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RESEARCH ARTICLE

Therapeutic Study of Saffron against Doxorubicin Toxicity in the Management of Cancer Chemotherapy

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Abstract

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. Doxorubicin is an essential component of treatment for childhood solid tumors and aggressive lymphomas and shows activity in acute lymphoblastic or myeloblastic leukemias. The use of the drug induced cardio toxicity and affects the immune functions. This toxic side effect makes the problem during cancer chemotherapy causes myelosuppression, mucosal ulceration, alopecia and diarrhoea etc.

The aim of the study was to evaluate therapeutic impact of saffron in animal model for ameliorating the toxic side effects being produced during doxorubicin administration.

In the present investigation saffron @ 8 mg/kg b.w. was used against administration of doxorubicin @ 5 mg/kg b.w. in rats. Red Blood Cell Count, White Blood Cell count, Platelets, and Absolute Lymphocyte Count were observed on day 5th. A marked reduction in total count of RBC, WBC, ALC, & Platelets were observed on day 5th. When Saffron (8mg/kg b.w.) administered five days prior to Doxorubicin administration and continued for 10 days, on day 15th blood extracted for haematological analysis. Significant increase in total count of WBC, ALC and Platelets were observed while there is no significant ($P > 0.05$) statistical difference was observed in RBC.

Thus findings of present investigation showed that therapeutic potency of saffron help to ameliorate the toxicity produced during cancer chemotherapy.

INTRODUCTION

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). However, its clinical use is limited due to its side effects in high and repeated doses. The use of the drug induced cardiotoxicity and affects the immune functions (Santos et al., 2010). Reportedly, doxorubicin suppressed the production of IL-2, INF-gamma, lymphocyte proliferation and CD4+/CD8+ ratio in tumour-bearing mice (Zhang et al., 2005).

Since Doxorubicin is a very good anti-cancer drug which is used for the treatment of solid tumors, 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. Thereafter

receiving the doxorubicin as a chemotherapy cycle, patients looses their immunity so fast, which in turns patients unable to receive another cycle of doxorubicin. Thus present investigation was aimed to evaluate therapeutic potency of Saffron in animal model to ameliorate the toxicity produced during cancer chemotherapy. The out come of the study could be implementing for clinical use so that patient could easily get full cycle of cancer chemotherapy.

Botanical name of Saffron is *Crocus sativus* (*C. sativus*) and it is perennial stemless herb of the Iridaceae family . Cultivation of saffron is widely practice in Iran and Spain and other countries, such as Italy, India, and France are producing at low scale. Active principle of Saffron have shown the presence of constituent such as crocin, croetin, safranal and picrocrocin (Abolhasani et al., 2005). Petals of Saffron contains anthocyanins, glycosides, and flavonoids (Gil et al., 2002). Kaempferol glycoside is the major flavonols (84.0% of total flavonol content) in saffron (C. sativus) (Goupy et al, 2013). Saffron have antitumor (Abdullaev et al., 2004; Abdullaev 2001; Das et al., 2004), antioxidant (Ranmadan et al., 2010; Assimopoulou et al., 2005; Sanchez -vioque et al., 2010; Goli et al., 2010) property. The immune system has been involved in the etiology and pathology of many diseases and immune modulation helps to improve as well as controlling the diseases.

Materials and Methods

Animals

In the present investigation, experiments were performed on 14-16 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.

Saffron (*Crocus sativus*) : Saffron was purchased from Haridwar Medicinal Store, Haridwar, Uttrakahand, India. The identity of the medicinal plant was confirmed by Dr. Shikha Choudhury (Botanist), Department of Botany, S.N.S.R.K.S College, Saharsa, Bihar, India. The stigma of *Crocus sativus* is used in herbal medicines, and the stigma extract was prepared as follows:

Extract of stigmas were prepared using maceration with ethanol (80%, v/v) for 3 days and subsequently, the mixture was filtered and concentrated under reduced pressure at 35 °C. Finally, the extracts were dissolved in normal saline (Hossienzadeh et al., 2002; Akhondzadeh Basti et al., 2007) for further study.

Methodology

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 intra peritoneally @ 5 mg/kg b.w. Group C: Saffron (8mg/kg b.w.) administered five days prior to Doxorubicin administration and continued for ten days. Blood extracted from control and treated group of rats on day 5^{th} after Doxorubicin administration. In third group Saffron (8mg/kg b.w.) administered five days prior to Doxorubicin administration and blood taken on 15^{th} day for total count of WBC, RBC, Platelets & ALC.

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 haematological (WBC, RBC, Platelets & ALC) study.

White Blood Cell Count (WBC) - A 1:20 dilution of blood was made by adding 10 µl of blood to 200 µl of wbc diluting fluid in a plastic tube. After tightly corking the tube the suspension was well mixed by rotation. The improved Neubauer counting chamber was loaded with the diluted blood by means of pasteur pipette. The loaded counting chamber was allowed for two minutes for cells to settle, after which the preparation was viewed under the microscope 10 mm objective. The cells were counted in the 4 large corner squares of the counting chamber. The calculation of total white blood cells was made using the formula N x 2.5 x 20

Red blood cell count (RBC) - A 1:200 dilution of blood was made by adding 10µl of blood to 2000 µl of wbc diluting fluid in a plastic tube. A clean dry improved Neubauer counting chamber with cover slip already in position

was loaded with diluted blood using pasteur pipette. The chamber was left undisturbed for 2 minutes for the cells to settle. The cells were counted under the microscope using 40 mm objective. Cells were counted in 80 small squares in the central ruled area of the counting chamber. The calculation of Red blood cells was made using the formula Nx50 x 200.

Platelets Count – Thin film of blood smear was made and stained by Leishmann's stain. Observation was made at 100 x magnification. Number of thrombocytes observed at five fields and after averaging of five fields, calculated value was multiplied by 20,000. (N x 20,000)

Absolute Lymphocyte Count – Absolute lymphocyte count was made by multiplying the total number of WBC with percentage of lymphocyte. (ALC = Total no. of wbc x % of lymphocyte)

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

Analysis of Haematological Parameters

Table - 1

Parameter	Control (n = 6) Gr. I	Doxorubicin (n = 6) Gr. II	Saffron + Doxorubicin Treated (n = 6) Gr. III
RBC (10^6 / cumm)	4.093 ± 0.1116	3.258 ± 0.1647	3.456 ± 0.0312
WBC (cumm)	8400 ± 4843.6	4100 ± 266.7	7500 ± 432.6
ALC (lymphs/mm3)	4900 ± 212.7	1978 ± 212.4	4654 ± 321.3
PLT (cumm)	3,89,252 ± 17911	1,67,876 ± 15985	3,56,674 ± 8434

Values are expressed as Mean ± SEM, one way ANOVA followed by Dunnet's Test, Treated groups are compared with control group. RBC = Red Blood cells, WBC = White Blood Cells, ALC = Absolute Lymphocyte Count , PLT = Platelets.

There was significant statistical difference ($p < 0.001$) was observed in the WBC of Doxorubicin treated group with compare to control. A significant increase however was seen in the *Saffron* treated group of WBC. There was also significant statistical difference ($p < 0.001$) were observed in the Absolute Lymphocyte Count (ALC), & Platelets (PLT), with compare to control. A marked significant statistical increase ($p < 0.001$) was observed in the ALC, WBC, & PLT during the period of the study in the *Saffron* treated group except RBC count. There was no significant statistical difference ($p>0.05$) in the RBC count was seen in all groups with compare to control during the period of the study.

Discussion

Crocus sativus L. (C. sativus) commonly known as saffron, has been undertaken for the study of immunomodulation against Doxorubicin toxicity.Various studies have shown that saffron extract has antioxidant (Tajali et al., 2008), antinociceptive and anti-inflammatory (Hosseinzadeh, et al, 2002), and antidepressant (Akhondzadeh Basti et al., 2007; Hossienzadeh et al., 2007) effects. But effects of alcoholic extract of saffron on immune system have not been reported. Thus the aim of the present investigation was to evaluate the effects of Saffron extract in animal model for the management of cancer chemotherapy by mitigating the toxicity on immune system.

There are various herbal preparations which alter immune function and display an array of immunomodulatory effects (Sarang et al., 2010). In various in vitro and in vivo studies, herbal medicines have been reported to modulate cytokine secretion, histamine release, immunoglobulin secretion, cellular co-receptor expression, lymphocyte activation, and phagocytosis (Patwadhan et al., 2005; Plaeger, 2003). Despite of that their clinical utility is limited and associated with complications (Wieland et al., 2005). This investigation is done for the first time with saffron on hematological parameters fro the management of cancer chemotherapy was evaluated in rats.
The results obtained from this study indicated that use of saffron stigmas at a dose of 8 mg/kg has a clear immunostimulatory effect as it was shown by the significant increase in Absolute Lymphocyte count (ALC). The results of our study indicated that use of saffron stigmas at the doses of 8 mg/kg has promising effect on hematological parameters. In the present investigation blood thickening was not observed as Doxrubicin itself responsible for thickening of blood which causes difficulties during cancer chemotherapy, therefore Saffron played a vital role in thinning of blood. The present result showed that, use of saffron causes an increase in the number of white blood cells (WBC) in treatment groups and also control group which indicates immunemodulatory activities of saffron which is essential for the management of cancer chemotherapy. RBC count showed no significant difference ($p>0.05$) between the control and treated groups with extract of saffron. The results indicated that use of saffron at dose of 8 mg/kg causes an increase in antibody response. The effects of flavonoids of Saffron on immune responsiveness might have a variety of pathways for activating the immune cells. Activated immune cells generate free radicals and increase oxidative stress (Costantini, 2006; Horak et al., 2007), while T cell and B cell based immune reactions are highly sensitive to oxidative stress(Von Schant et al., 1999). Saffron help to activate network of immune response regulated by the T helper (Th) cells which can stimulate the B lymphocyte to produce IgG. In this way saffron probably activated some of these effects by stimulating the secretion of Th cell cytokines such as IFN-γ which is the main cytokine to stimulate the B lymphocytes for IgG production. Thus saffron helps to increase the secondary immune responses (IgG) against doxorubicin toxicity and helps to combat the toxic side effects of cancer chemotherapy.

Conclusion

Doxorubicin is a good anti cancer drug and being used in variety of cancer cases but its toxicity causes myelosuppresion, mucosal ulceration and alopecia etc, hence management of cancer chemotherapy has become challenge. The present investigation was aimed to combat the toxicity of Doxorubicin through ethnolic extract of immunomodulator plant like Saffron as a adjuvant therapy. Saffron (8 mg/kg b.w.) administered five days prior to Doxorubicin administration and continued for ten days then significant increase in total count of WBC, ALC and Platelets were observed after treatment but there is no significant statistical difference in the RBC count was seen in all groups during the period of the study.
. Thus findings of present investigation showed that therapeutic potency of Saffron ameliorate the toxicity produced during cancer chemotherapy by mitigating the bone marrow depression.

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