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Effects of autohemotherapy and β-Glucan Extract from Saccharomyces cerevisiae on hematological responses in mice

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Abstract:

Autohemotherapy and β-Glucan extract are a types of treatment that have showed its efficiency by the medical community for many reasons. In this study we aimed to evaluate the effects of authohaemotherapy and β-glucan on hematological response. We used mice (number =30, weighing =40-60g). The study consisted in a control group, and a treatment groups autohemotherapy and β-glucan extract group, blood samples were collected at the first day and at the eighth day after the application. In the all groups we collected 100 µl of blood from tail each mouse through a syringe with a previously prepared solution of sodium citrate 2%. Autohemotherapy group was direct injected 50 µl tail blood for them in the quadriceps muscle, while β-glucan extract group was injected 50 µl β-glucan extracted from *Saccharomyces cerevisiae* in the quadriceps muscle. the control group was injected 50 µl normal saline 0.9%. The complete blood count (CBC) done calculated through using Auto-hematology analyzer device. In the all groups we observed increased production of erythrocytes, hemoglobin, Leukocytes and platelet ($p<0.05$). Autohemotherapy and β-glucan extract did Significantly of hematological responses in mice.

Key words: Autohemotherapy, β-glucan, *Saccharomyces cerevisiae*, blood, hematological responses.

INTRODUCTION

Autohemotherapy, referring to the immediate intramuscular or subcutaneous reinjection of blood. Since the introduction of this method by Ravaut in 1913 **[1],** autohemotherapy has been employed in a wide range of disease. Several articles on the subject have been published in medical journals , which used against psoriasis, malaria, ebola, AIDS, Urticaria , Eczema ,allergic diseases and therapy in some treatments of erythrocytes are erythropoiesis and immune stimulators **[2,3,4,5]**, Also Autohemotherapy was applied in patients with systemic lupus erythematosus resistant to costicoesteroids [**6**]. Autohemotherapy has also been proposed as a preventive action, the reported beneficial action of autohemotherapy has been attributed to the presence of antigens in the blood which stimulate the production of antibodies when injected into the tissues , the action of autohemotherapy may be similar to that of an autogenous vaccine [**7, 8**]. In recent years, increasing attention has been to Beta glucans isolated from the cell wall of yeast such as *Saccharomyces cerevisiae*, β-glucans have been widely used in research for medical purposes **(9,10,11,12).** β-glucans are not synthesized by humans, so these compounds are recognized by our immune systems as non-self-molecules , inducing both innat and adaptive immune responses [**13**]. β-glucans belong to a class of compounds which are described as biological response modifiers , they can modulate the immune system by stimulating phagocytosis and production of pro-inflammatory cytokines [**14,15**]. They stimulate the defense mechanisms of the host against disease instead of attacking the infectious agent, so these agents remain non-toxic to the cells of the host organism [**16**]. The main functions of beta-glucans, related to stimulation of the immune system include increased host resistance to viral, bacterial, fungal and parasitic infections, as well as an anti-tumor adjuvant effect and prevention of the carcinogenicity **[17]**, Also they are used as adjuvants in the development of various types of vaccines, by boosting the cellular immune response without the toxicity exhibited by other adjuvants (**18,19**). Therefore, we aimed to evaluate the effects of autohemotherapy and β –Glucans extract from *Saccharomyces cerevisiae* injection on hematological responses in mice.

MATERIALS AND METHODS

Animals

The experiments were performed in mice (number =30, weighing =40-60g). mice were housed individually in plastic cages under standard laboratory conditions. We divided the mice into three groups: Control (n=10), Beta-glucan extract (n=10) and autohemotherapy (n=10) groups. They were kept under a 12-h light/dark cycle and had free access to food and water **[20].**

β-glucan

Beta-glucan was purified from the cell wall of *Saccharomyces cerevisae* according to Byron, 1993 **[21]**

Blood Sampling, Injection and Analysis

Blood samples was collected (100-200) µl via the tail vein with a needle injection of 1 ml with a previously prepared

solution of sodium citrate 2%, looking for a 1: 10 ratio of the volume of anticoagulant in the volume of blood collected. The animals were anesthetized in order to avoid stress during the procedure using a chloroform solution. In the autohemotherapy group we collected 50 µl of blood from tail vein each mice through a syringe was direct injected in the intramuscular. The application of blood was considered the autohemotherapy treatment. In the β-Glucans extract group we collected 50 µl of blood from each mice, and injected 50 µl β-glucan. In the control group we collected 50 µl of blood from tail vein each mice, and injected 50 µl normal saline 0.9%. this procedure was performed once in each mice. In the eighth day after the treatment of experiment, the mice were anesthetized and blood collected via jugular vein for laboratory analysis [**6,22]**. Blood samples were analyzed through complete blood count (CBC) used Auto-hematology analyzer device, Human Germany **[23].**

Statistical Analysis

All the experiments were conducted in triplicate and analysed using one way ANOVA [**24**,**25**].

RESULTS AND DISCUSSION

Hemoglobin, packed cell volume and Erythrocytes

Table 1 Showed the treatment had effect on the Hemoglobin, PCV and Erythrocytes of the mice. The control, β-glucans and Autohemotherapy injection groups of Hemoglobin were 15.0, 15.7 and 16.0 g/dl respectively, when the PCV were 50 ,52 and 53% respectively , also the Erythrocytes were 12.5 ,13.1 and 13.8 /mm^3 respectively. We observe that all parameters were significantly increase ($p<0.05$) in the treatment groups compared with the control group.

Table 1 Effects of β-glucans and autohemotherapy on Hemoglobin, PCV and Erythrocytes counts in mice.

Parameter / Groups	HB g/dl	PCV %	Erythrocytes × 10^6 /mm^3
Control	15.0±0.40 b	49.5±0.50 b	12.5±0.90 c
β-glucans	15.7±0.50 a	52.0±0.55 a	13.1±1.02 b
Autohemotherapy	16.0±0.44 a	53.0±0.78 a	13.8±1.00 a

Different lettersverticallymeansignificant differenceat thelevel of significance(P <0.05). The values representmean ±S.E.

In treated mice groups it was observed that a significantly increase occurred in hemoglobin, PCV and erythrocytes levels, β-glucans treatment group for mice has been agreement with El-Kashoury *et al* (2016**) [26] ,** there was a increase may be to the β-glucan of different origin has been demonstrated to be potent antioxidants, also there are some reports on the immune antioxidant activity relationship of glucan which may result in proliferation of bone marrow stem cells as indicated by increased in bone marrow cell count [**27,28]**. We observed significantly increase of hemoglobin, PCV and erythrocytes in autohemotherapy group for mice has been agreement with previous studies in this field on laboratory animals, They agreed with Aline *et al* (2013) **[6]** there was a increase in the hemoglobin, PCV and erythrocytes of rats compared with control group. The increase in hemoglobin, PCV and erythrocytes levels may be to the blood removal and injected is responsible for inducing blood cell production, since blood loss lead to decreased tissue oxygenation and it is a stimulus for erythropoiesis [**29**].

As shown table 2 the treatment groups had effect on Platelet of mice. The control, β-glucans and autohemotherapy groups of Platelet was 220, 300 and 350/mm^3 respectively. We observe that Platelet was significantly increase ($p<0.05$) in the treatment groups compared with the control group.

Table 2 Effects of β-glucans and autohemotherapy on Platelet in mice.

Parameter / Groups	Plt (× 10^3 /mm^3)
Control	220±1.70 c
β-glucans	300±2.00 b
Autohemotherapy	350±2.33 a

Different lettersverticallymeansignificant differenceat thelevel of significance(P <0.05). The values representmean ±S.E.

In treated mice groups it was observed that a significantly increase occurred in Platelet, β-glucans treatment group has been agreement with Kotrbacek *et al*.,(2016) **[30]** where observed Influence of β-Glucan on the aggregation of platelets in pigs. They stated that beta glucan supplementation may be beneficial in the prevention of excessive blood platelet activation-related diseases, such as cardiovascular or inflammatory diseases. We observed that autohemotherapy increased blood platelet levels, a previous study reported opposite findings in humans, where evaluated the effects of autohaemotherapy on the platelet function in chronically haemodialysed patients. They found that autohemotherapy induce platelet aggregation [**31].**

Table 3 Showed the treatment groups had effect on the total and differential leukocytes in mice. We observe that total Leukocytes were significantly increase ($p<0.05$) in the treatment groups compared with the control group, while We observe that Neutrophils, Lymphocytes and Monocytes were significantly increased ($p<0.05$) , Eosinóphils was significant decreased, also Basophils was no significant in the treated groups compared with the control group.

Table 3 Effects of β-glucans and autohemotherapy on Total and differential Leukocytes in mice.

Parameter / Groups	Leukocytes × 10^3/µL	Neutrophils %	Lymphocytes %	Monocytes %	Eosinóphils %	Basophils %
Control	8.70±1.00 b	23.0±0.08 b	73.0±0.02 b	2.0±0.03 b	1.7±0.02 a	0.3±0.06 b
β-glucans	10.2±1.11 a	24.0±0.04 a	73.5±0.03 a	2.1±0.03 b	0.4±0.07 b	0.0±0.04 b
Autohemotherapy	10.0±1.03 a	23.8±0.05 a	73.4±0.03 a	2.4±0.02 a	0.3±0.05 b	0.1±0.08 b

Different lettersverticallymeansignificant differenceat thelevel of significance(P <0.05). The values representmean ±S.E.

The reason significantly increased for Leukocytes may be due to beta glucans immunostimulatory agents of the Pattern Recognition Receptors can deliver the antigen into cells and induce an antigen-specific immune response [**19**]. beta-glucans present interesting immunomodulating properties as for vaccine development and further research should test the limits for its applications. Beta-glucans extracted from *Saccharomyces cerevisiae*are important bioactive compounds for animal and human health, glucan could stimulate animal cells proliferation, promote cytokine secretion and enhance antibody titer of vaccine **[32]**. The immunological potency of beta-glucans also can be associated with their ability to activate leukocytes **[33]** In general, it has been suggested that beta-glucans of high molecular weight can directly activate leukocytes, stimulating their phagocytic **[34]**.

The results of autohemotherapy group agrement with Silva *et al* (2009) **[12]**, were found an increase in leukocyte count in mice compared to the control group. The autohemotherapy is able to increase the differential count of leukocytes, clearly demonstrating the contribution to the defense system of the organism **[35] .** However, not always the amount of leukocytes directly influences in the effectiveness of the immune system, because there is also action of mediators, cytokines, growth factors, antibodies **[36].**

CONCLUSION

Autohemotherapy and β-glucans injection had effect on the hematological responses in mice.

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