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Frankincense Derivatives Enhanced Bone Marrow and Spleen Cellularity Following Busulfan - Cyclophosphamide Conditioning Regimen

Sulaiman Al-Hashmi*, Saeid Vakilian, Fatemeh Jamshidi-adegani, Juhaina Al-Kindi, Abdulaziz Al-Dhuhli, Mohammed Al-Broumi, Ahmed Al-Harrasi

Laboratory for Stem Cell & Regenerative Medicine, Natural and Medical Science Research Center (NMSRC), University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman.

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***CORRESPONDING AUTHOR**
Dr. Sulaiman Al-Hashmi
0000-0002-4010-116X

Email: sahashmi@unizwa.edu.om

ABSTRACT

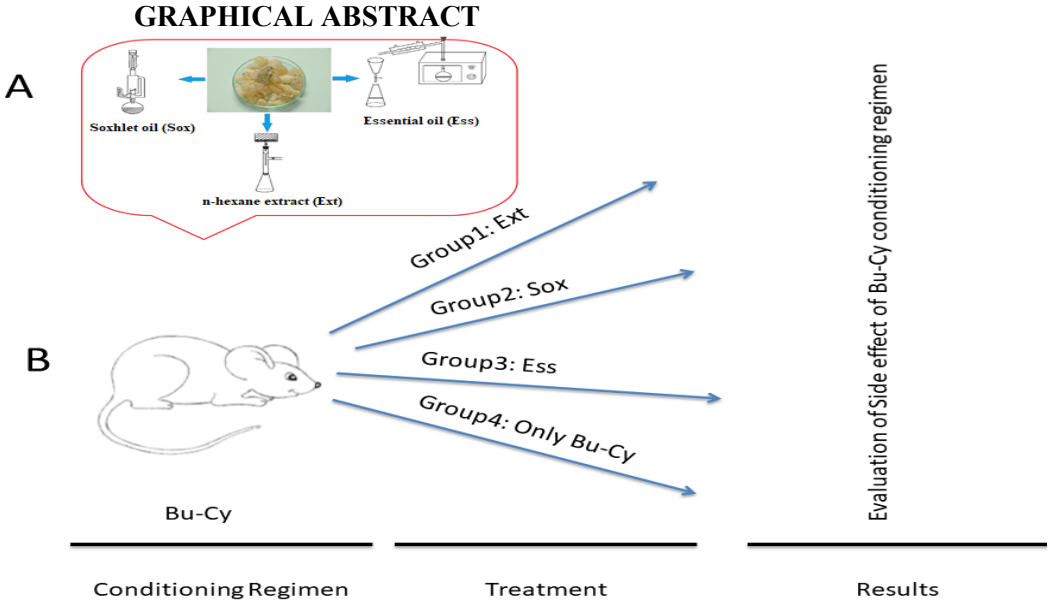
Background and Aim: The power of chemotherapy on prolonging and improving the life of patients is undeniable, but it often consists of various side effects. Therefore, developing of effective strategies for reduction of chemotherapy drugs toxicity is necessary. Natural products including frankincense - derived products have been demonstrated to reduce chemotherapy side effects. The aim of this study was to diminish the side effects of Busulfan - Cyclophosphamide (Bu - Cy) regimen as chemotherapeutic agents with the aid of frankincense derivatives administration in the mice model.

Methods: The chemotherapy conditioning regimen was created in Female Balb / c mice by intraperitoneal injection of 60 mg Busulfan and 150 mg Cyclophosphamide per kg of mice. Frankincense derivatives including essential oil (Ess), soxhlet oil (Sox) and n-hexane extract (Ext) were extracted and injected intraperitoneally to chemo - conditioned mice.

Results: As expected, mice treated with Bu - Cy chemotherapy had lower bone marrow cells count and spleen index compared to the control, whereas frankincense derivatives helped reducing side effects and modifying immune system and general health.

Conclusion: Despite that all three extracts have decreased the side effects of chemotherapy; Ext could simultaneously increase the spleen index and the bone marrow cells count and also improve the health in comparison with the negative control group.

GRAPHICAL ABSTRACT



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INTRODUCTION

Cancer is a widespread disorder and is one of the leading causes of death. Chemotherapy is most commonly used to treat various types of cancers, but may cause myelosuppression such as a decrease in peripheral blood, spleen index and bone marrow cells, which may lead to anemia, haemorrhage and infection (1). Since myelosuppression is considered as a side effect of chemotherapy, the treatment of that can have numerous benefits for cancer therapy. In order to accelerate the hemopoietic recovery during therapy, hematopoietic growth factors (HGF), such as erythropoietin (EPO), granulocyte - macrophage colony - stimulating factor (GM - CSF), and thrombopoietin (TPO) can be used. But the mentioned regimens will be costly and cause unwanted effects like allergic reaction (2, 3).

Busulfan (Bu) and Cyclophosphamide (Cy) are in the class of most successful antineoplastic alkylating agents as well as they have immunosuppressive roles and commonly have been used for cancer treatment like chronic myeloid leukemia. Cyclophosphamide is routinely administered with Busulfan (Bu) as a conditioning regimen for bone marrow transplantation and treatment of wide variety of cancers including various types of leukemia, ovarian cancer and breast cancer etc. but this conditioning regimen was reported to cause early and long-term toxicities, leading to a high amount of transplantation-associated death (4, 5) and also patient who are undergoing chemotherapy are susceptible to different types of bacterial and fungal infection (6-9). Since numerous people are suffering from chemotherapy drugs, the reduction of side effect of these drugs is necessary.

Frankincense is an aromatic resin derived from Boswellia trees. Frankincense derivatives (oils, extracts, pure resin, etc.) reported with potent medicinal applications including their anti-mutagenic and apoptotic properties. In addition to their ability to diminish cancer cells, they promote health and vitality in neighboring non-cancerous cells. Either that active component is boswellic acid or other chemicals in the derivative extracts, researchers found that these compounds alongside conventional chemotherapy may act as a novel treatment for ovarian cancer. Also some studies have proven similar effects on bladder, breast, colon, skin, stomach and pancreatic cancers (10-15).

The benefits of frankincense essential oil may go beyond its anti - tumor effects. In particular, it can manage the side effects of chemotherapy, which can be worse than the symptoms of cancer itself. It has demonstrated that frankincense can be used as a treatment for cerebral edema in brain cancer patients, avoiding the side effects of steroid therapy. The essential oil is also useful to remove struggles related to immune system, oral health and stress / anxiety. Researchers discovered that multiple levels of immune systems were stimulated by Boswellia serrata injection including: delayed hypersensitivity reaction, IgG, IgM, cytokines and T-cell interactions (15-18).

Frankincense derivative compounds have been shown to be beneficial for controlling side effects related to either cancer or chemotherapy. Chemotherapy (Bu - Cy) has influential side effects, including the suppression of immune system and bone marrow. So, the present study aimed to use the established mouse model of Busulfan and Cyclophosphamide conditioning regimen (19, 20) and to study the impact of frankincense derivative compounds on the side effect of this regimen.

METHODS

Plant material collection

Royal Hougari White (RHW) frankincense (*Boswellia sacra*) was collected from Hougar Mountain, Salalah in 2012 and identified by a taxonomist, Natural and Medical Sciences Research Center, University of Nizwa. The voucher specimen (RHW-02 / 2012) of the resin was deposited in the herbarium of the Natural & Medical Sciences Research Center, University of Nizwa, Oman. The air dried gum resin of frankincense (10 g) was ground to a fine powder and used to extract ESS, Sox, and Ext.

Extraction and isolation

The extraction and isolation was done according to the *Al-Harrasi et.al.* publication (21). Before extraction, all resin samples were dried under subdued light, grounded to fine powder and kept in sterile containers. Air-dried regular grade resin of hougari frankincense was processed using methanol extraction method. After evaporation of the extract, it was loaded to vacuum liquid column chromatography over a silica gel column (1000 g, 70-230 mesh, Merck) with the aid of pure n-hexane as a solvent mobile phase to obtain n-hexane extract (Ext). Soxhlet apparatus was applied to extract the Soxhlet oil (Sox) using n - hexane. Essential oils (Ess) was isolated from the resin of hougari frankincense using microwave hydro - distillation method.

Animal study

Female Balb / c mice (10-14 weeks old) were obtained from the laboratory animal house of The University of Nizwa. The animals were allowed to acclimatize for two weeks before the start of the experiments. Animals were randomly organized into five groups, each of five mice. All mice were received standard food and water ad libitum in the pathogen - free animal house under controlled conditions. Group 1 received PBS as a standard control. The other four groups of mice received condition regimen as previously reported (19, 20). In brief, Busulfan (Bu, Sigma - Aldrich), 20 mg / kg

body weight, was injected intraperitoneally once daily for three consecutive days. After Bu administration, the animals received intraperitoneal injections of Cyclophosphamide (Cy, Sigma - Aldrich), 75 mg / kg body weight, once daily for two consecutive days. The mice then kept for two days followed by an intraperitoneal injection of Ess, Sox or Ext each of 200 mg / kg divided for three days. The mice's weight and general health were followed until the experiment end point. After three weeks, all mice were scarified and spleen and one femur were harvested from each mouse for evaluating weight and cellularity, respectively.

Bone marrow cellularity

Each mouse femur was carefully harvested to avoid bone fragmentation. The bone was then cleaned of surrounding tissues. A small incision was made on the hip side of the bone. The marrow was flushed out from the spongy tissue of the bone using a 21G needle connected to a 5ml syringe filled with PBS. The flushed marrow was processed into a single-cell suspension, and the total mononuclear cells were counted using a hemocytometer.

Spleen index

Following the dissection of the mice, each spleen was harvested, and all connected tissues were carefully removed. The spleens were subsequently dried using filter paper to eliminate any surface fluid. Finally, the spleens were weighed using an analytical balance (Sartorius, Germany).

Statistical Analysis

The variables were expressed as the mean ± standard deviation (SD). The Kuskal - Wallis test was employed to assess differences between the groups, and the specific mean ranks that exhibited a significant difference ($p < 0.05$) were determined using a one-way analysis of variance (ANOVA).

RESULTS

Effects of frankincense derivatives on the body weight of mice

The relative weight change of mice at different time points was calculated in comparison with the initial weight. The control group did not indicate any significant change in the weight, whereas noticeable depletions were observed in Bu - Cy - Sox group. In the Bu - Cy group, a decline in mouse weight was observed starting 11 days after the initiation of the conditioning regimen. However, 8 days thereafter, the mice began to regain their weight. Consistent reductions were observed across all treated groups using frankincense derivative compounds. Notably, these groups exhibited greater weight reduction when compared to the Bu - Cy group. However, statistically significant weight reduction was only observed in the Bu - Cy - Sox group on days 15, 17, and 21 ($P \leq 0.05$). This higher reduction in the weight of Bu - Cy - Sox - treated mice may be due to the metabolic effect. There was no noticeable difference between Bu - Cy - Ess and Bu - Cy - Ext. The most dramatic reduction in the relative weight of the mice was observed in the Bu - Cy - Sox group; further studies are needed to evaluate this finding.

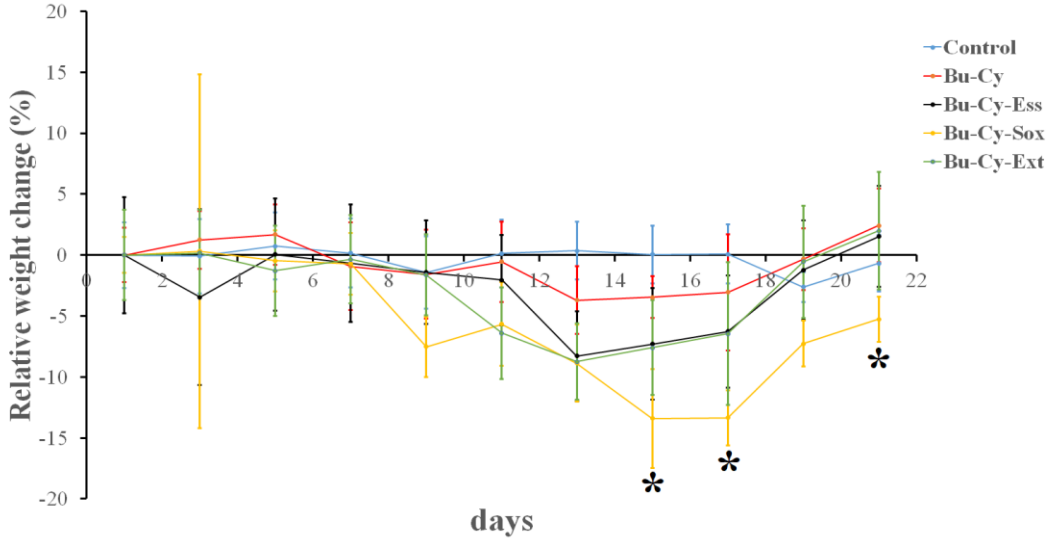

FIGURE 1. Relative weight changes of mice in the control group, Busulfan - Cyclophosphamide conditioning regimen (Bu - Cy), Bu - Cy regimen with intraperitoneal injection of essential oil (Bu - Cy - Ess), Bu - Cy regimen with intraperitoneal injection of n-hexane extract (Bu - Cy - Ext), and Bu - Cy regimen with intraperitoneal injection of soxhlet oil (Bu - Cy - Sox) groups over a period of 21 days. The asterisk (*) denotes statistical significance compared to the control group at a significance level of $P \leq 0.05$ (N = 5).

Investigation of bone marrow cells count

Bone marrow hematopoietic stem cells are responsible for adequate production of blood cells that play an important role in the immune system. Bone marrow is vulnerable to chemotherapy and one of the side effects of chemotherapy is

bone marrow suppression which in this study was reflected in the counts of the bone marrow cells as shown in figure 2. While the number of bone marrow cells in Bu - Cy group was less than control, in all groups treated with frankincense derivative compounds the number of MSCs was significantly more than the control group ($P \leq 0.05$). As well as, the highest number of bone marrow cells was obtained in both Bu - Cy - Ext and Bu-Cy-sox ($P \leq 0.05$), however, there was no significant difference between these two groups.

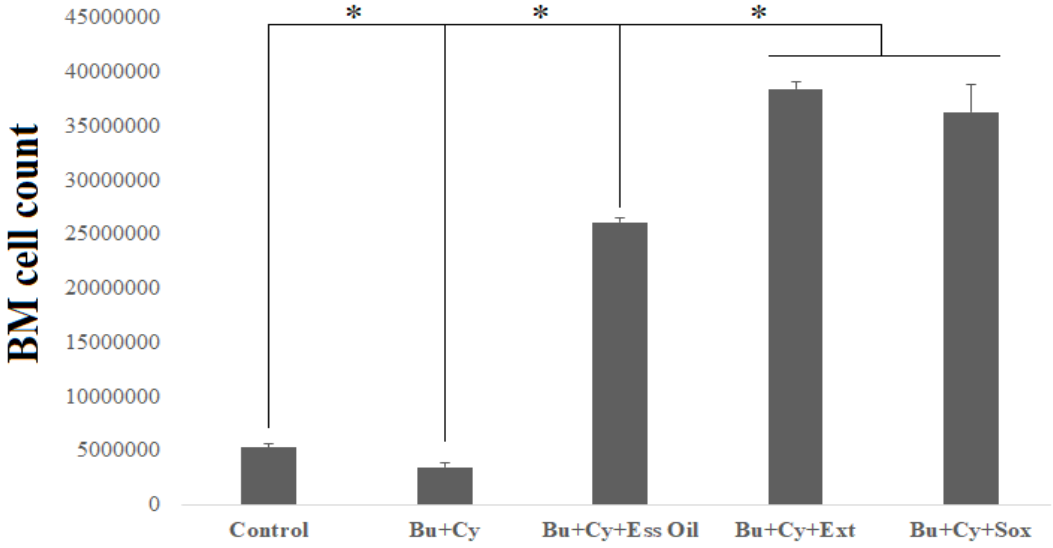

FIGURE 2. Bone marrow cells count of mice in the control group, Busulfan - Cyclophosphamide conditioning regimen (Bu - Cy), Bu - Cy regimen with intraperitoneal injection of essential oil (Bu - Cy - Ess), Bu - Cy regimen with intraperitoneal injection of n - hexane extract (Bu - Cy - Ext), and Bu - Cy regimen with intraperitoneal injection of soxhlet oil (Bu - Cy - Sox) groups. The asterisk (*) denotes statistical significance compared to the control group at a significance level of $P \leq 0.05$ (N = 5).

Spleen index evaluation

As shown in figure 3, spleen index in control group were 0.48±0.02, while that in Bu - Cy group was 0.31 ± 0.02. The spleen index in mice was significantly decreased by the chemotherapy treatment ($P \leq 0.05$). It is predicted that during chemotherapy, spleen will lose its weight mainly from the reduction of lymphocytes cells (B and T cells). All groups treated with frankincense derivative compounds significantly increased the spleen index in comparison to the Bu - Cy chemotherapy group. Among other groups, Bu - Cy - Ext spleen index is significantly more than the control group ($P \leq 0.05$), suggesting that Ext can reverse the atrophy of lymphoid organs (also known as secondary lymphoid organs).

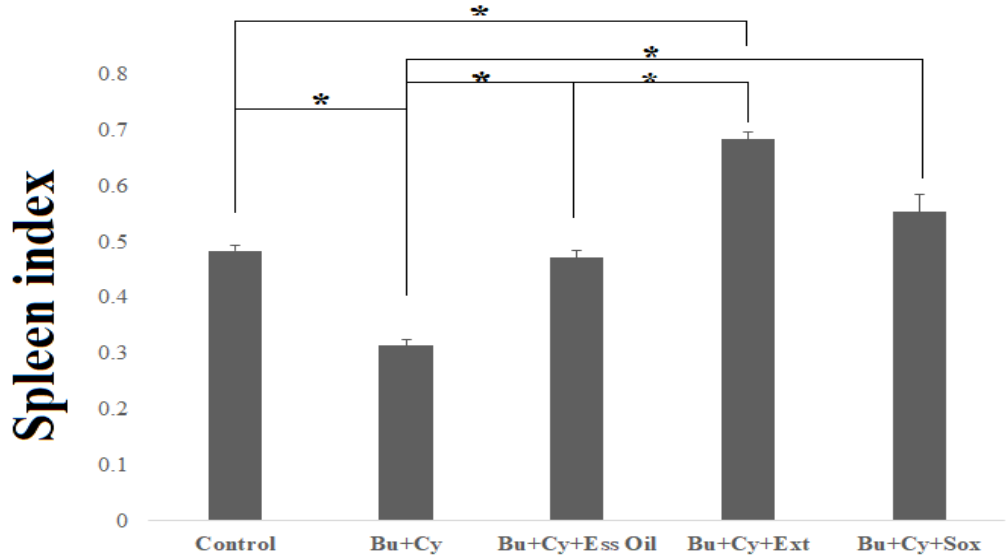

FIGURE 3. Spleen index of mice in the control group, Busulfan - Cyclophosphamide conditioning regimen (Bu - Cy), Bu - Cy regimen with intraperitoneal injection of essential oil (Bu - Cy - Ess), Bu - Cy regimen with intraperitoneal injection of n-hexane extract (Bu - Cy - Ext), and Bu - Cy regimen with intraperitoneal injection of soxhlet oil (Bu - Cy - Sox) groups. The asterisk (*) denotes statistical significance compared to the control group at a significance level of $P \leq 0.05$ (N = 5).

DISCUSSION

The herbal medicine has been shown to be effective in the reduction of chemotherapy induced myelosuppression (22). Liu et al. demonstrated the hematopoietic effects of Fufang E'jiao Jiang in mice with chemotherapy-induced myelosuppression (23). Moreover, Bhatia et al. have represented the protective efficacy of S - allylcysteine against cyclophosphamide - induced bladder hemorrhagic cystitis in a murine model (24). Frankincense is a resin that obtain from the trees of the genus Boswellia and has several medicinal applications. Many studies showed anti-inflammatory, anti - mutagenic and apoptotic properties of frankincense (12, 13). In this study, we investigated the effect of frankincense for the improvement of bone marrow and spleen cellularity.

Both Ext and Sox maximized bone marrow cell count and spleen index, while there was no significant difference between them. The maximal decrement in the weight of the mice was belonged to the Bu - Cy - Sox group, which may reflect possible toxicity of Sox. Therefore, it seems that Ext is the most appropriate frankincense derivative for improving bone marrow cell count and spleen index, and herby managing the chemotherapy side effects.

Furthermore, the study demonstrated that frankincense derivatives exhibit potential immunetropic properties. The findings of this investigation offer a novel perspective on the advantageous effects of frankincense derivatives in relation to bone marrow transplantation outcomes. Additionally, enhancing bone marrow and spleen cellularity subsequent to bone marrow transplantation could lead to reduced engraftment time, thereby decreasing the risk of infection and hospitalization. Further studies are warranted to assess the potential toxicity associated with frankincense derivatives and to apply these extracts in a bone marrow transplantation model for comprehensive evaluation.

CONCLUSION

In conclusion, the effects of three derivatives of frankincense on myelosuppression have been investigated in a mouse model of Busulfan/Cyclophosphamide conditioning regimen. This study presented an interesting finding, as the injection of Ext after the chemotherapy induction enhances stem cells proliferation and improves immune system with the minimal weight loss. Further studies are warranted to see if this approach would be beneficial for stem cell transplantation.

DATA STATEMENT

We declare here that all data belonging to the represented research are reproducible and clear. All the raw data are available for sharing upon request.

ACKNOWLEDGEMENTS

Not declared.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

ETHICAL CONSIDERATIONS

The animal experiments were performed according to the guidelines of the National Committee of Bioethics and the institutional Animal Ethics Committee (Ethical approval code: VCGSR/AREC/04/2022). The animals were kept under standard laboratory conditions for housing, feeding, and breeding.

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