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ANTI-LEUCOPENIC EFFICACY OF ROYAL JELLY DURING CYCLOPHOSPHAMIDE TREATMENT

Syeda Hina Kausar and V. R. More
Research student, Department of Zoology,
Government Arts, Science College, Aurangabad (M.S.) India.

E.mail:hina33113@gmail.com

Abstract:

Current study is effort to find out the potential antileucopenic efficacy of royal jelly against leucopenia induced by cyclophosphamide in male albino mice. Male Swiss albino mice of 20±5gms were unevenly divided into six groups; G1: normal control group 0.9% saline solution I.P. weekly, G2: RJ (100mg/kg/d) CMC suspended administered by orally, G3: cyclophosphamide(50mg/kg/week) was injected intraperitoneally, G4: I.P. cyclophosphamide(50 mg/kg/week) along with royal Jelly (100mg/kg/d), G5: I.P. cyclophosphamide(50 mg/kg/week) with royal Jelly (250mg/kg/d), G6: I.P. cyclophosphamide(50 mg/kg/week) and royal Jelly (500mg/kg/d). Experiment lasted for 12 weeks. The TLC and DLC were performed using automated hematology system. Cyclophosphamide treated mice exhibit leucopenia, lymphocytopenia, neutropenia and decline in monocyte count as compared to control group. The administration of royal jelly to CPA treated mice, according to the present experimental plan significantly improves the alterations induced in leukogram. It was suggested that royal jelly ameliorate cyclophosphamide-induced decrease in leucocytes, thus it might be used as a dietary protective natural remedy during the chemotherapy.

Keywords: Royal jelly, cyclophosphamide, Swiss albino mice, leukocytes, neutropenia.

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Introduction:

Cyclophosphamide (CPA) is an alkylating agent most commonly used in the treatment of different types of haematological and solid malignancies, autoimmune disorders like rheumatoid arthritis, systemic lupus erythematous, and multiple sclerosis and other medical conditions (Lu. *et.al.*, 2015).

However, drug has a number of side effects and toxicities like nausea, vomiting, alopecia, bone marrow suppression, hepatotoxicity, nephrotoxicity, urotoxicity, cardio toxicity, immunotoxicity, mutagenicity, teratogenicity, and carcinogenicity (Nafees, *et.al.*, 2015).

Royal jelly is secreted from the hypo pharyngeal and mandibular glands of worker honeybees containing varieties of nutritional components like proteins, lipids, carbohydrates, vitamins and minerals (Stocker *et.al.*, 2005). It has a lots of properties such as anti-tumor and anti-inflammatory properties, anti-fatigue and hypotensive activity, (Nagai & Inoue, 2004) antioxidant activities, antibacterial effects and enhancement of immune activity (Sver, *et.al.*, 1996). Due to these exclusive properties, royal jelly has become very important for human beings.

So, we considered the beneficial properties of RJ against cyclophosphamide induced leucopenia in mice.

Material and methods:

Chemicals:

The tested compound CPA were bought from Zydus Cadila, (G.Rem). RJ were purchase from the apiculture farm of Hi-tech Natural product (India) Ltd. From colonies of Apis mellifera in the lyophilized form. Food pallet was bought from VRK Nutritional solution, Pune, Maharashtra (India). All other chemicals used in this experiment were of analytical grade from Merck (India) Ltd, Mumbai, India. The dose has been selected on the basis of previously published studies and by acute toxicological study.

Preparation of royal jelly and cyclophosphamide:

At the proportion of 100 mg/kg/d/mice of royal jelly were dissolved in 5% CMC (Carboxy methyl cellulose) suspension administered through an intragastric tube through the mouth. While CPA was injected through intraperitoneally rout at the proportion of 50 mg/kg/mice by dissolving in distilled water ,for combine dose different proportions of RJ as 100mg/kg, 250mg/kg and 500mg/kg were

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suspended in CMC. The doses were weighed on SF-400 digital LCD balance.

Animals:

A total of 36 Pathogen free male Swiss albino mice, with a weight of 20±5 g were obtained from the Laboratory of Wokhardt Research Institute Aurangabad M.S. (India). The experimentation performed was in full compliance with the guidelines of the committee for the purpose of control and supervision of experiments on the animal (CPCSEA) Act of 2007 Govt. of India on animal welfare.

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy, Aurangabad and (MH) India. (Ref. No. CPCSEA/IAEC/Pcology-53/2017-18/134.)

Housing conditions:

The mice were accommodated in standard polypropylene cages with a size of 32 X 11 cm food palate and a water bottle held on a stainless steel grill top .The bedding material of the cages was changed daily. Maximum of 6 mice housed per polypropylene cage.

All mice were maintained under standard laboratory conditions (25 ± 1°C temperature; 12:12 h light/dark and 55-65 % humidity) and isolated for 7 days prior to the start of the study. Standard rodent chow diet and water were provided ad libitum to the experimental animals.

Experimental design & Plan:

36 adult male Swiss albino mice of 8-9 week age and with 20±5g weight were randomly allocated into 6 groups; each group consisting of 6 mice and the experiment lasted for 12 weeks. After an acclimatization period of one week, (G1-G6).

G1: Normal Control Group (C): served as healthy control. Mice fed only with basal diet and water and was administered with 0.9% Normal saline (10ml/kg/week), for 12 weeks.

G2: Royal jelly Group (RJ): mice were administered with Royal jelly (100 mg/kg) CMC suspended orally everyday between 10 AM to 11 AM for each mice for 12 weeks.

G3: Cyclophosphamide Group (CPA): was injected with CPA 50 mg/kg/week, for 12 weeks (once in a week) by intraperitoneally.

G4: CPA & Low dose RJ Group (CPA+RJ1): were injected with CPA (50 mg/kg/week) followed by RJ administered orally (100 mg/kg /day) respectively, for 12 weeks.

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G5: CPA & Medium dose RJ Group (CPA+RJ2): mice were injected with CPA (50 mg/kg/week) followed by RJ administered orally (250 mg/kg /day) respectively. for12 weeks.

G6: CPA & High dose RJ Group (CPA+RJ3): mice were injected with CPA (50 mg/kg/week) followed by RJ (500 mg/kg /day) respectively, for 12 weeks.

Collection of blood:

After 12 weeks of exposure approximately 1 ml of blood samples were obtained using mice bleeding tubes from retro-orbital sinus plexus in an EDTA-containing tubes. All blood samples were labelled and immediately analyzed for blood parameters.

Measurements of parameters:

Total leukocyte count (TLC) and differential leukocyte count (DLC) were analyzed using the automated haematology method with the "Haematology auto analyzer Sysmex x100.The blood analyzer was periodically calibrated.

Statistical analysis:

All Values are expressed as means ± SEM. Graph pad PRISM 6.01 for windows computer program was used for statistical analysis of the results.

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A value of ***P<0.001, **P<0.01, *P<0.05 was considered to be statistically significant.

Results:

The Groups (G2 and G3) were compared with normal control (G1) group while combined treatment groups (G4 to G6) were compared with Cyclophosphamide (G3) group for the period of 12 weeks and obtained results are summarized in (Table 1).

Total leucocyte count:

Current study demonstrated that the royal jelly treatment (G2). non-significantly increased the total leukocyte count (TLC) compared to control group (G1). However A significant reduction (P<0.001) in leukocyte count in a Cyclophosphamide injected mice (G3) were observed as compared with the control (G1).

Similarly, there is a non-significant difference in the TLC were seen in CPA with low dose (100mg/kg) royal jelly group (G4) compared to CPA-injected mice of (G3). Interestingly, the mice that injected with CPA along with medium (250mg/kg) and high dose (500mg/kg) of RJ (G5 and G6) displayed a significant increase (P<0.05, P<0.01) in TLC compared to CPA-

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injected group (G3).

Lymphocyte count:

Animals treated with Royal jelly alone (G2) exhibiting a non-significant difference in lymphocyte count towards control group (G1).whereas in (G3) cyclophosphamide a highly significant decrease ($P<0.001$) were recorded. (G4 and G5) group animals have statistically similar percent of lymphocytes as (G3) CPA treated animals. But animals of (G6) revealed an increased in lymphocyte percent ($P<0.05$) compared to (G3).

Monocyte count

Non-significant difference were recorded in a (G2) animals by means of Monocyte count while a significant decrease were seen in Cpa (G3) group compared to control (G1).

Animals treated with CPA with 100mg/kg Rj (G4) and CPA+ 250mg/kg Rj (G5) does not exhibit any significant difference in monocyte count but high dose CPA with 500mg/kg (G6) exhibit significant increase ($P<0.05$) compared to CPA treated mice(G3).

Neutrophil count:

In RJ treated animals of (G2) NEUTROPHIL count was significantly similar while a decline was seen in CPA injected mice (G3) compared to (G1).

In (G4) count was statistically similar but (G5) exhibited a significant difference ($P<0.05$) while an increase was ($P<0.01$) recorded in (G6) animals compared to CPA (G3).

Discussion:

Cyclophosphamide (CPA) is widely used in the treatment of different malignancies Geno toxicity is another severe toxic effect induced by CP which leads to mutagenicity, teratogenicity and carcinogenicity (Mirkes, 1985). Initially in liver the CPA is activated by microsomal oxidation system enzyme cytochrome p450 converting CPA into 4-hydroxy CPA, a cytotoxic metabolite, then, 4- hydroxy CPA is further converted to some other cytotoxic metabolites as phosphoramide mustard and acrolein (Pratheeshkumar & Kuttan, 2010). Phosphoramide mustard the active metabolite of CPA forms DNA crosslinks which lead to DNA strand breaks and subsequently to chromosomal Breaks (Schneider *et.al.*, 1977). Acrolein, the other metabolite interferes with tissue antioxidant defence mechanism through producing highly reactive oxygen free radicals that further react with DNA causing its damage (Yoshida, *et.al.*, 2009).

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Current study demonstrated the impact of RJ on leukocyte of mice intoxicated by cyclophosphamide. A clear decrease was detected in the TLC and DLC.

CPA produces increased ROS in a bone marrow, which is the leading cause of severe DNA damage of lymphocytes (Bhattacharjee, *et.al.*, 2017). the plasma membranes of blood elements are made up of polyunsaturated fatty acids (PUFA) which is damaged by the oxidative stress produced by ROS. In addition, the decrease in WBCs count recorded in the CP-injected mice is due to oxidative stress which induced lipid peroxidation and damage of blood cell (Chew & Park, 2004). CPA treatments cause the alteration in the bone marrow, by means of the destruction of stem cells and failure to regenerate new blood cells (Sanjeev, *et.al.* 2012).

Leukopenia has been observed in the CPA treated mice might be the result of oxidative stress-induced lipid peroxidation and damage of their cell membranes. Similar result were found by many researchers. CPA injection in normal mice resulted in moderate leukopenia (Ray *et.al.*, 2000). (Duggina *et al.*2015) reported that leucopenia are frequently seen in patient during CPA treatment. (Elshater, *et al*, 2018 reported CP is well-known as a cellular immunosuppressive agent (El Tarabany, 2017).

In the present study leukocytes returns to the normal value because of the co administration of RJ with a CPA. It has been found that a natural substance RJ has excess number of bioactive compounds, such as protein, carbohydrates, vitamins minerals, fatty acids. It is a rich source of antioxidants that opposed to myelo suppressive effects induced by CPA.

The mechanism might be related to recovery of hematopoiesis by means of modulating the bone marrow activity, as well as enhanced immune functions. The Treatment with RJ brought back all the parameters to near normal levels, indicating the protective effects of royal jelly on the hematopoietic system. It seems likely that 10-HDA (and probably other fatty acids) selectively bind to the cell membrane of B lymphocytes and, thus, could interfere with antibody secretion by these cells.

When administered, RJ provide normalization of the all blood elements in the peripheral blood of mice, thus preventing bone marrow depletion. The number of peripheral blood lymphocytes was increased in the RJ treated mice as R J-

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induced restoration of cellular immunity. El-Tarabany, 2017 reported Royal Jelly has increasing the erythrocyte and leukocyte count in in laying hens at the late stage of production.

Conclusion:

Cyclophosphamide caused leucopenia, neutropenia, lymphocytopenia and a significant decrease in monocyte also, while RJ is significantly improved and normalized all leucocytes altered during cyclophosphamide treatment. Thus RJ is a useful product alone or in combination with chemotherapeutic agent during leucopenic condition to improve all leukocyte parameters.

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Table 1. Effect of Royal jelly on CPA-induced changes in TLC and DLC in male albino mice.

Groups ▸ Parameters	G1: Cont.	G2: RJ	G3: CPA	G4: RJ_1+CPA	G5: RJ_2+CPA	G6: RJ_3+CPA
TLC (10^3/µl)	5.46± 0.38	6.14± 0.54	1.49± 0.44***	2.14± 0.52	3.84± 0.52†	5.03± 0.52††
Lymphocytes %	65.69± 2.54	68.50± 2.90	32.40± 2.98***	42.16± 2.81	50.78± 2.25	58.18± 3.21†
Monocytes %	3.27± 0.40	4.14± 0.90	0.58± 0.14**	1.69± 0.18	1.93± 0.33	2.83± 0.38†
Neutrophils %	20.07± 1.30	20.85± 2.59	6.24± 1.28***	9.70± ±1.21	11.84± 1.31†	15.86± 1.23††

1. The values signify mean ±SEM, number of samples is 6 mice per group.
2. Values are significant at ***P≤0.001, **P≤0.01, *P≤0.05 compared with normal control (G1) group.
3. Values are significant at †††P≤0.001, ††P≤0.01, †P≤0.05 compared with cyclophosphamide (G3) group.

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