH+RIF+PZA	5.51±0.26 aA	4.04±0.28 Ab	10.52±0.40 cA	9.12±0.17 cB	12.41±0.34 a	11.50±0.65 a	39.59±0.71 a	38.48±0.72 a
3. INH+RIF+PZA+ENA	6.40±0.28 b	7.53±0.15 b	12.58±0.48 b	13.06±0.47 b	13.04±0.15 b	13.62±0.35 b	42.68±0.39 b	42.50±0.53 b
4. INH+RIF+PZA+PMJ	6.67±0.23 b	7.99±0.22 b	12.98±0.39 b	13.85±0.45 b	13.06±0.37 b	13.85±0.36 b	43.25±0.24 b	43.16±0.44 b

Values are Mean ± SE (n=6); One way ANOVA (SPSS) Means with different alphabets as superscripts differ significantly ($p<0.05$) among the groups at respective time intervals.
Alphabets with lowercase represent vertical comparison Alphabets with uppercase represent horizontal comparison

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