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Full Length Research Paper

Effect of ginger rhizome extract on lymphocytopenic guinea pig with azathioprine

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The objectives of this study were to investigate quantitative alterations in leukocytes and immunosuppressive effects of ginger rhizome associated with oral administration of Azathioprine (AZA) on guinea pig model. This study was carried out at the Al Jouf University during the period from March to April 2013. A total of twelve guinea pigs randomly divided into three groups (four guinea pigs each) were used in this study. Group I, received daily oral administration of 50 mg/kg body weight of AZA in combination with 50 mg/ml of ginger rhizome extract for five days; Group II was treated with 50 mg AZA daily for five days, while Group III was used as a control group. Hematological alterations associated with ginger rhizome and AZA were measured using standard hematological and data analysis techniques. Ginger rhizome in combination with AZA was found to enhance the reduction in the lymph (%) compared to AZA treatment ($P < 0.05$). Lymphocyte was significantly lower in group II (AZA) and group I (AZA + ginger) ($P < 0.05$), 13 and 18%, respectively than the control group, 38%. This may be due to either inhibition in one of the lymphopoiesis growth factors or shortening the life span of lymphocytic cells by the action of AZA. The results of the present study indicated an immune suppressive effect of ginger rhizome extract associated with immunosuppressive agent (AZA) with mainly lymphocytopenia. The current findings suggest that ginger may have a role in either the stimulation of lymphopoiesis or discontinue the effect of AZA, as the percentage of lymphocytes was significantly higher in group II versus group I after mixing up AZA + ginger. Accordingly, further studies would be useful to estimate lymphocytic growth factors to know the relationship between ginger, AZA and lymphopoiesis.

Key words: Azathioprine, ginger rhizome, blood cell counting, hematological alterations.

INTRODUCTION

Azathioprine (AZA) is an immunosuppressive drug used in organ transplantation and autoimmune diseases and belongs to the chemical class of purine analogues (American Society of Health-System Pharmacists, 2012). When an immunosuppressive agent such as AZA is given to living beings, it will develop a leukocytopenia condition as reported by Kumar et al. (1989). This model can be utilized as immunocompromised hosts to study a to study a variety of bacterial and viral infections as well as aspects related to immune suppression. The alterations

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which happened in the hematological parameters due to such treatments, can be used as indicators of health and disease. Particularly, the changes in leukocytic parameters measure the immunity and immune suppression conditions. Ginger root which is the rhizome of the plant *Zingiber officinale* commonly known as ginger belongs to the family Zingeberaceae. The rhizome of *Z. officinale* is commonly known for its protective effect on health when used in traditional medicine. It has been used to treat headaches, rheumatism, burns, peptic ulcer, dyspepsia, depression and impotence (Tyler and Robbers, 1999). The oleoresin from the rhizome has been shown to have anti-inflammatory, antipyretic, antihepatotoxic, analgesic and cardiotonic properties (Surh, 1999). Mustafa et al. (1993) found it is able to inhibit the activity of cyclooxygenase and lipoxygenase and hence decrease the pain in rheumatism and headaches. Another effect of *Z. officinale* found by Topic et al. (2002) showed that it could inhibit lipid peroxidation by maintaining the levels of antioxidants. Several studies had also found the antiemetic, anticholesterolaemic and antiplatelet effects of *Z. officinale* (Guh et al., 1995; Tanabe et al., 1993; Mowrey and Clayson, 1982). According to a real world study of Imuran and Ginger drug interactions, a study created by eHealthMe© based on reports from the Food and Drug Administration (FDA) showed that there is no interaction effect found between AZA and ginger. However, the interaction effect of ginger rhizome with immunosuppressive agents such as cyclosporine and AZA on investigated subjects was not affirmed. AZA strongly affects proliferating cells, such as the T and B cells of the immune system because it acts as a pro-drug for mercaptopurine, inhibiting an enzyme that is required for the synthesis of DNA (Maltzman and Koretzky, 2003; Patel et al., 2006). The main adverse effect of AZA is bone marrow suppression, which can be life-threatening, especially in people with a genetic deficiency of the enzyme thiopurine S-methyltransferase (Evans, 2004). It is listed as a carcinogenic to humans by the International Agency for Research on Cancer (1987). AZA is used to prevent rejections following kidney or liver transplantation, usually in conjunction with other immunosuppressive therapy, corticosteroids, and local radiation therapy (Nuyttens et al., 2005; Remuzzi et al., 2004). This study was carried out on the leukocytopenic model in guinea pig to know drug interactions between AZA and ginger through observing the hematological changes that happened to the model animal as a result of the AZA and ginger treatments.

METHODOLOGY

This study was carried out at the animal research facility and laboratories of the College of Applied Medical Sciences (CAMS) at Al Jouf University during the period of March to April, 2013. Hematological alterations associated with ginger rhizome and AZA were measured using standard hematological and data analysis techniques.

Extraction preparation

A total of 0.5 kg fresh ginger rhizomes were washed with water and then sliced to small pieces and then squeezed with an electric squeezing machine. One milliliter of the obtained liquid was evaporated under reduced pressure at 70°C. The weight of the dried powder was found to be 50 mg/ml. This was used as the concentration of the ginger extract.

Animal care and use

A total of twelve guinea pigs with average weight of 1.0 kg were used in this study. All procedures were performed in compliance with relevant laws and National Committee of Bioethics (NCBE) guidelines and as per Al Jouf University Institutional Bioethics Local Committee. The total blood volume of a guinea pig equals 4.5 to 8.3% of the body mass being blood (69 to 75 ml/kg body mass). The animals were randomly divided into three groups (four guinea pigs each); Group I received daily oral administration of 50 mg/kg body weight of AZA in combination with 50 mg/ml of ginger rhizome extract for five days, Group II was treated with 50 mg/kg body weight of AZA daily for five days, and Group III was a control group which received no AZA nor ginger. The animals were kept individually and all of them were examined on a daily basis for signs of morbidity and mortality rate if any.

Blood collection and analysis

All guinea pigs were anesthetized with chloroform and then sacrificed by decapitation. About 4 ml of blood was collected in EDTA tubes for the Complete Blood Count (CBC). CBC was measured by automated blood cell counter SYSMEX SE-9500 at the hematology laboratory at the College of Applied Medical Sciences (CAMS). The data of the hematological parameters were analyzed for variable data using t test between different treatments and groups by GRAPHPAD software. A two-tailed p value < 0.05 was considered statistically significant.

RESULTS

The leukocytic count in the three groups showed significant variations in most of the hematological parameters as shown in Table 1. The level of white blood cells (WBCs) and lymph% in treated animals with AZA, AZA plus ginger rhizome, and the control group are shown in comparison with normal reference ranges in guinea pigs. Ginger rhizome in combination with AZA was found to enhance the reduction in the lymph% compared to AZA treatment ($p<0.05$). An increase in platelets counts was also observed with treatment of ginger rhizome extract in combination with AZA compared with the AZA alone.

The variations of the blood cell count between the AZA and control groups were found to be statistically significant in certain leukocytic parameters including Lymph, Gran, and Mid, but not in total WBC. Comparison between the AZA group and AZA plus ginger group showed significant variations in WBCs, Lymph%, and Gran% as shown in Tables 1 to 4 and Figure 1. The mean levels of cell counts showed variations with statistical significant in WBCs, Lymph%, Gran%, and Mid%.

Table 1. Leukocyte count after oral administration of 50 mg/kg body weight of AZA. Mean values ±SD of the hematological parameters in control group and AZA treatment group.

Parameter	Mean ± SD			*p value
	AZA	AZA + ginger	control	
WBCs/µl	8.73 ± 0.19	14.90 ± 3.16	8.40 ± 0.60	>0.05*
Lymph%	18.33 ± 1.25	13.13 ± 2.03	37.88 ± 4.93	<0.05*
Gran%	57.28 ± 1.80	61.53 ± 2.43	41.98 ± 2.40	<0.05*
Mid%	2.30 ± 0.14	3.33 ± 1.00	1.678 ± 0.25	<0.05*

*****Significance was considered at p value <0.05.

Table 2. Comparison between alterations which happened in different treatments categorized based on type of leukocytic parameters (treatments include AZA treatment versus control, and AZA plus ginger versus azathioprine).

Treatment	Category/Parameter	*p value
AZA versus control	Leukocytes	<0.05
AZA + ginger versus AZA	Leukocytes	<0.05

*****Significance was considered at p value <0.05.

Table 3. Normal and abnormal level of the blood parameters for groups given AZA alone, AZA plus ginger versus control group.

Parameter	AZA	Control	AZA + Ginger
WBCs 10^9/µl	Normal (8.73)	Normal (8.40) (2 to 14)	Abnormal (14.90)
Lymph%	Abnormal (18.33)	Normal (37.88) (28 to 84%)	Abnormal (13.13)

*Normal range are based on reference ranges for LABOKLIN© laboratory parameters in Guinea pigs.

Table 4. Mean level of the blood parameters for animals given AZA plus ginger rhizome versus control group.

Parameter	Mean ± SD		p value
	Group 2 (AZA)	Group 3 (control)	
WBCs 10^9 μl^{-1}	8.73 ± 0.19	8.40 ± 0.60	>0.05
Lymph%	18.33 ± 1.25	37.88 ± 4.93	<0.05
Gran%	57.28 ± 1.80	41.98 ± 2.40	<0.05
Mid%	2.30 ± 0.14	1.678 ± 0.25	<0.05
	Group 1 (AZA + ginger)	**Group 3 (control)**	
WBCs 10^9 μl^{-1}	14.90 ± 3.16	8.40 ± 0.60	<0.05
Lymph%	13.13 ± 2.03	37.88 ± 4.93	<0.05
Gran%	61.53 ± 2.43	41.98 ± 2.40	<0.05
Mid%	3.33 ± 1.00	1.678 ± 0.25	<0.05
	Group 1 (AZA + ginger)	**Group 2 (AZA)**	
WBCs 10^9 μl^{-1}	14.90 ± 3.16	8.73 ± 0.19	<0.05
Lymph%	13.13 ± 2.03	18.33 ± 1.25	<0.05
Gran%	61.53 ± 2.43	57.28 ± 1.80	<0.05
Mid%	3.33 ± 1.00	2.30 ± 0.14	>0.05

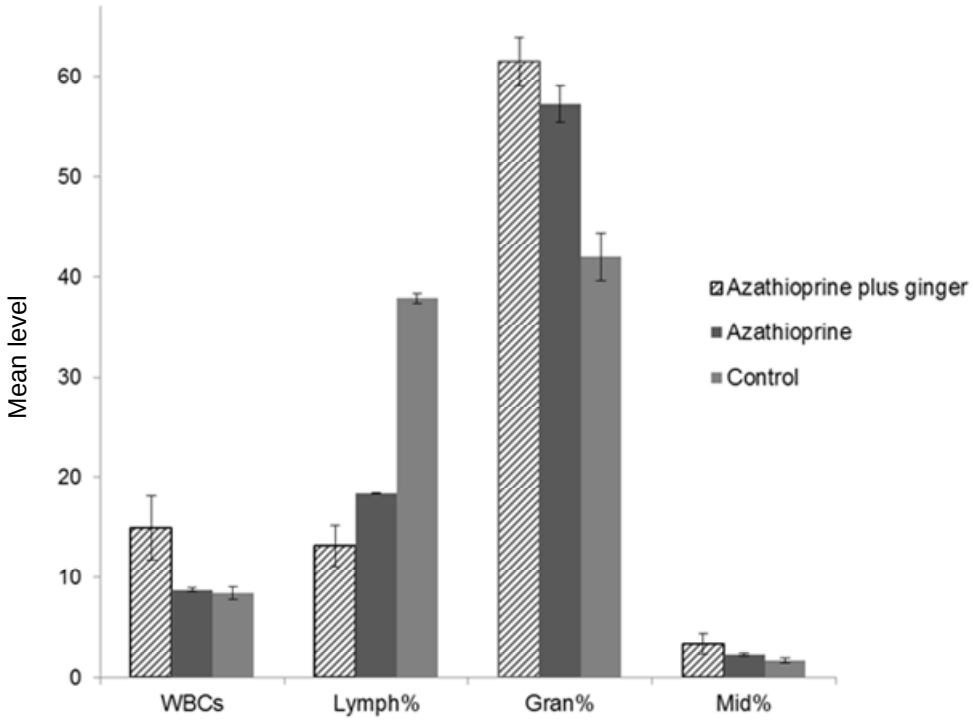

Figure 1. Mean levels of leukocytic parameters for subject with AZA, AZA plus ginger and control group.

DISCUSSION

The observed hematological alterations associated with ginger treatment revealed information about the enhancing immune suppressive effect of ginger rhizome associated with the immunosuppressive agent (AZA). The results of the present study suggest that an extract of ginger rhizome enhances the immune suppression effect on the lymphocytopenic guinea pig model with AZA. Lymphocytopenia was very obvious when we have added doses of ginger to the AZA treatment. The variations in differential WBCs due to the use of AZA and ginger rhizome are statistical significant in WBCs, Lymph%, Gran%, and Mid%. These variations revealed information about the enhancement effect of the extract of ginger rhizome with immunosuppressive agents. Leukocytic blood changes could be interpreted as a result of bacterial and viral infection. Lymphoid haemopoiesis is the process in which lymphocytes (B cells and T cells) develop from progenitor cells. B cell is completed in the bone marrow (BM), whereas T cell lymphopoiesis occurs in the thymus. Their proliferation process is well regulated by growth factors for instance cytokines, insulin-like growth factor-1 and steroid hormones (Clark et al., 1993; Ichii et al., 2008). For example, interleukin-7 interacts with stem cell factor to start this process successfully and steroid hormones influence steady-state rates of lymphocyte production in BM (Hirose et al., 2002). The alterations in these growth factors might affect lymphopoiesis by either elevation or diminishing the lymphocytes count. Here, lymphocyte was significantly lower in the group-2 (azathioprine) and group-I (azathioprine + ginger) ($p<0.05$), 13 and 18%, respectively than the control group, 38% (Table 1). This may be due to either inhibition in one of the lymphopoiesis growth factors or shortening the life span of lymphocytic cells by the action of AZA.

The current findings suggest that ginger may have a role in either the stimulation of lymphopoiesis or discontinue the effect of AZA, as the percentage of lymphocytes was significantly higher in Group II versus Group I after mixing up AZA + ginger. Accordingly, further studies would be useful to estimate lymphocytic growth factors to know the relationship between ginger, AZA and lymphopoiesis with a higher sample size and detailed differential WBCs for warranted and confirmed results.

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Conflict of Interest

The author(s) have not declared any conflict of interests.

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