Original Article

AMELIORATIVE ROLE OF BEE HONEY AND ROYAL JELLY AGAINST CISPLATIN INDUCED ALTERATION IN HEMATOLOGICAL PARAMETERS IN MALE WISTER ALBINO RAT

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ABSTRACT
Objective: This study aims to investigate the ameliorative role of dietary bee honey and royal jelly against cisplatin-induced alterations in hematological parameters in male wistar albino rat.

Methods: Male wistar albino rats of same age and weight were randomly divided into four groups; G. I: control group which was given 0.9% saline, G. II: cisplatin (7 mg/kg/d) was injected intraperitoneally for 15 d, G. III bee honey with royal jelly (500 mg/kg/d of honey and 100 mg/kg/d of royal jelly) fed orally daily for 15 d, G. IV: cisplatin (7 mg/kg/d) was injected intraperitoneally and honey (500 mg/kg/d) and royal jelly (100 mg/kg/d) fed orally daily for 15 d. The hematological parameters like total number of white blood cells (WBCs), red blood cells (RBCs), platelets, percentage of hemoglobin (Hb), and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were measured by using automated hematology system.

Results: Cisplatin treated rats revealed a significant decrease in total number of white blood cells (WBCs), red blood cells (RBCs), platelets, percentage of hemoglobin (Hb), and mean values of packed cell volume (PCV), corpuscular volume (MCV) and corpuscular hemoglobin concentration (MCHC) as compared to control group. Royal jelly and honey treated group of rats revealed a significant increase in all blood parameters compared to control group. Dietary bee honey with royal jelly along with cisplatin-treated rats revealed significant increases as compared to animals treated with cisplatin (G.III) and the computed significant values for the above parameters are 10.00, 2.30, 8.54, 12.00, 35.00, 47.40 and 32.30 respectively.

Conclusion: Bee honey and royal jelly could be used as dietary preventive natural products against cisplatin-induced hematological alterations during the treatment of cancer.

Keywords: Wister albino Rats, Hematology, Cisplatin, Honey, Royal jelly, Ameliorative

INTRODUCTION

Cisplatin is one of the most cytotoxic agents and is widely used to treat a variety of cancers, but it is associated with toxic side effects. The oxidative stress through the formation of free radicals is one of the mechanisms of cisplatin-induced toxicity [1]. The free radical scavengers, or which prevent the formation of the reactive hydroxyl free radicals, can provide protection against cisplatin-induced hemotoxicity, especially blood parameters [2]. Different natural products and dietary compounds have been recently investigated and evaluated as potential protective antioxidant agents against cisplatin-induced toxicity [3]. Honey and royal jelly are natural dietary substances, which previously tested to ameliorate the toxic side effects of a different substance, through their antioxidant, radical scavenging and antiperoxidative activity [4,5].

Blood delivers requisite materials such as nutrients and oxygen and carries waste products from the cells. It contains RBCs, WBCs and platelets, which are suspended in a fluid medium; plasma [6, 7]. The measurement of hematological parameters erythrocytes, leukocytes, platelets and concentration of hemoglobin are some of the most frequently performed clinical laboratory tests in which variations in the count of blood cells signal regarding diseases or ill health of human body. For overall health assessment and diagnosis of many disorders, complete blood count is required.

Consequently, the aim of the present study was to investigate the ameliorative role of dietary bee honey and royal jelly against cisplatin-induced alterations in hematological parameters in male wistar albino rat.

MATERIALS AND METHODS

Animals

Healthy male wistar albino rats weighing 200-250 gm (10-12 w age) were obtained from the animal house of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-India. All the experimental procedures were carried out in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on the animal (CPCSEA). All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of RCPEER, Shirpur (Reg No.-651/PO/ReBi/ S/02/CPCSEA).

Housing conditions

The rats were housed in standard plastic cages. The bedding material of the cages was changed every day. Maximum of 3 rats housed per polypropylene cage having a size of 32 X 11 cm with stainless steel grill top mesh having facility for holding food palate and a water bottle. The rats were allowed free access to food, diet and water throughout the experimental period. All animals were housed in an air-conditioned room at a temperature range between 22-25 °C, relative humidity in between 30%-60% and with a 12-hour light-dark cycle.

Acclimatization

Selected rats were randomly divided into four groups containing 6 rats in each group and were allowed to acclimatize to laboratory conditions for 7 d prior to experimentation.

Water

Water processed by reverse osmosis and Ultraviolet (UV) light was supplied ad libitum to the rats.

Chemicals

Cisplatin was purchased from (Cipla Ltd company-Goa-India). Bee honey and royal jelly collected directly from the Apis mellifera colonies located in the university campus. Food pallet was...
purchased from Nutrivet Life sciences, Pune, Maharashtra, India. All other chemicals used in the experiment were of analytical grade.

### Preparation of royal jelly and honey

500 mg/kg/d of honey and 100 mg/kg/d of royal jelly were dissolved in distilled water and administered through an intragastric tube through the mouth. The doses were weighed on digital scales, where the dose relies on animal weight, in which each single gram of the experimental rat should receive 0.5 mg of honey and 0.1 mg of royal jelly.

### Experimental design

For the study, 24 adult male wister albino rats of 10-12 w age and with 200-250g weight randomly divided into 4 groups; each group consisting of 6 rats and were treated for 15 d as below:

- **Group I (Control):** 0.9% (10 ml/kg/d) saline solution was administrated for 15 d.
- **Group II (Cisplatin):** Cisplatin (7 mg/kg/d) intraperitoneal injection for 15 d [8, 9].
- **Group III (bee honey+royal jelly):** Bee honey (500 mg/kg/d)+Royal jelly (100 mg/kg/d) orally administrated for 15 d [8, 10].
- **Group IV (Cisplatin+bee honey+royal jelly):** 7 mg/kg/d of cisplatin intraperitoneal injection along with 500 mg/kg/d of honey+100 mg/kg/d of royal jelly orally were through an intragastric tube for 15 d.

### Blood collection

After 15 d of treatment, blood samples were collected via retro-orbital puncture under light ether anesthesia. Blood collected was put in tubes, containing a substance of Ethylenediaminetetraacetic acid (EDTA) (15-20 IU per ml of blood), to check the number of WBC, RBC, PLT, PCV, Hb, MCV, MCH and MCHC [11].

### Hematological assay

The hematological parameters like a total number of WBCs, RBCs, platelets, % hemoglobin (Hb), and mean values MCV, MCH and MCHC were measured by using automated Hematology System, Sysmex Exigo, Box 42056, SE-126 13 Stockholm, Sweden.

### Statistical analysis

All data were expressed as mean±S. E. M and statistically analyzed using Graph Pad Prism 7 for Windows (Prism Inc, Chicago, IL, U. S. A). The statistical significance of differences among different study groups was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison tests as a post hoc test. P value of 0.05 or less was taken as a criterion for a statistically significant difference.

### RESULTS

Effect of treatment of cisplatin (G, II), bee honey and royal jelly (G, III), and the combined treatment of cisplatin with bee honey and royal jelly (G, IV) on hematological parameters of male wistar albino rats were evaluated in comparison with control group (G, I) for the period of 15 d and obtained results were summarized in (table 1).

The results demonstrated that cisplatin treated rats, (G, II), exhibited significant decrease in the total number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin% (Hb) and mean values of packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) contents and the percentages decreased was 22.7%, 55.5%, 36.25%, 14.9%, 34.1%, 19.3% and 24.9%, respectively as compared to the control group rats.

In the present study, it was observed that after honey and royal jelly treatment (G, III), total number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (Hb)% and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of experimental rats showed a significant increase as compared to the control group and the percentages of increase was 8.3%, 8.9%, 11.82%, 11.8%, 2.43%, 2.68% and 0.57%, respectively.

After combining treatment of cisplatin along with honey and royal jelly (G, IV), the total number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), haemoglobin (Hb)% and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of experimental rats was significantly increased as compared to animals treated with cisplatin (G, II). The percentage was 17.64%, 36.4%, 42.5%, 11.1%, 29.6%, 12.51% and 23.2%, respectively.

### DISCUSSION

In the present study it was observed that due to cisplatin treatment rats for 15 d, the total number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin Hb%, and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly decreased. Similar results were reported by many authors [12-9].

The results demonstrate that after 15 d of cisplatin treated rats, the total number of white blood cells (WBC) was significantly decreased. This might be due to infection and inflammation during cisplatin

### Table 1: Haematological parameters of control and treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (G, I)</th>
<th>Cisplatin (G, II)</th>
<th>Honey and royal jelly (G, III)</th>
<th>Cisplatin with Honey and royal jelly (G, IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC</td>
<td>11.00±0.088</td>
<td>8.50±0.17***a</td>
<td>12.00±0.30a</td>
<td>10.00±0.19**b</td>
</tr>
<tr>
<td>×10^12/Cmm</td>
<td>#±22.7%</td>
<td>#±8.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total RBC</td>
<td>3.80±0.421</td>
<td>1.69±0.244*</td>
<td>4.14±0.439a</td>
<td>2.30±0.65*</td>
</tr>
<tr>
<td>×10^12/Cmm</td>
<td>#±55.5%</td>
<td>#±8.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets x (10^3/mm)</td>
<td>9.39±0.202</td>
<td>5.99±0.489***a</td>
<td>10.50±0.420a</td>
<td>8.54±0.362***b</td>
</tr>
<tr>
<td>Haemoglobin (%)</td>
<td>12.70±0.251</td>
<td>10.80±0.5779***a</td>
<td>14.20±0.209a</td>
<td>12.00±0.29b</td>
</tr>
<tr>
<td>(%)</td>
<td>#±14.9%</td>
<td>#±11.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>41.00±0.43</td>
<td>27.00±2.4***a</td>
<td>42.00±0.32a</td>
<td>35.00±1.4*</td>
</tr>
<tr>
<td></td>
<td>#±3.4%</td>
<td>#±2.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (%)</td>
<td>52.20±0.850</td>
<td>42.10±0.186***a</td>
<td>53.60±0.837a</td>
<td>47.40±1.14*</td>
</tr>
<tr>
<td></td>
<td>#±19.3%</td>
<td>#±2.68%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>34.90±0.196</td>
<td>26.20±1.20*</td>
<td>35.10±0.479a</td>
<td>32.30±0.93***b</td>
</tr>
<tr>
<td>(gm/dl)</td>
<td>#±2.4%</td>
<td>#±0.57%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. *indicate SD of three observations, 2. # (+) or (-) indicates percent variation over control. 3. w (+) or (-) indicates percent variation over cisplatin. 4. Values are significant at *P<0.001, **P<0.01, ***P<0.05, 5. NS (Not significant). 6. a = P<0.001, **P<0.01, ***P<0.05 values compared with normal control. 7. b = P<0.001, **P<0.01, ***P<0.05 values compared with cisplatin group. 8. WBC = White blood cell, 9. RBC = Red blood cells, 10. PCV = Packed cell volume, 11. MCV = Mean corpuscular volume, 12. MCHC = Mean hemoglobin concentration.
treatment. The main molecular mechanism of cisplatin is myelotoxicity is due to its ability to bind with cellular DNA and render the cell incapable of replication [20]. Beside this, another mechanism of cisplatin is its ability to induce oxidative stress [21]. Reactive oxygen species are toxic to bone marrow cells and probably can trigger apoptosis and affect cell cycle, causing anemia and a decrease in leukocyte count [22]. Myelosuppression resulting in leukopenia and thrombocytopenia is a frequent and a major complication of cancer chemotherapy [23]. Many authors [24-8, 14-5] observed that the number of WBC was decreased after exposure to cisplatin administration.

After 15 d of cisplatin treatment to rats, the platelet count was significantly decreased compared to control group. This might be due to cisplatin inhibiting bone marrow activity or could be due to decreased production or increased consumption of platelets or due to the increased platelets aggregation [29]. Cisplatin causes oxidative stress in human platelets and lymphocytes, which might reflect on their life expectancy, the induction of apoptosis, and thereby ultimately reduced the number of these cells in the blood of experimental animals. However, the decrease in the WBCs number could be the consequence of infection and inflammation during cisplatin treatment and cisplatin metabolism in the experimental rats. [30]. Cisplatin reduced the platelet count in rats under experiment [25] and depleted the platelet number and caused cumulative anemia in rats [13].

After 15 d of cisplatin treatment rats, the total number of RBC and Hb% were significantly decreased as compared to control. Similar results were reported by many authors [12-4, 16, 18, 25, 28]. The previous results suggested that there was an etiological relationship between anemia and cisplatin treatment. This relation could be explained through different mechanisms, including the destruction of bone marrow cells or increased osmotic fragility of RBC. Thus, cisplatin therapy might lead to anemia as a result of either suppression of the activity of hematopoietic tissues, impaired erythropoiesis, and accelerated RBCs destruction because of the altered RBCs membrane permeability, increased RBCs mechanical fragility, and/or defective iron metabolism [13].

The reduction of RBC and Hb% attributed to the hemorrhage or hemolysis or because of impaired blood formation in bone marrow due to cisplatin toxicity and that led to imbalance between production and loss, inhibition of DNA synthesis in bone marrow precursor cells, leaving both RNA and protein synthesis intact and inhibition of many steps of heme biosynthesis in rats, as result of cisplatin use [31]. The reduction in the Hb% is related to suppression of erythropoiesis and iron supply to erythroblasts [32, 33].

It also seems less likely that the reduced RBC count was a result of hematopoietic colony forming unit (CFU-E) maturation disturbances. Haemolytic anaemia has been reported in patients treated repeatedly with Cisplatin [34-6]. Antibodies reacting with Cisplatin-RBC-membrane complexes [34] can also cause hemolysis.

It was also found that cisplatin therapy inhibited the production of renal erythropoietin, which resulted in a lower RBC production. The nephrotoxic effect of cisplatin showed a negative effect on erythropoiesis that resulted in the low production and the count of RBCs [12]. Cisplatin is said to cause anemia by interfering in the iron metabolism [37].

Due to cisplatin treatment rats for 15 d, mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were significantly decreased as compared to control group. Similar results were reported by a number of authors [26, 16, 18].

Following the treatment with cisplatin, decreased MCV, MCH and reduced MCHC, suggest that a microcytic hypochromic anemia was due to the suppression of erythropoiesis [37]. It is reported that reactive oxygen species (ROS) increases hemoglobin glycation and erythrocyte fragility and bone marrow can be damaged by direct oxidation [38, 39]. More specifically haemoglobin-derived iron might contribute to the pathogenesis of cisplatin by inducing the oxidative stress [31, 40].

Thus, in the present study, it was observed that the decrease in the MCV, MCH and MCHC after exposure to cisplatin was attributed to the production of erythrocytes with lower MCV, MCH and MCHC and these parameters closely related to Ht levels and Hb. Hb data could be strongly influenced by MCV, MCH and MCHC values [41].

[42-3], it was found that cisplatin was more toxic to earlier haemopoietic progenitor cells than the mature ones. It was suggested that anemia could be due to the difference in time of maturation of the erythroid series. However, in a recent study [34] hemolysis was blamed for the production of anemia [44].

The above-mentioned effects of cisplatin could be due to their ability to form free radicals [45]. This fact may ensure the hypothesis of the ability of cisplatin to form free radicals, which have been implicated as playing a role in the etiology of many alterations [46].

The success of a chemotherapy is dependent not only on effectively removing tumor cells but also on reducing the related immunosuppressive complications that are primarily caused by apoptosis of circulating leukocytes cells (leucopenia). Rat’s response to cisplatin chemotherapy caused severe immunosuppressive conditions, as reflected in a lower WBC count.

In the present study it was observed that after honey and royal jelly treatment (G, III), the total number of white blood cells (WBC), red blood cells (RBC), hemoglobin % (Hb), platelets (PLT), and the mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of experimental rats were significantly increased as compared to control group.

Antioxidants can prevent cell damage caused by the action of reactive oxygen species (ROS) and free radicals [47]. The antioxidant activities are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl [48]. Recently, royal jelly has received particular attention as a highly efficient antioxidant and has the free radical scavenging capacity [49]. It contains many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, minerals, and vitamins [50]. Honey is a natural antioxidant, which may contain flavonoids, ascorbic acid, tocopherols, catalase, and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals [51-54].

In the present study it was observed that after combined treatment of cisplatin along with honey and royal jelly (G, IV), the total number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin % (Hb) and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of experimental rats were significantly increased as compared to animal treated with cisplatin (G, II). Honey and royal jelly have a protective role against many drugs, wherever [55] the honey protective effects on organs through the important antioxidant parameter (RBCs, WBCs and Platelets) because of decreased lipopolysaccharide in rats. Honey is reported to attenuate the hematological, effects induced by gentamicin [56, 45]. Natural honey significantly (P<0.05) restored Hb content, RBC, PCV, platelet and WBC close to control values in wistar albino rats fed hydrocarbon contaminated diets [57].

The royal jelly has a hematopoietic role against azathioprine [17]. Honey has a hematopoietic role against zinc [58] and also it is reported that honey effects on amikacin-induced toxicity on hematological parameters [19].

The present study showed the improvement in the tested blood parameters as erythrocytes, hemoglobin, leukocytes, platelets and the mean value of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) indicates that honey and royal jelly administration prevented blood cell damage by maintaining the integrity of cells. Administration of royal jelly to rats ameliorated the effect of radiation that induced oxidative stress and hematological alterations [56].

CONCLUSION

Cisplatin caused a decrease in the total number of red blood cells (RBC), blood platelets (PLT), hemoglobin (HB) % and mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), whereas honey and royal jelly
reversed these decreases. A high significant protective and curative effect on the studies on blood parameters due to honey and royal jelly indicates that honey and royal jelly should be supplemented to the patient when cisplatin chemotherapy is executed.

AUTHORS CONTRIBUTIONS
All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

CONFLICT OF INTERESTS
Declared none

REFERENCES


