

**PROTECTIVE EFFECT OF BEE PROPOLIS AGAINST ANTI-TUBERCULOSIS DRUGS (RIFAMPICIN AND ISONIAZID)-INDUCED HEMATOLOGICAL TOXICITY IN SPRAGUE DAWLEY RATS**UMESH BHARTI<sup>1</sup>, NEELIMA R KUMAR<sup>2</sup>, JASPREET KAUR<sup>3</sup><sup>1</sup>Department of Zoology, PG Govt. College for Girls, Sector-11, Chandigarh, Punjab, India. <sup>2</sup>Department of Zoology, Panjab University, Chandigarh, Punjab, India. <sup>3</sup>Department of Biotechnology, UIET, Panjab University, Chandigarh, Punjab, India. Email: dbhv90@gmail.com

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**ABSTRACT****Objective:** Protective effect of bee propolis against anti-tuberculosis (TB) drugs (rifampicin and isoniazid)-induced hematological toxicity in Sprague Dawley (SD) rats.**Methods:** Experimental male SD rats weighing 180±20 g were randomly assigned into eight groups (n=6), the Group 1 served as control; Group 2 received 200 mg bee propolis/kg body weight; Groups 3, 5, and 7 were treated with drugs 100 mg rifampicin/kg body weight, 50 mg isoniazid/kg body weight, and 100 mg rifampicin+50 mg isoniazid/kg body weight, respectively. Groups 4, 6, and 8 were treatment groups receiving 200 mg bee propolis/kg body weight+100 mg rifampicin/kg body weight, 200 mg bee propolis/kg body weight+50 mg isoniazid/kg body weight, and 200 mg bee propolis/kg body weight+100 mg rifampicin+50 mg isoniazid/kg body weight, respectively. All the treatments were given for 30 days, and then, the rats were sacrificed under light esthesia by cervical dislocation and blood was collected for physiological studies.**Results:** Bee propolis supplementation (200 mg/kg body weight) showed increased level of hemoglobin with respect to rifampicin (15.45%), isoniazid (11.34%), and rifampicin plus isoniazid (5.04%) administered groups after 30 days of treatment. Moreover, the decreased level of red blood cell count and white blood cell count by anti-TB drugs rifampicin, isoniazid, and rifampicin plus isoniazid together was also elevated in treatment group with bee propolis.**Conclusion:** Coadministration of propolis (200 mg bee propolis/kg body weight) with drugs helped modulate the toxic effects by restoring tested values to near normal.**Keywords:** Propolis, Rifampicin, Isoniazid, Hemoglobin, Red blood cell count, White blood cell count.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i3.15991>**INTRODUCTION**

Propolis (bee glue) by honey bees from the bark of the trees and flower buds is an adhesive, resinous substance which is transformed with bee wax and salivary secretions and used by honey bees to seal crevices in their hive and prevent the entry of intruders. Bee propolis is rich in phenols, tannins, polysaccharides, flavonoids, terpenes, aromatic acids, and aldehydes. Propolis has imperative pharmacological properties which can be used for a wide range of purposes as anti-inflammatory, antioxidant, and antibacterial agents [1]. Various plant derived drugs (such as *Pongamia pinnata*, *Embllica officinalis*, and *Rubia cordifolia*) were reported to be the best alternative drug for the anti-tuberculosis (TB) drug-induced toxicity [2,3].

TB is a communicable disease and is a major global health problem. In 2014, 9.6 million new TB cases were reported, out of which 5.4 million were men, 3.2 million were women, and 1.0 million were children. There were 1.5 million TB deaths [4]. Drugs used for the treatment of TB have been reported to cause major adverse reactions and significant morbidity leading to a compromised treatment regimen. Side effects such as hepatitis, dyspepsia, exanthema, and arthralgia were responsible for termination of therapy in up to 23% of patients during the intensive phase [5]. Several studies have reported that leukopenia, eosinophilia, hemolytic anemia along with hepatotoxicity, fatigue, dizziness, headache, and dyspnea, occurred after rifampicin and isoniazid administration [6] (Baciewicz *et al.*, 2008). Regular education and monitoring of patients were also required to control the disease in developing nations [7]. The aim of this study was to evaluate the protective effect of dietary bee propolis supplementation against

toxicity induced by anti-TB drugs rifampicin and isoniazid in Sprague Dawley (SD) rats.

**METHODS****Experimental animals**

Male SD rats of body weight in the range of 180±20 g were obtained from the central animal house of Panjab University, Chandigarh, vide institutional animal ethics committee approval letter no. PU/IAEC/2013/18. Animals were kept in polypropylene cages bedded with rice husk in 12 hrs light/dark cycle under hygienic conditions. They were fed with standard pellet feed and water *ad libitum*.

**Collection and preparation of bee propolis extract**

Bee propolis was collected from Langstroth bee hives maintained in an apiary at Majri village near Old Panchkula, Haryana. Ethanolic extract of propolis was prepared.

**Drugs**

Rifampicin and isoniazid drugs were purchased from Himedia.

**Experimental protocol**

The animals (male SD) were randomly assigned into eight groups of six rats each and treated as follows:

- Group A: Control group with normal water
- Group B: 200 mg bee propolis/kg body weight
- Group C: 100 mg rifampicin/kg body weight
- Group D: 200 mg bee propolis/kg body weight+100 mg rifampicin/kg body weight

- Group E: 50 mg isoniazid/kg body weight
- Group F: 200 mg bee propolis/kg body weight+50 mg isoniazid/kg body weight
- Group G: 100 mg rifampicin+50 mg isoniazid/kg body weight
- Group H: 200 mg bee propolis/kg body weight+100 mg rifampicin+50 mg isoniazid/kg body weight.

Oral gavages using a metal oropharyngeal cannula and syringe were employed in the administration of the bee propolis extract, rifampicin, and isoniazid once daily for 30 days. The animals were sacrificed by cervical dislocation after 30 days of treatment.

### Blood collection

Blood samples were taken from each rat by terminal bleeding from the vena cava with syringe rinsed with EDTA and transferred into a sterile EDTA container.

### Hemoglobin estimation

#### Principle

Hemoglobin reacts with N/10 HCl and gets converted into golden brown acid hematin. The intensity of color depends on the amount of hemoglobin in the blood. Sahli's hemoglobin meter was used to estimate hemoglobin (g/dl) [8].

### Red blood cell count (RBC count)

#### Principle

Blood is diluted 200 times with the help of RBC diluting fluid (Hayem's solution). Neubauer hemocytometer was used to count the number of RBCs under light microscope [8]. The final result was expressed as the number of cells per cumm of blood using the formula below:

$$\text{RBC count} = \frac{\text{Number of red blood cells counted} \times \text{Dilution factor} \times 4000}{80(\text{Number of small squares counted})}$$

### Total white blood cell (WBC) count

#### Principle

The blood is diluted 20 times with the help of WBC diluting fluid (Turk's Fluid). Neubauer hemocytometer was used to count WBC's under light microscope [8]. The number of cells present in the 4 corner areas (1 mm<sup>2</sup> each) was counted.

$$\text{WBC count} = \frac{\text{Number of cells counted} \times \text{Dilution factor} \times 10}{4(\text{Number of 1 squares ml. counted})}$$

### Statistical analysis

Data were presented as mean±standard deviation and analyzed using one-way analysis of variance. p<0.05 were considered statistically significant.

## RESULTS AND DISCUSSIONS

### Effect of bee propolis on hemoglobin

Hemoglobin level of the animals given bee propolis extract (200 mg/kg body weight) was 14.52±0.17, which was lower than the control group (15.1±0.18) at p<0.005. A significant decrease (23.70%) in the level of hemoglobin was observed with (100 mg/kg body weight) rifampicin, there was 18.34% decrease in isoniazid (50 mg/kg body weight), and 12.05% decrease in combination of rifampicin (100 mg/kg body weight) plus isoniazid (50 mg/kg body weight)-treated groups.

When, however, the treatment was supplemented with bee propolis (200 mg/kg body weight) drastic increase was noticed with respect to rifampicin (15.45%) 100 mg/kg body weight, isoniazid (11.34%) 50 mg/kg body weight, and rifampicin plus isoniazid (5.04%) 100 mg/kg+50 mg/kg body weight administered groups after 30 days of treatment. The results were expressed as mean ±standard deviation

(n=6). The data were analyzed using one-way analysis of variation (Fig. 1).

### Effect of bee propolis on RBC count

The RBC count of control rat was 8.15±0.21 million/cumm. When anti-TB drugs were given, significant decrease was observed, which was 4.19% with rifampicin, 4.05% with isoniazid, and 3.81% with rifampicin plus isoniazid together. On treatment with bee propolis at a dose of 200 mg/kg body weight with rifampicin (100 mg/kg body weight), the level were increased to 7.39±0.14 million/cumm, with isoniazid (50 mg/kg body weight) 7.65±0.21 million/cu mm and with rifampicin and isoniazid (100 mg/kg+50 mg/kg body weight) together red blood cell count was 7.735±0.21 million/cumm.

Rise in RBC count on treatment with bee propolis was statistically significant at p≤0.05 with respect to rifampicin, isoniazid, and rifampicin and isoniazid together as shown in the Fig. 2. The results were expressed as mean±standard deviation (n=6). The data were analyzed using one-way analysis of variation.

### Effect of bee propolis on WBC count

The total leukocyte count (TLC) for normal rat was found to be 18925±250. Leukopenia was observed in drug-treated rats with decrease of 37.78% (rifampicin), 26.81% (isoniazid), and 37.51% (rifampicin and isoniazid together).

After 30 days of treatment with 20 mg/kg body weight of bee propolis significant increase of 22.92% (100 mg/kg body weight of rifampicin), 22.90% (50 mg/kg body weight of isoniazid), and 45.87% (rifampicin, 100 mg/kg body weight + isoniazid 50 mg/kg body weight together) was seen in WBC count. The data were statistically significant at p≤0.05 as shown in Fig. 3. The results were expressed as mean±standard deviation (n=6). The data were analyzed using one-way analysis of variation.

TB is a leading health problem in developing countries and is the major cause of death worldwide (almost 40% of the world's TB cases). Rifampicin and isoniazid are important first-line drugs used against TB [4]. Since the drugs used in treatment of TB have been reported to show toxic side effects (hypersensitivity reactions, hepatitis, acute interstitial nephritis neuropsychiatric, hepatic, and gastrointestinal problems in patients with chronic renal failure) [9], studies have been performed to attempt to reduce the toxicity using various herbal products such as vitamin E [9], silymarin [10], ocimum [11], bee pollen [8], and garlic [12]. The polyphenolic compounds present in propolis [13] have protective effect for the RBC cell membrane [14]. Administration of propolis to diabetic mice significantly elevated hematological parameters such as the total number of red blood cells,

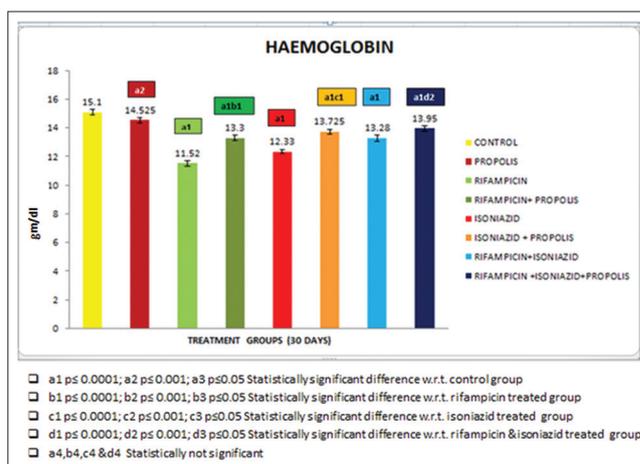


Fig. 1: Effect on hemoglobin level in eight treatment groups (n=6) (mean±standard deviation)

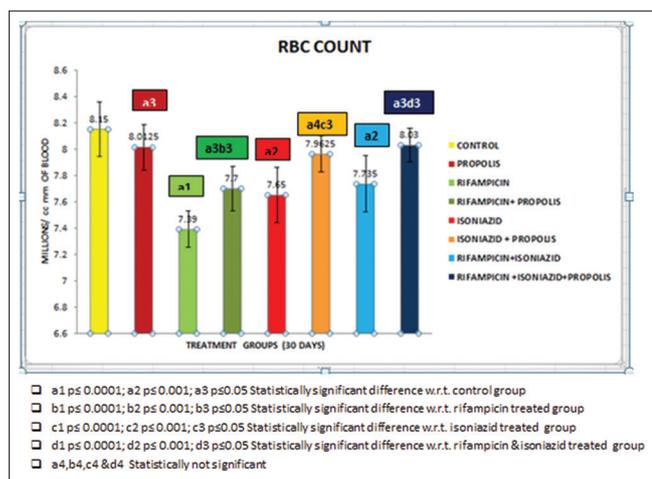


Fig. 2: Effect on red blood cell count in different treatment groups (n=6) (mean±standard deviation)

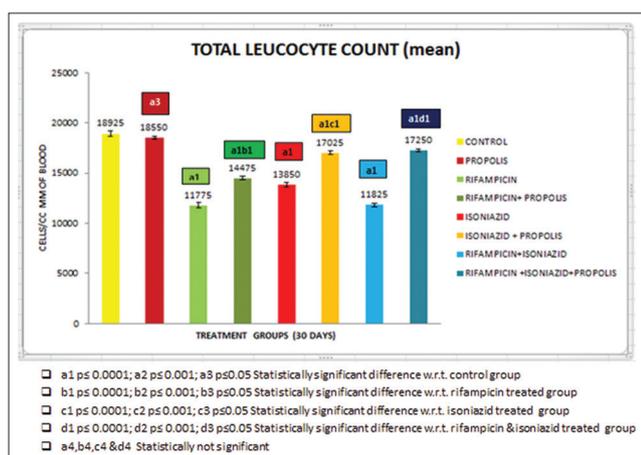


Fig. 3: Effect on total leukocyte count in different treatment groups (n=6) (mean±standard deviation)

hemoglobin, and hematocrit level [15]. Antioxidant and free-radical scavenging properties of propolis [16] due to the presence of flavonoids and phenolic antioxidants were responsible for protective effect against anti-TB drugs. Increased hemoglobin level, RBC count and WBC count revealed in the present study therapeutic properties of propolis against anti-TB drugs rifampicin and isoniazid toxicity.

## CONCLUSION

The generation of hematological response was calculated through hemoglobin estimation, RBC count and TLC in different groups of SD rats. Anemia was detected in anti-TB drug-treated animals. There was

severe decline in hemoglobin count, RBC count, and WBC count when animals were treated with rifampicin, isoniazid, and rifampicin and isoniazid in combination. On supplementing bee propolis with anti-TB drugs, significant escalation in the level of hemoglobin, RBC count and WBC count were recorded. The treatment with propolis could recover cellular membrane and organ functioning more profoundly, thereby maintaining all hematological parameters near normal values. This supports the hypothesis that bee propolis shows protective effect by normalizing the levels of treated blood parameters.

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