

REVERSAL OF PACLITAXEL INDUCED NEUTROPENIA BY *WITHANIA SOMNIFERA* IN MICE

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Abstract : The effect of aqueous extract of *Withania somnifera* (L. Solanaceae) was studied against paclitaxel induced neutropenia in mice. After paclitaxel 1 mg/kg, i.v. administration significant fall in total WBC and absolute neutrophil count was observed on day 3 and day 5. *W. Somnifera* (200 mg/kg, p.o.) per se produced significant increase in neutrophil counts. *W. somnifera* (200 mg/kg, p.o.) when administered for 4 days before paclitaxel treatment and continued for 12 days caused significant reversal of neutropenia of paclitaxel. The findings of the study suggest the potential of *W. somnifera* as an adjuvant during cancer chemotherapy for the prevention of bone marrow depression associated with anticancer drugs.

Key words : paclitaxel
mice

neutropenia
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INTRODUCTION

Paclitaxel is an effective anticancer agent from the bark of *Taxus brevifolia* Nut (Taxaceae) and is useful in treating several solid tumours and soft tissue sarcomas (1). Myelosuppression is a major limiting side effect of paclitaxel. It induces troublesome neutropenia of grade 3 to 4 in the dose range of 150-250 mg/m² in more than 50% of the patients. To prevent paclitaxel induced

myelosuppression the current approach is to use colony stimulating factors e.g. GM-CSF or G-CSF. However, the high cost is the major limitation in their use.

W. somnifera (Ashwagandha), an Indian herbal plant is being used in many indigenous preparations as a health tonic and an immunostimulant (2, 3). It has been reported to have antiinflammatory and anti-arthritic activity (4). It has been shown to

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increase the hemoglobin, total red blood cells and white blood cells in mice with chemically suppressed immunity, and stimulate total WBC in normal Balb/c mice and also reduce leucopenia induced by sublethal dose of gamma radiation (5). Recently, in an experimental study in mice, ashwagandha prevented myelosuppression induced by cyclophosphamide, azathioprine or prednisolone (6, 7, 8). However, effect of *W. somnifera* on paclitaxel induced neutropenia has not been evaluated. Therefore, in the present study, effect of aqueous extract of *W. somnifera* was investigated against paclitaxel induced neutropenia in mice.

METHODS

The study was conducted on healthy (with no apparent infection) male Swiss albino mice (23–25 g). They were housed under standard laboratory conditions for acclimatization before the experiment and fed standard laboratory dry pallet diet (Golden Feed Company, Delhi) and water *ad libitum*.

Selection of animals: Total WBC count and differential leukocyte count (DLC) of mice were done on 3 consecutive days and only those animals were included in the study which showed consistent Total WBC and DLC. The mice which showed any sign of infection were excluded from the study.

Drugs and preparation of the solutions: Paclitaxel (courtesy Dabur Research Foundation, India) and Recombinant Human Granulocyte Monocyte Colony Stimulating

Factor (rHuGM-CSF) (Sandoz Pharma Limited, Switzerland) were diluted with sterile saline and administered intravenously and subcutaneously respectively in a volume of 0.1 ml. *W. somnifera* aqueous extract (courtesy Dabur Research Foundation, India) was suspended in 1% gum acacia and administered orally by intragastric tube in a volume of 0.5 ml. All the drug solutions were freshly prepared before administration.

Mice were divided randomly into different treatment groups. Each group had 10–12 mice each. Blood (0.2 ml) was collected by retro-orbital venous sinus puncture with a capillary tube for measurement of total WBC and absolute neutrophil counts.

Effect of paclitaxel on total WBC and absolute neutrophil counts: A single dose of paclitaxel (1 mg/kg) was administered intravenously. Total WBC and absolute neutrophil counts were measured prior to paclitaxel treatment and on day 0, 3, 5, 8, 12 and 18 after paclitaxel administration.

Effect of W. somnifera on paclitaxel induced changes in total WBC and absolute neutrophil counts: In group of animals treated with *W. somnifera* (200 mg/kg, p.o. for 4 days, total WBC and absolute neutrophil counts were measured at the beginning of the treatment. After 4 days of *W. somnifera* treatment a single dose of paclitaxel (1 mg/kg, i.v.) was injected. The administration of *W. somnifera* (200 mg/kg) continued until 8 days of post paclitaxel injection. Total WBC and absolute neutrophil counts were measured on day 0,

3, 5, 8, 12 and 18 after paclitaxel administration.

Effect of GM-CSF on paclitaxel induced changes in total WBC and absolute neutrophil counts: GM-CSF (25 mcg, s.c.) was administered after 5 min. of paclitaxel administration. It was continued until 8 days of post paclitaxel injection. Total WBC and absolute neutrophil counts were measured on day 0, 3, 5, 8, 12 and 18 after paclitaxel administration.

The total WBC and absolute neutrophil counts of *W. somnifera* and GM-CSF treated group were compared with paclitaxel control group. Total WBC and absolute neutrophil counts values were represented in mean \pm sem and results were analysed with ANOVA and students paired "t" test was used to calculate statistical significance.

RESULTS

Effect of paclitaxel on total WBC and absolute neutrophil counts: After intravenous injection of a single dose of paclitaxel 1 mg/kg a significant fall in total WBC count was observed on day 3 (8875 ± 1259) and day 5 (9260 ± 1843) as compared to counts before paclitaxel administration (12950 ± 1285) (Fig. 1). It did not return to normal even after day 18 of paclitaxel injection. Paclitaxel also caused significant fall in absolute neutrophil counts on day 3, 5 and day 8 to 385 ± 79 , 736 ± 118 and 1052 ± 191 as compared to counts before paclitaxel administration (2056 ± 103) respectively (Fig. 2). All the animals survived till the study period (18 days).

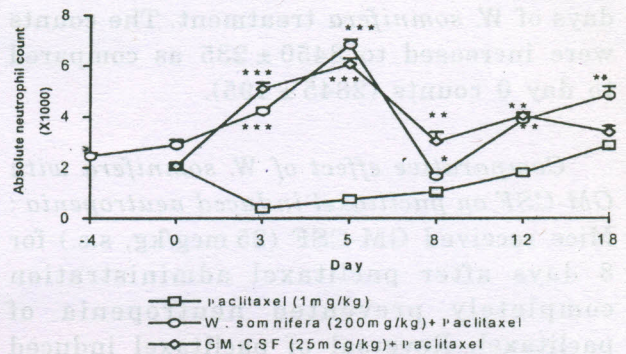


Fig. 1: Effect of *W. somnifera* aqueous extract and GM-CSF on paclitaxel induced changes in total WBC counts in mice. Each value represents Mean \pm SEM of total WBC Counts. *P<0.05, **P<0.01 and ***P<0.001 Compared to paclitaxel control.

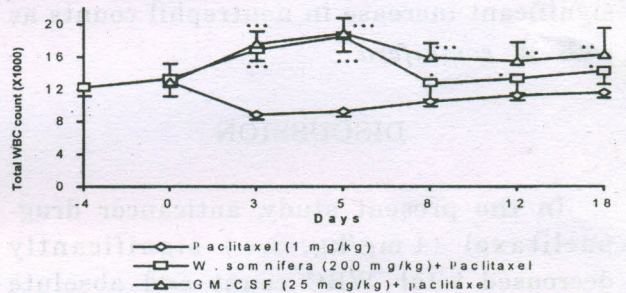


Fig. 2: Effect of *W. somnifera* aqueous extract and GM-CSF on paclitaxel induced neutropenia in mice. Each value represents Mean \pm SEM of absolute neutrophil Counts. *P<0.05, **P<0.01 and ***P<0.001 Compared to paclitaxel control.

Effect of W. somnifera on paclitaxel induced neutropenia: Mice that received *W. somnifera* (200mg/kg, p.o.) for 4 days before and 8 days after paclitaxel administration showed significant increase in absolute neutrophil counts on day 3 (4226 ± 121), day 5 (6845 ± 188) and day 8 (1657 ± 147) as compared to paclitaxel treated group (385 ± 79 , 736 ± 118 and 1052 ± 191) respectively (Fig. 2). *W. somnifera* (200 mg/kg, p.o.) per se produced significant increases in absolute neutrophil counts. Maximum increase was observed after 8

days of *W. somnifera* treatment. The counts were increased to 8450 ± 235 as compared to day 0 counts (2845 ± 195).

Comparative effect of W. somnifera with GM-CSF on paclitaxel induced neutropenia: Mice received GM-CSF (25 mcg/kg, s.c.) for 8 days after paclitaxel administration completely prevented neutropenia of paclitaxel. Reversal of paclitaxel induced neutropenia by *W. somnifera* (200 mg/kg, p.o.) was similar to GM-CSF (25 mcg/kg, s.c.) (Fig. 2). Similar reversal was observed on paclitaxel induced changes in total WBC counts. GM-CSF per se also produced significant increase in neutrophil counts as with *W. somnifera*.

DISCUSSION

In the present study, anticancer drug-paclitaxel (1 mg/kg, i.v.) significantly decreased total WBC count and absolute neutrophil counts. Aqueous extract of *W. somnifera* per se produced significant increase in neutrophils counts. Others have also shown that *W. somnifera* induces neutrophilia (5). Four days pretreatment followed by 8 days posttreatment with *W. somnifera* significantly reversed paclitaxel induced neutropenia and changes in total WBC counts. It has been reported that ashwagandha increased the haemoglobin, total red blood cells and white blood cells in mice with chemically suppressed immunity (5). Methanolic extract of ashwagandha significantly increased total WBC counts in normal Balb/c mice and reduced leucopenia induced by sublethal dose of gamma radiation (5). Recently it has been suggested that ashwagandha

significantly increases bone marrow cellularity (8). It has ability to enhance stem cell proliferation and also stimulate spleen and thymus cells and thus enhance the immune system. Ashwagandha inhibited tumour growth and increased tumour free survival in a dose dependent manner in mice (5). Ashwagandha treatment before irradiation synergistically increased survival time to 120 days even in advanced tumour (9). Withaferin A, a steroidal lactone from the roots of *Withania somnifera* also has antitumor activity against Ehrlich ascites carcinoma cells (10, 11) and radiosensitizing effect on experimental mouse tumours (12, 13). Ashwagandha prevented myelosuppression induced by cyclophosphamide, azathioprine or prednisolone (7). In the present study, *W. somnifera* prevented paclitaxel induced leucopenia and neutropenia. Though the exact mechanism of action of *W. somnifera* is still unknown, the enhanced production of growth factors such as GM-CSF by *W. somnifera* has been postulated (8).

Protective effect of *W. somnifera* against paclitaxel induced neutropenia indicates that it is a potential agent to be evaluated clinically for its effect on anticancer treatment induced myelosuppression. The presently used agents GM-CSF and G-CSF though effective, have a major limitation due to their exorbitant cost. *W. somnifera* could be an indigenous and a economic alternative to reduce side effects of anticancer drugs. Additionally its immunostimulant property will help in improving cancer treatment strategies.

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