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HEMATOLOGICAL PROFILE OF RATS ADMINISTERED WITH PROPOLIS FOLLOWING ANTITUBERCULOSIS DRUGS INDUCED TOXICITY

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ABSTRACT

Antituberculosis drugs (ATD) used as standard drugs for the treatment of deadly disease tuberculosis, cause blood disorders. The present study was conducted to investigate the efficacy of propolis against ATD induced alteration in blood parameters in rats. Rats were administered with ATD for 8 weeks (3 days/week) followed by propolis at three different doses (100, 200 and 400 mg/kg) conjointly for 8 weeks (3 days/week) orally. Silymarin (50 mg/kg) was used as positive control in the study. After 8 weeks, animals were euthanized; blood was collected by retro-orbital sinus method for analysis of hematological parameters. The results of this study show a decrease in red blood cells, hemoglobin, hematocrit, mean corpuscular volume, red blood cell distribution width alongwith increase in the number of lymphocytes in ATD induced rats. Treatment with propolis extract encountered ATD induced blood parameter alteration which was evident by significant reversal in hematological indices towards control in a dose dependent manner. Thus, it can be concluded that propolis may be an agent of therapeutic choice in case of ATD induced hematological alterations.

Figure : 00 References : 18 Table : 01

KEY WORDS : Antituberculosis drugs, Hematological parameters, Propolis, Rats

Introduction

World Health Organization (WHO) declared tuberculosis (TB) as one of the leading cause of death from an infectious disease after the HIV worldwide[7]. A multi-therapy of first-line anti-tuberculosis drugs isoniazid (H), pyrazinamide (Z), rifampicin (R) and ethambutol (E) are used for treatment of tuberculosis, for 2 months followed by a combination of isoniazid/rifampicin for 8 months[16]. These ATDs can cause various minor and major adverse reactions in the body with the varying degree of severity[4]. The most frequent adverse effects include skin reactions, gastrointestinal disorder, neurological disorder, vertigo and the most fatal one is hepatotoxicity[2]. Less scientific information is available on ATD induced alterations in hematological parameters. Thus, present study was undertaken to investigate therapeutic potential of propolis against anti-tuberculosis drugs induced alterations in hematological parameter.

Natural remedies from medicinal plants and natural products are considered to be effective and safe alternative treatment for blood borne diseases. Propolis is a natural substance produced by honey bees (*Apis mellifera* L.) from resins, balms and gums; it is a complex resinous mixture of salivary bee secretions and wax[3]. The chemical composition of propolis sample is quite complex and over 300 different chemical compounds have been identified from various parts of the world[9]. An important part of biological and pharmacological activities of propolis is due to the presence of high content of polyphenolic content such as phenolic acids and flavonoids[12]. It has a wide range of biological activities such as anti-carcinogenic[1], antioxidative[17], immunomodulatory[5] and hepatoprotective[18], wound healing properties[13]. Thus it was hypothesised that propolis may be helpful in ameliorates anti-tuberculosis drugs induced alteration in blood parameters in rats.

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TABLE -1: Therapeutic effect of propolis on blood parameters against anti-tuberculosis drugs induced toxicity.

Groups	RBC(10^6/mm^3)	Hb(g/dL)	HCT(%)
Control	7.30±0.39	15.3±0.91	38.9±2.61
Propolis *per se*	7.40±0.45	15.2±0.91	38.6±2.56
ATD	6.39±0.39	12.2±0.68^{ø}	31.2±2.85
ATD+ Propolis 100 mg/kg	6.59±0.50	14.3±0.84	34.1±2.84
ATD+ Propolis 200 mg/kg	6.94±0.35	13.6±0.84	34.8±2.77
ATD+ Propolis 400 mg/kg	7.10±0.45	14.2±0.79	35.3±2.93
ATD+ Silymarin 50 mg/kg	7.20±0.43	15.0±1.11*	38.1±2.76
ANOVA	0.92	1.82	1.25

Data are mean ± S.E. of n = 6; ^{ø}ATD vs Control at P £ 0.05, *ATD + Therapy vs ATD at P £ 0.05, §Significant at 5% for ANOVA.

Abbreviations: RBC = Red blood cells; Hb = Hemoglobin; HCT = Hematocrit; ATD = Anti-tuberculosis drugs.

Materials and Methods

Animals and Chemicals

Female albino rats of Wistar strain (150±10 g body weight), housed under standard husbandry condition (25±2º C temp, 60-70% relative humidity, 14 h light and 10 h dark) were used in study. The animals were fed on standard pelleted diet and drinking water *ad libitum.* Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India. The study was carried out with the approval of the institutional animal ethics committee (994/Ere/Go/06/CPCSEA). The antituberculosis drugs isoniazid, rifampicin, ethambutol and pyrazinamide were generously obtained from the Government TB hospital, Bilaspur (C.G.). Crude propolis was generously gifted by Prof. O.P. Agarwal, senior entomologist, School of Studies in Zoology, Jiwaji University who collected it from the hives of *Apis mellifera.*

Therapeutic potential of propolis against ATD

Rats were divided into seven groups of six animals each. Group I served as control and served with vehicle only. Group II was administered with propolis *per se* at the dose of 400 mg/kg orally. Group III to VII were administered with above mentioned doses of ATD for 8 weeks (3 alternative days in a week) and group III served as experimental control. Animals of groups IV, V and VI were given propolis at the doses of 100, 200 and 400 mg/kg, p.o, for 8 weeks (3 alternative days in a week considering every next day of ATD treatment) respectively. Group VII was given silymarin 50 mg/kg, p.o, as positive control. After 8 weeks of treatment, blood was drawn from retro orbital venous sinus by the conventional method[14]. Blood samples collected in ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes (8.5%) were quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Hematological parameters were analyzed: red blood cells (RBC), hemoglobin (HB), haematocrit (HCT), volume a mean corpuscular erythrocyte (MCV), red blood cell distribution width (RDW) and the number of lymphocytes (LYM). All hematological parameters were analyzed by using the automated method with the automatic analyzer "Haematology auto analyzer Hema 2062+".

Statistical analysis

The results were expressed as mean ± SE of six animals used in each group. The differences between the mean values was calculated by one-way analysis of variance (ANOVA) considered significant at P d" 0.05 level followed by student's t-test[8].

Results and Discussion

Blood acts as a pathological indicator of the status of the exposed animals to toxicants and other conditions[11]. The parameter usually measured are red blood cells, hemoglobin, mean corpuscular volume, hematocrit and lymphocytes. Hematological studies are

useful in the diagnosis of root cause of many diseases as well as investigation of the extent of damage to blood[6,15]. Hematological disorders indicating the abnormal condition in the profile of blood parameters, due to changes in metabolism.

Red blood cells serve as a carrier of hemoglobin. Hemoglobin that reacts with oxygen carried in the blood to form oxyhemoglobin during respiration. Red blood cell and hemoglobin are involved in the transport of oxygen and carbon dioxide in the body[10]. In the present study decrease in the hemoglobin content in the rats treated with ATDs might be due to increased catabolism and degradation of bilirubin which leads to decreased red blood cells number which in turn indicates anemic condition. Hematocrit is the percentage (%) of red blood cells in blood and is involved in the transport of oxygen and absorbed nutrients. In the present study, significant alterations were observed in blood parameters such as decrease in red blood cells, hemoglobin and hematocrit (*P* d" 0.05) after ATDs exposure (Table 1). All the three doses of propolis maintained the red blood cells, hematocrit and hemoglobin level near to control. Hemoglobin content in the propolis treated groups were higher. This increase may arise as a result of increase red blood cell count and possibly the mineral content of the propolis especially iron content, an increase in iron supply is necessary to elevate the hemoglobin content. This suggests that the propolis may be useful in anemic conditions. Propolis capable to reverse the hematocrit content towards control so, increased hematocrit shows a better transportation[6].

Mean corpuscular volume (MCV) is a measure of the average volume of a red blood cell. A low MCV is an indication of microcytic anemia. The result suggests that the ATDs treated groups may have anemia as MCV is significantly lower (*P* d" 0.01) as compared to control. Propolis at the dose of 400 mg/kg restored the MCV content and reduced the anemic condition in rats towards rats (*P* d" 0.01). The main role of lymphocytes is to fight infections. Present observation indicates that ATDs groups have low immune system as they have elevated level of lymphocytes in comparison to control groups (*P* d" 0.01). Increased level of lymphocytes showed that rats are more prone to have disease. Propolis decreased lymphocytes and improved body's defense mechanism.

Conclusion

In view of these results, it appears that ATD induced hematological disturbances in rats. . It can be concluded that propolis has potential to reverse ATD induced blood parameters alterations thus, can be used as an excellent protective agent during ATD regimen.

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