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RESEARCH ARTICLE

CELLULAR IMMUNITY OF ASHWAGANDHA AGAINST DOXORUBICIN TOXICITY

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ABSTRACT

Doxorubicin is one of the important antitumor agents having a variety of therapeutic potency against variety of human tumors including soft tissue sarcoma, breast cancer, small cell carcinoma of the lung and acute leukemias. Similarly it has toxic effect on various parts of the body especially on immune system and heart. Whenever this drug is used on cancer patients, its toxicity acts on immune system of the patients by depressing the bone marrow. It has been suggested that Ashwagandha plays an important role in immune system regulation, but its impact on toxicity produced by cancer chemotherapy is still obscure. In present investigation to evaluate the efficacy of Ashwagandha against Doxorubicin (anti cancer drug) toxicity lymphocyte subpopulations activity was examined in Rats. After administration of Doxorubicin @ 5 mg/kg b.w. Intraperitoneal (I.P.) to rats marked reduction in the number of $CD8^+$ cells and in the proportion of $CD4^+$ cells were observed on day 21st. When Ashwagandha (300mg/kg b.w.) administered five days prior to Doxorubicin administration and continued for 21 days ((21 days was counted from the first exposure of Doxorubicin)) then significant increase in the number of $CD8^+$ cells and in the proportion of $CD4^+$ cells were observed. Thus findings of present investigation showed that Ashwagandha acts as an immunomodulator for cellular immunity and ameliorate the toxicity produced during cancer chemotherapy by mitigating the bone marrow depression.

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INTRODUCTION

There are several approaches used for treating cancer including chemotherapy. One of the most popular chemotherapeutics is doxorubicin. However, its clinical use is limited due to its side effects in high- and repeated-doses. The use of the drug induced cardio toxicity and affected the immune functions (Santos *et al*., 2010). Doxorubicin is a member of the Anthracyclin drug family and one of the most frequently used drug to treat many forms of cancer such as leukemia, lymphoma and solid tumors (Singal *et al*., 1995). Doxorubicin is one of the important antitumor agents having a variety of therapeutic potency against variety of human tumors including soft tissue sarcoma, breast cancer, small cell carcinoma of the lung and acute leukemias. Doxorubicin is one of the most popular chemotherapeutics (Tan *et al*., 2009). Reportedly, doxorubicin suppressed the production of IL-2, INF-gamma, lymphocyte proliferation and CD4+/CD8+ ratio in tumour-bearing mice (Zhang *et al*., 2005). Chemotherapy is assumed to be immunosuppressive; Doxorubicin with combination chemotherapy caused a significant and persistent decrease in B-cell numbers (Sara *et al.,* 1999). Humans have variable effects on different components of the immune system. For example, lymphocyte depletion in human patients undergoing chemotherapy has been reported, but the degree of lymphocyte depletion appeared to be dependent on the particular chemotherapy protocol (Harris *et al*., 1976) Lymphocyte depletion, specifically, depletion of CD4+ T cells, may persist long after completion of chemotherapy (Azuma *et al*., 1998). A series of animal studies show ashwagandha to have profound effects on the haematopoietic system, which acts like a immunoregulator and chemoprotective agent (Kuttan *et al.,* 1996). Thus present investigation was aimed to evaluate cellular immunity of Ashwagandha to ameliorate the toxicity produced during cancer chemotherapy so that the patient could easily get full cycle of chemotherapy.

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MATERIALS AND METHODS

Animals

In the present investigation, experiments were performed on 16 -18 weeks old healthy charles foster rats. For the optimal growth and development, the rats were kept in ideal condition under a well regulated light and dark (12h:12h) schedule at 23±1°C in the animal house, Mahavir cancer Institute & Research centre, patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) and the experiment was duly approved by the IAEC. Animals were given food and water *ad libitum*.

Doxorubicin

Drug was procured from pharmacy of Mahavir cancer institute.

Ashwagandha

Dry root of *W. somnifera* (Ashwagandha) were purchased from Haridwar Medicinal Store, Haridwar, Uttrakahand, India. The identity of the medicinal plant was confirmed by Prof. Ashok Kumar Ghosh (Botanist), Department of Environmental Science, A. N. College, Patna. Preparation of aqueous root extract : 10g of root powder was dissolved in 100ml of distilled water in a conical flask and boiled at 100·C in water bath for 6 hrs and then filtered through Whatmann no.1 filter paper. The filtrate was then stored at room temperature for further study.

MATERIALS AND METHODS

Study Design

Eighteen rats were used in the study and were grouped into three groups.

Group A: 6 untreated rats kept as control and served with equal volume of distilled water by gavage method.

Group B: rats treated with Doxorubicin @ 5 mg/kg b.w.

Group C: Ashwagandha (300mg/kg b.w.) administered five days prior to Doxorubicin administration and continued for 21 days (21 days was counted from the first exposure of Doxorubicin). Blood extracted from control (Gr. A), Doxorubicin treated group (Gr. B) and (Gr. C) Doxorubicin (5 mg/kg b.w) along with Ashwagandha (300mg/kg b.w.) of rats on day 5^{th} and day 21^{st} for CD4 & CD8 count.

Collection of Blood

The blood from the control and treated rats were obtained from heart puncture. Rats were anaesthetized for this purpose. Collection of blood from heart puncture is one of the most effective methods, which causes least stress to the animal. The blood was collected in EDTA vaccutainer tube for CD4 & CD8.

Protocol for CD4 & CD8

Take 4 FACS tube and labelled each tube as IC, CD4, CD8 and CD4+CD8. Take 100 µl blood in each labelled FACS tube. Add 1 µl CD4 Antibody in CD4 labelled FACS tube, 1 µl CD8 Antibody in CD8 labelled FACS tube and 1-1 µl from both CD4 & CD8 Antibody in CD4+CD8 labelled FACS tube. Incubate for 30 minutes at Room Temp in dark. Further add 1 ml FACS lysis buffer in each tube. Again incubate for 20 minutes at Room Temp at dark. Centrifuge at 1500 RPM for 10 minutes. Decant and wash with 2 ml PBS. Suspend in 400 µl PBS. Then Acquire at FACS (Fluorescence Activated Cell Sorting) machine.

Statistical analysis

Data were analyzed with statistical software (Graphpad Prism 5) and values were expressed as Mean ± SEM. And differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) using the Dunnett's test.

RESULTS AND DISCUSSION

Analysis of CD4 & CD8 count for cellular immunity of Ashwagandha.

There was significant statistical difference ($p < 0.001$) was observed in the CD4 & CD8 count of Doxorubicin treated group in 21 days treatment with compare to control. A significant increase however was seen in the count of CD4 & CD8 of Doxorubicin along with *Ashwagandha* treated group in 21 days treatment. CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. CD4+ T helper cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells. They are called helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle.

Table 1. Effects of Doxorubicin and *Withania somnifera* on CD4 count

Control Gr. I	DOX (5 mg/kg) Day 5th Gr. II	DOX (5 mg/kg) Day 21st Gr. II	DOX (5mg/kg) + W.S. (300mg/kg) Day 5th Gr. III	DOX (5mg/kg) + W.S . (300 mg/kg Day 21st Gr. III
47.37 ± 1.737	45.65 ± 1.056	38.03 ± 1.264	42.25 ± 1.264	43.40 ± 1. 650

Values are expressed as Mean ± SEM one way ANOVA followed by Dunnet's test, Treated groups are compared with control group.

Table 2. Effects of Doxorubicin and *Withania somnifera* on CD8 count

Control Gr. I	DOX (5 mg/kg) Day 5th Gr. II	DOX (5 mg/kg) Day 21st Gr. II	DOX (5mg/kg) + W.S . (300 mg/kg) Day 5th Gr. III	DOX (5mg/kg) + W.S. (300 mg/kg Day 21st Gr. III
18.15 ± 1.207	17.10 ± 0.096	11.65 ± 0.668	12.08 ± 0.131	17.81 ± 0. 727

Values are expressed as Mean ± SEM one way ANOVA followed by Dunnet's test, Treated groups are compared with control group.

Thus present investigation was aimed to evaluate the efficacy of Ashwagandha against Doxorubicin (anti cancer drug) toxicity, lymphocyte subpopulations (CD4 & CD8) activity was examined in Rats. In the present investigation ashwagandha showed marked increases in the CD4 & CD8 count after bone marrow suppression induced by Doxorubicin. This increase in the CD4 & CD8 count was observed through out in one cycle of 21 days. The actions of WS on the immune system are subtler than simply suppressing the immune/ inflammatory response. WS modulates the immune response, increasing the expression of T-helper 1 (Th1) cytokines, as well as CD4 and CD8 counts, and natural killer (NK) cell activity (Bani *et al*., 2006, Davis *et al*., 2002, Khan *et al*., 2006) which is in support of my work. Recent research suggests a possible mechanism behind the increased cytotoxic effect of macrophages exposed to W. somnifera extracts (Davis *et al* 2000). Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganisms and tumor cells. Iuvone *et al* demonstrated Withania somnifera increased. No production in mouse macrophages in a concentration-dependent manner. This effect was attributed to increased production of inducible nitric oxide synthase, an enzyme generated in response to inflammatory mediators and known to inhibit the growth of many pathogens (Iuvone *et al*., 2003). Ashwagandha exhibited stimulatory effects, both in vitro and in vivo, on the generation of cytotoxic T lymphocytes, and demonstrated the potential to reduce tumor growth The chemopreventive effect was demonstrated in a study of ashwagandha root extract on induced skin cancer in Swiss albino mice given ashwagandha before and during exposure to the skin cancercausing agent 7,12-dimethylbenz[a]anthracene. An in vitro study showed withanolides from Withania somnifera inhibited growth in human breast, central nervous system, lung, and colon cancer cell lines comparable to doxorubicin. Withaferin A more effectively inhibited growth of breast and colon cancer cell lines than did doxorubicin. These results suggest Withania somnifera extracts may prevent or inhibit tumor growth in cancer patients, and suggest a potential for development of new chemotherapeutic agents (Prakash *et al*., 2002)

Conclusion

It is known that doxorubicin is a potent anti-cancer drug, which is used in chemotherapy, but on the other hand it exerts toxicity on body parts and weakens the immune system of the patient, therefore patients suffers from other ailments like fever, low haemoglobin & platelet counts, and gastrointestinal disorders etc & unable to receive another cycle of chemotherapy. Thus findings of present investigation showed that Ashwagandha acts as an immunomodulator for cellular immunity against cancer chemotherapy drug. By doing this we could enhance the efficacy of the drug & also will ease the burden of the treatment.

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